Bacheloroppgave

NTNU
Norges teknisk-naturvitenskapelige universitet
Fakultet for naturvitenskap
Institutt for bioingeniørfag

Eileen Lau

Accuracy of bromocresol green (BCG) method for plasma albumin

Riktigheten av bromokresolgrønn (BCG) metoden for albumin i plasma

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Veileder: Kristin Nørsett, Kristin Graven, Arne Åsberg

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Abstract

Albumin concentration can be measured using several methods such as bromocresol green method and immunonephelometric method. Results measured by different methods may vary to some extent especially at lower albumin concentration. It is thus important to know how much it is differed from the immunonephelometric (reference) method. The aim of this study is to investigate and assess albumin concentrations measured by bromocresol green method (AlbBCG) and immunonephelometric method (AlbNEPH).

A total of 204 anonymous patient samples were selected randomly and measured in 2 analytical instruments. Advia Chemistry XPT applies BCG method while Atellica NEPH 630 applies immunonephelometric method in the albumin analysis. Other parameters such as age, gender and creatinine concentrations were noted. Statistical analysis was performed by using MedCalc to analyse correlation between the variables and regression analysis.

The mean of AlbBCG and AlbNEPH was significantly different based on the t-test analysis. There was also a strong correlation ($r = 0.944$, $p<0.0001$) between both methods. Passing-Bablok regression model and Bland-Altman analysis showed that there was a systematic error which led to a difference of AlbBCG and AlbNEPH. Further investigations using multiple linear regression showed that there was a linear relationship with good correlation ($r = 0.59$, $p<0.0001$) between difference of AlbBCG and AlbNEPH and mean of both methods. There was no correlation between age, gender, creatinine concentration and difference of AlbBCG and AlbNEPH. Difference of AlbBCG and AlbNEPH was possibly due to the overestimation by AlbBCG which produced positive mean difference. With the difference in the albumin results, it is important to evaluate the different albumin measurement methods used in laboratories.

Sammendrag

Albuminkonsentrasjon kan måles ved bruk av flere metoder, så som bromokresolgrønn metode og immunefelometrisk metode. Resultater målt ved forskjellige metoder kan variere til en viss grad, spesielt ved lavere albuminkonsentrasjon. Det er dermed viktig å vite hvor mye det skiller seg fra immunefelometrisk- (referanse) metoden. Målet med denne studien var å undersøke og vurdere albuminkonsentrasjoner målt ved bromokresolgrønn metode (AlbBCG) og immunefelometrisk metode (AlbNEPH).

Totalt 204 anonyme pasientprøver ble valgt tilfeldig og målt i 2 analyseinstrumenter. Advia Chemistry XPT bruker BCG-metoden, mens Atellica NEPH 630 anvender immunefelometrisk metode i albuminanalysen. Andre parametere som alder, kjønn og kreatininkonsentrasjoner ble notert. Statistisk analyse ble utført ved å bruke MedCalc for å analysere sammenheng mellom variablene og regresjonsanalyse.

Gjennomsnittet av AlbBCG og AlbNEPH var signifikant forskjellig basert på t-test analysen. Det var også en sterk korrelasjon ($r = 0,944$, $p < 0,0001$) mellom begge metodene. Passing-Bablok regresjonsmodell og Bland-Altman-analyse viste at det var en systematisk feil som førte til en forskjell mellom AlbBCG og AlbNEPH. Ytterligere undersøkelser ved bruk av multippel lineær regresjon viste at det var en lineær sammenheng med god korrelasjon ($r = 0,59$, $p < 0,0001$) mellom forskjellen mellom AlbBCG og AlbNEPH og gjennomsnittet av begge metodene. Forskjell på AlbBCG og AlbNEPH skyldtes muligens overvurderingen av AlbBCG som ga positiv middelforskjell. Med en slik forskjell i albuminresultatene, er det viktig å evaluere de forskjellige albuminmålingsmetodene som brukes i laboratorier.

Acknowledgement

I would like to sincerely thank all the staff in Department of Clinical Biochemistry in St Olav Hospital, I appreciate all your help in analysing the samples. I would also like to thank Kristin Graven for gathering all the data and giving some helpful advices. I am really grateful for Arne Åsberg for his statistical guidance and supervision.

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Most of all, I am very thankful to my parents and family and Albert who have been continuously supporting me throughout my work.

List of abbreviations

4-AAP	4-aminoantipyrine
HMMPS	N-(3-sulfopropyl)-3-methoxy-5-methylaniline
BCG	Bromocresol Green
NEPH	Immunonephelometry
AlbBCG	Albumin concentration measured by Bromocresol Green method
AlbNEPH	Albumin concentration measured by Immunonephelometric method
CI	Confidence interval

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1.0 Introduction

The human blood is divided into two major components; about half of it consists of plasma proteins and another half consists of blood cells. Plasma proteins is one of the most important biological components. There are thousands of different proteins with unique sizes, molecular structure, solubility as well as function and they are made up of organic compounds called amino acids. Amino acid sequence are the ones that determine different types of proteins and they are bound together by peptide bonds to form longer amino acid chains. Some of these amino acid chains will undergo protein folding, which is the interaction and binding within the protein to form different structure. As a result, their functions are determined structurally. Fibrous protein and globular protein are the two main protein classification. Globular proteins such as plasma proteins, enzymes, haemoglobin and peptide hormones gain more clinical interest these days (1).

One of the most common globular proteins being tested is albumin and this can be tested through blood drawn from patients. These tests are performed automatically in clinical laboratories on the analytical instruments and it is closely monitored by laboratory personnel. It is crucial that every laboratory establishes their own standard procedure in order to ensure quality in all laboratory results. One of the challenges that is common in a laboratory setting is quality and it includes accuracy and precision of test results.

1.1 Albumin

1.1.1 Biochemistry and Function

Albumin is a small, water soluble, globular protein circulating in the human blood plasma. It has a molecular mass of 66.3kD, consists of 585 amino acid with a negative charge at normal pH and has binding sites for other molecules, see figure 1.

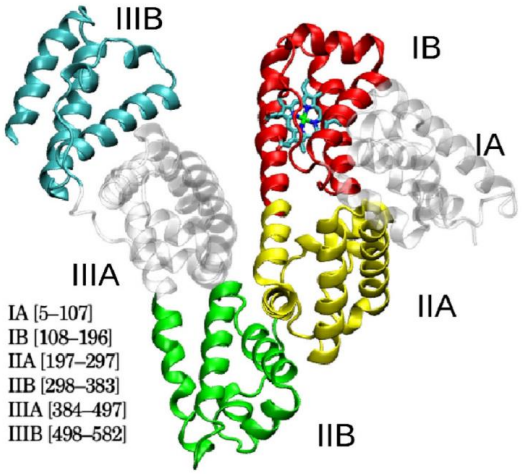


Figure 1: The molecular structure of albumin (2)

Albumin is synthesized in the liver and is excreted into the bloodstream. Around 60% of albumin can be found in the extravascular fluids, for example cerebrospinal fluid, interstitial fluid, and amniotic fluid. The main function of albumin is to maintain colloidal osmotic pressure between vascular and extravascular space. It is structurally equipped with binding sites for other molecules such as fatty acids, bilirubin, calcium, and hormones. These albumin-bound molecules are then transported between the blood vascular system (1,3,4).

1.1.2 Clinical Significance

Laboratory results are important in clinical diagnosis. High albumin concentration in the blood or hyperalbuminemia indicates acute dehydration with no known clinical significance. Albumin level has been used to monitor and detect the patient's nutritional status. A study mentions that albumin is shown as a highly sensitive marker for patient's nutritional status (4).

On the contrary, a low albumin concentration in the blood or hypoalbuminemia indicates acute and chronic inflammation. A decrease in albumin level is seen in most cases of hepatic diseases, kidney diseases and inflammatory disease of the intestinal tract. Besides, there will also be a decrease in albumin concentration in some situation where patients develop edema or ascites.
Albumin is an indicator and biochemical marker for patients with chronic kidney disease and there are also studies conducted on patients under dialysis treatment by analysing their albumin levels (5,6). In a healthy individual, albumin remains in the bloodstream and is not eliminated through

urine. As mentioned earlier, albumin is a small-sized molecule and has the potential to leak out into the urine. One of the reasons is caused by kidney disease or nephrotic syndrome where the glomerular basement membrane is damaged. An increasing protein in urine or proteinuria will result in hypoalbuminemia. This is the reason why patients with kidney disease who are undergoing dialysis treatment, should routinely monitor their albumin level. Calcium is another component frequently tested among these patients because it is highly affected by the albumin concentration and the fact is that around 50% of the calcium is bound onto albumin. Calcium concentration is adjusted based on the corrected calcium formula especially when albumin concentration is abnormally low. Studies have been conducted on the relationship between calcium and albumin, for instance, the importance of using albumin in adjusting calcium levels. There are a few published corrected calcium formulas which is widely used in most laboratories. One of them is derived from albumin-bromocresol green method in the 70s by Orell et al. (7). They found out that low albumin levels affect the concentration of total calcium and therefore it is common to measure total calcium and correct it based on the albumin concentration. However, there are studies that claim the albumin-adjusted calcium formula as unnecessary in certain groups of patients. A study shows that adjusted calcium concentration is not reliable in the intensive care setting and alternative measurement method should be used instead (8).

1.1.3 Analytical methods

Common laboratory testing for albumin is based on automated dye-binding or colorimetric method and immunonephelometric method. Bromocresol green (BCG) and bromocresol purple (BCP) assays are more widely used than immunonephelometric assays in laboratories to measure albumin levels. In BCG and BCP assays, albumin molecules are bound by the dye molecules and this causes a change in absorbance. The absorbance is then detected by a spectrophotometer with specific wavelength range to determine the albumin concentration. Immunonephelometric method is based on antigen and antibody reaction and the amount of complex molecules is detected by light scattering or nephelometry.

1.1.4 Pros and Cons of using different analytical methods

The three types of assays mentioned are the currently used methods but there seems to be a lack of standardization of the albumin assay. Studies have shown that there are quite a few limitations between the different methods (9,10). Research are still ongoing, and results are studied in order to come up with a standardized analytical methodology for albumin assay.

Albumin analysis by bromocresol green (BCG) method (AlbBCG)

AlbBCG is common and widely used as a routine test because it is relatively less costly. It can perform large number of samples at the same time (11). Albumin molecule has a high affinity towards the binding site of BCG dye molecule (1).

However, some studies showed that AlbBCG overestimates albumin concentration especially at low albumin concentration (1,5,6,11–14). A study also claims that higher levels of albumin tend to be underestimated by AlbBCG (11). In addition, AlbBCG produces positively biased results especially in hypoalbuminemia (5,12,15). These inaccurate results will have an effect in clinical decision-making such as inappropriate diagnosis and treatment (14).

Analytical interference is the main factor of the inaccuracy in AlbBCG, according to several studies (1,13,15,16). One of the earliest studies claims that α-, β-globulins and bilirubin can interfere with the binding of BCG and albumin (13). Bruns et al. (1) claimed that when the overall serum protein pattern is abnormal, it will lead to inaccurate results. Besides, it also mentions that the cause of inaccuracy is possibly due to presence of fibrinogen and heparin in the sample. The same study also suggests the use of immunochemical quantification for better accuracy (1). Recent study by Garcia et al. (15) indicated that α-globulins which are the acute phase proteins, are the cause of interference. In a healthy individual, plasma proteins consist of mostly albumin and a considerably small amount of globulin. Albumin levels will not be accurate and are potentially overestimated in conditions such as patients who have hypoalbuminemia and those who are experiencing inflammation when there is an increase their serum globulin. In addition, the study further identified which type of α-globulins that contributes to the factor; the subtypes of the α-globulins: α_1- and α_2-globulins. A study that involves patients with nephrotic syndrome with hypoalbuminemia, shows that α_2-macroglobulin and haptoglobins (which are α_2-globulins) can bind onto BCG molecules (16).

Albumin analysis by immunonephelometric method (AlbNEPH)

AlbNEPH is known to be able to estimate albumin accurately when the level is low. It is also more specific than AlbBCG and there is less interference as well as cross reactivity from other proteins (6,14). It is widely known that AlbNEPH is used as a reference method in comparison with other methods to analyse albumin results because of its accuracy. However, AlbNEPH is less commonly used in most laboratories in routine testing due to the high cost and it requires more sophisticated instrumentation (6). Although AlbNEPH is more accurate and precise compared to AlbBCG, the analysis time for AlbNEPH is longer than that of AlbBCG (17).

Table 1: Overview of pros and cons of using AlbBCG and AlbNEPH.

Method	Pros	Cons
AlbBCG	Low Cost Short analysis time Able to test large amount of sample	Low specificity Low accuracy Affected by interference
AlbNEPH	High specificity High accuracy Less affected by interference	High cost Longer analysis time Not able to test large amount of sample

Correlation between AlbBCG and AlbNEPH

A lack of standardization in albumin assay has resulted in several studies trying to find correlation between the 2 methods (5,16). Some studies have consistently showed that there is a good correlation between AlbBCG and AlbNEPH (6,12,16). Good correlation between AlbBCG and AlbNEPH has also been observed for different patient conditions; with normal kidney functions, with nephrotic syndrome and patients undergoing dialysis treatment (12,16).

1.2 Creatinine

In clinical diagnostic, it is common and necessary to perform several tests from each blood sample drawn or even more blood samples to ensure that the test results are reliable before a conclusion is drawn. In addition, tests that are relevant and inter-related are included in a blood test panel

which is a series of tests necessary to assess health condition of a given person. Renal panel which is an important test panel to assess kidney's condition consists of both albumin and creatinine tests.

1.2.1 Biochemistry, Function and Clinical Significance

Creatinine is a cyclic anhydride molecule with a molecular mass of 113D. It is produced primarily in the kidney as a final product that resulted from the degradation of creatine and phosphocreatine. Creatinine is widely used to assess renal function. Healthy individuals do not excrete creatinine into the urine because it is reabsorbed by the glomerulus. There are cases where patients who develop kidney failure exhibit abnormal creatinine level. This is due to the small creatinine size, it can easily pass through the damaged glomerulus and excreted out into the urine. Therefore, creatinine is used as a biomarker or indicator of kidney function (1). Creatinine values are also used to predict estimated glomerular filtration rate or eGFR. eGFR and albumin levels are somewhat correlated in a study and the study claims that albumin concentration exhibits positive correlation with eGFR (18). Creatinine can also be used to measure muscle mass as the level is directly proportional to the level of free creatine in muscle (19).

1.3 Influence of age and gender on albumin concentration

Age and gender are the two main biological variables that may influence albumin concentration (20). A change in albumin concentration can be seen in some cases especially in older people, but the findings are however quite inconsistent (21–23). One of the factors may be due to patient sampling in which studies may include some older people with underlying sickness such as hypoalbuminemia, and some with perfect health condition. Several studies show that there is a weak negative correlation between albumin concentration and age i.e. an increase in age leads to a decrease in albumin levels (21–23). However, a study reveals that age does not contribute to the decrease in albumin (24). There is still lack of studies regarding the effect of gender and albumin levels. A related study between mortality and albumin levels with the association of gender differences indicates that men tend to have higher predictive value of low albumin than women (21).

1.4 Problem

The objective of this study is to identify accuracy of albumin concentration measured by bromocresol green method (BCG) and immunonephelometric method (NEPH). This is done by studying 204 patient blood samples tested both by BCG and NEPH. Correlation between the two methods is to be investigated. Other variables such as age, gender and creatinine are also considered in the investigation.

Questions for the investigation when measuring plasma albumin level:

- Is there a difference between BCG method and NEPH method?
- Is there any bias from the methods?
- Is the BCG method and NEPH method correlated?
- Do other variables (age, gender, creatinine) have influence towards albumin concentration?

2.0 Materials and Methods

2.1 Samples

In this study, a total of 204 patient blood samples were randomly selected and analysed in the Department of Clinical Chemistry in St Olav's Hospital, Trondheim (Appendix 11). All patient information remained anonymous but only the year of birth and gender were disclosed. The blood samples were labelled by a series of numbers, separated into plasma aliquots, and analysed accordingly in two analytical instruments, namely Advia Chemistry XPT and Atellica NEPH 630[1].

2.2 Analytical instruments

2.2.1 Advia Chemistry XPT

Advia Chemistry XPT (Siemens), see figure 2, is an automated clinical chemistry instrument based on spectrophotometric principle.

Figure 2: Advia Chemistry XPT (Siemens) is one of the analytical instruments used to process and analyse samples presented in the current study. (25)

For Advia Chemistry XPT albumin analysis, the reagent used is Bromocresol Green (BCG) which binds to the albumin molecules and produces albumin-BCG-complex at pH 4.2. The albumin-BCG-complex is measured in absorbance at wavelength 596nm and it is directly proportional to the albumin concentration. Unlike albumin, the creatinine analysis is based on enzymatic and colorimetric method. It follows a stepwise reaction where creatinine is first hydrolysed into

[1] All laboratory work was done by the laboratory personnel in the Department of Clinical Chemistry at St Olav's Hospital Trondheim.

creatine by creatininase. Creatine is then hydrolysed into sarcosine by creatinase. Sarcosine is converted into glycine, formaldehyde, and hydrogen peroxide in the presence of oxygen and sarcosine oxidase. The hydrogen peroxide, together with 4-aminoantipyrine (4-AAP) and N-(3-sulfopropyl)-3-methoxy-5-methylaniline (HMMPS), are catalysed by peroxidase to form blue coloured complexes which are then measured at the wavelength of 596nm. The coloured complex is directly proportional to the creatinine concentration in the sample (26–28). Detailed analytical procedures in the instrument can be found in manufacturer manual (26).

2.2.2 Atellica NEPH 630

Atellica NEPH 630 (Siemens), see figure 3, is an automated instrument based on immunonephelometric principle.

Figure 3: Atellica NEPH 630 (Siemens) is one of the analytical instruments used to process and analyse samples in the current study (29).

For Atellica NEPH 630 albumin analysis, albumin molecules form complexes with specific antibodies in an antigen-antibody reaction. Based on the nephelometric measuring principle, these immune complexes scatter the light that passes through the sample. The scattered light intensity is detected between the angles of 13° – 24° and these are proportional to the albumin concentration in the sample (30,31). Detailed analytical procedures in the instrument can be found in manufacturer manual (31).

2.3 Reagents

The reagents, controls and calibrators used are different for both instruments. R1, the main reagent containing bromocresol green dye and sodium azide, NaN_3 (Siemens) was used in the albumin analysis in Advia Chemistry XPT (Siemens). Siemens Chemistry Calibrator and Autonorm Clin Chem (L2 & L3) were used as standard and controls, respectively. In the same instrument, creatinine analysis was performed by using the reagents R1 (creatinase, sarcosine oxidase, HMMPS) and R2 (creatininase, 4-AAP, peroxidase, NaN_3) (Siemens). Siemens Chemistry Calibrator and Autonorm Clin Chem (L2 & L3) were also utilized as standard and controls, respectively. N-diluent, N-antiserum mot albumin, N-reaction buffer (Siemens) were the reagents used in the albumin analysis in Atellica NEPH 630. N-protein Standard SL was used as calibrator while Autonorm Clin Chem (L1 & L3) were used as controls (27,28,30).

2.4 Ethical Consideration

There was no informed consent from patients involved in the study. Samples were selected based on random selection from the laboratory and the samples were kept anonymous. All laboratory personnel have the duty of confidentiality.

2.5 Statistical methods

All results were recorded into Microsoft Excel spreadsheet and the statistical analysis was conducted mainly by using Excel and MedCalc statistical program[2]. Among other statistical methods, Passing-Bablok regression, Bland-Altman plot and Multiple regression applications were applied in this study.

[2] MedCalc is a statistical analysis tool which is downloaded from the website medcalc.org.

3.0 Result

The albumin concentration results extracted from both instruments were analyzed based on different parameters (Appendix 11). All albumin results analysed in Advia Chemistry XPT instrument were corrected by factor i.e. $Albumin_{corrected}$ = 0.95*Albumin (27,32). This correction is based on NORIP's reference range and has been introduced only to the Advia Chemistry XPT instrument which uses BCG method in albumin analysis (32). All calibrator and control results were approved before analyzing the samples. Result analysis was mainly done by observations through tables and figures generated by a combination of statistical programs, which were Excel and MedCalc.

Results were first processed by analyzing the mean of all the variables involved in this study, see Table 2. The mean age of the total sample was 58.8 ± 18.3 years old and this suggested that the samples consisted of large numbers of older people. A large variation in the creatinine concentration was observed in which the mean creatinine level was 119.17 ± 158.6 µmol/L. Reference range of albumin concentration varies with age and genders. The normal range of albumin is around 35 g/L to 50 g/L while concentration that is less than 30 g/L is considered hypoalbuminemia (33). Mean AlbBCG (36.8 ± 6.76 g/L) was slightly higher than mean AlbNEPH (31.2 ± 8.82 g/L) while the overall mean for both AlbBCG and AlbNEPH was 34.1 ± 7.66 g/L which was within the albumin reference range. Mean difference between AlbBCG and AlbNEPH was 5.7 ± 3.54 g/L.

Table 2: Mean for different variables based on gender.

Characteristics	Male (n = 104)	Female (n = 100)	Total (n = 204)
Mean age	60.2 ± 18.1	57.3 ± 18.4	58.8 ± 18.3
Mean creatinine level (µmol/L)	149.0 ± 198.0	89.1 ± 94.7	119.7 ± 158.6
Mean AlbBCG level (g/L)	37.7 ± 6.20	36.2 ± 7.25	36.8 ± 6.76
Mean AlbNEPH level (g/L)	31.9 ± 8.56	30.6 ± 9.08	31.2 ± 8.82
Mean of AlbBCG and AlbNEPH (g/L)	34.8 ± 7.26	33.4 ± 8.02	34.1 ± 7.66
Mean difference between AlbBCG and AlbNEPH (g/L)	5.8 ± 3.57	5.6 ± 3.51	5.7 ± 3.54

The focus of this study includes t-test, method comparison and multiple regression. T-test is used to analyze whether there is a difference between the albumin measurement methods. Method comparison studies involve mainly regression analysis while the multiple regression analyzes the relationship between various parameters.

3.1 T-test

Hypothesis test with student paired sample t-test was investigated to roughly estimate the differences between AlbBCG and AlbNEPH (Appendix 1). The null hypothesis showed that there was no difference between the mean albumin concentration measured by the AlbBCG and the mean measured by the AlbNEPH. The alternative hypothesis showed that there was a difference between both means. The t-test showed that the absolute statistic t value was larger than the absolute observed t value with the p-value lower than 0.05. This means that the null hypothesis was rejected since there was a significant difference between AlbBCG and AlbNEPH.

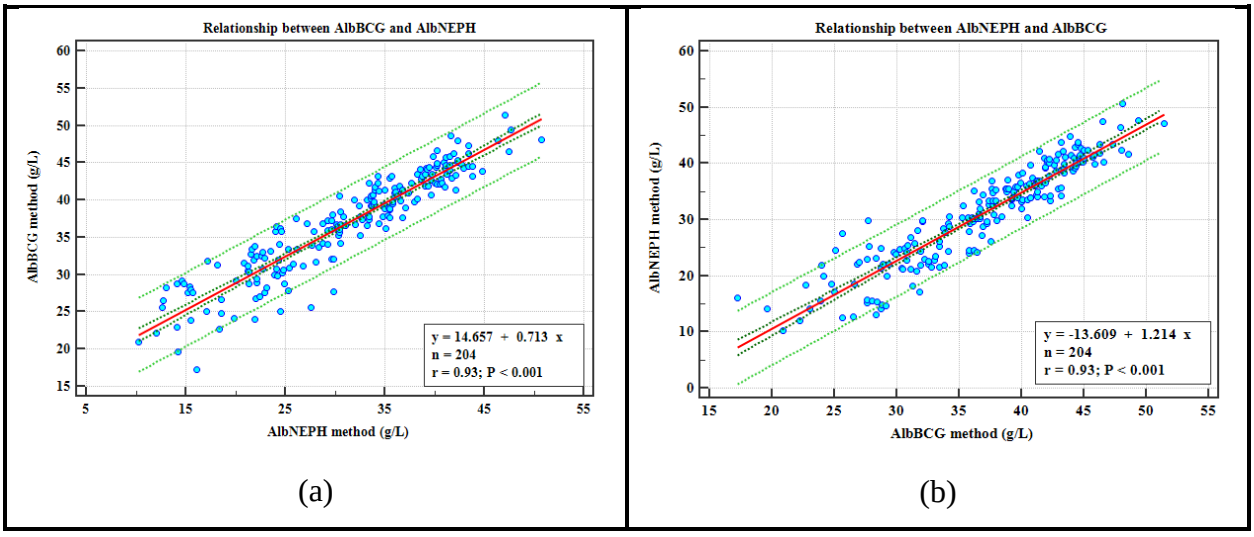
3.2 Method comparison

Regression analysis and scatterplots are commonly used in method comparison studies. There are several types of regression analysis, such as least squares method regression and Passing-Bablok regression. In this study, Passing-Bablok regression was applied in the analysis of AlbBCG and AlbNEPH. The least squares method of simple linear regression and multiple regression was used to analyse the relationship between mean of AlbBCG and AlbNEPH, age, gender, and creatinine concentration.

3.2.1 Simple linear regression

A preliminary analysis was attempted on MedCalc statistic program by using simple linear regression which was based on the least squares method. The reason for this analysis was to investigate whether AlbBCG and AlbNEPH was linearly correlated.

It was found that AlbBCG and AlbNEPH showed linear relationship and a high correlation coefficient (Figure 4 a and b). However, these analyses could not be approved. The reason being was that neither AlbBCG nor AlbNEPH had any influence on each other and therefore they were not categorized as independent or dependent variables. Thus, it was not suitable to apply the simple linear regression to estimate regression model which was based on least squares method. Passing-Bablok regression was recommended to analyze the highly correlated and linear relationship between AlbBCG and AlbNEPH.



(a) (b)

Figure 4: (a) Scatterplot based on simple linear regression showed AlbBCG plotted against AlbNEPH. (b) Scatterplot based on simple linear regression showed AlbNEPH plotted against AlbBCG.

3.2.2 Passing-Bablok Regression

The following analysis was based on Passing-Bablok regression where AlbBCG and AlbNEPH were assigned as both x- and y-variable. These produced different regression models whereas the correlation test remained the same in both analyses. It is known that AlbNEPH is often considered as the reference method in the accuracy testing of albumin concentration and it should therefore be set as an x-variable. However, according to the literature reviews, there is still a lack of standardization in albumin measurement. As a comparison purpose, AlbNEPH was assigned as the y-variable in another Passing-Bablok regression. This could possibly be used to predict AlbNEPH.

The regression analysis and scatterplots were observed and studied in different perspective. When AlbBCG (y-variable) was plotted against AlbNEPH (x-variable, and as reference method), AlbNEPH could be used to assess the accuracy of AlbBCG. On the other hand, AlbBCG (x-variable) could be used to estimate the value of AlbNEPH (y-variable) especially at abnormally low or high albumin concentration. The Passing-Bablok regression analysis showed the relationship between AlbBCG and AlbNEPH (Appendix 2, Figure 5 a and b).

The scatterplot was generated such that AlbBCG was set on the y-axis while the AlbNEPH was set on the x-axis. (Figure 5a). The regression model was estimated as follows:

$$AlbBCG = 0.746 * AlbNEPH + 13.732 \qquad \text{Equation 1}$$

The slope of the regression line was 0.746 (95% CI: [0.704 - 0.792]). It is understood that there is no proportional error and no significant difference between both methods for every 1 g/L increase in AlbNEPH which follows by 1 g/L increase in AlbBCG. On the contrary, this regression model predicted that each 1 g/L increase in AlbNEPH was associated with a 0.746 g/L increase in AlbBCG. Thus, it concluded that there was a significant difference in the slope value and a proportional error existed between AlbBCG and AlbNEPH.

The intercept of this regression model was 13.732 (95%CI: [12.054 – 15.221] g/L. It is understood that there will be no constant error if the intercept equals zero. However, this model would expect that when AlbNEPH is 0 g/L, it would have an average of 13.732 g/L of AlbBCG. Thus, it

concluded that there was a significant difference in the intercept and a constant error between AlbBCG and AlbNEPH.

The correlation coefficient, r = 0.944 (95% CI: [0.927 – 0.957], p<0.0001) indicated that AlbBCG and AlbNEPH has strong positive correlation, in other word, an increasing value in AlbNEPH was followed by an increasing AlbBCG value. As mentioned earlier, Passing-Bablok regression is only suitable to analyze highly linear correlated variables. In this case, even though AlbBCG and AlbNEPH were highly correlated, the correlation coefficient appeared to be unsuitable in the assessment of the method comparison. This is because a strong correlation does not indicate whether there is a difference in both methods.

The residual plot (Figure 5b) based on the Passing-Bablok regression showed the difference of predicted and observed AlbBCG plotted against AlbNEPH. It was observed that most of the datapoints were distributed along the regression line and within the 95% CI of the mean difference (mean +_1.96*residual standard deviation or RSD). There were only a few outliers that show large negative difference of AlbBCG (around [4 – 8] g/L) at lower AlbNEPH.

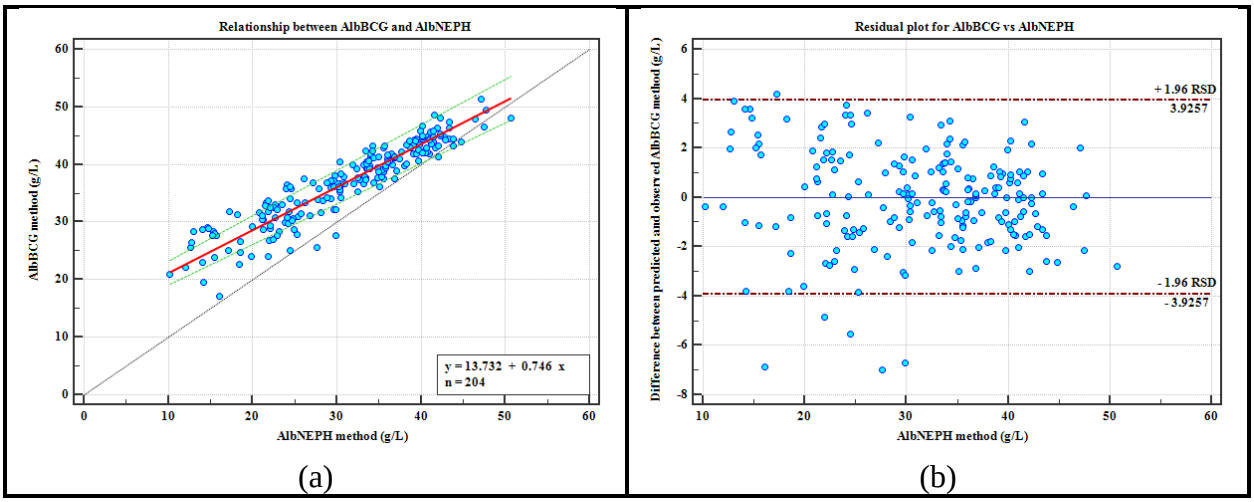


Figure 5: (a) Scatterplot showed AlbBCG plotted against AlbNEPH. An increase in AlbNEPH was followed by an increase in AlbBCG. (b) Residual plot showed the difference of predicted and observed values of AlbBCG plotted against AlbNEPH (the reference method).

The Passing-Bablok regression analysis showed the relationship between AlbNEPH and AlbBCG methods (Appendix 3, Figure 6 a and b).

The scatterplot was generated such that AlbNEPH was set on the y-axis while the AlbBCG was set on the x-axis. (Figure 6a). The regression model with the same correlation coefficient was estimated as follows:

$$AlbNEPH = 1.340*AlbBCG - 18.399 \qquad \text{Equation 2}$$

The slope of the regression line was 1.340 (95%CI: [1.263 – 1.421]). This regression model predicted that each 1 g/L increase in AlbBCG was associated with a 1.340 g/L increase in AlbNEPH. It is concluded that there was a significant difference in the slope value and a proportional error between both methods.

The intercept of the regression line was -18.399 (95%CI: [-21.633 – -15.220] g/L). Based on this model, it is predicted that a negative result was achieved when the albumin concentration measured by AlbBCG was 0 g/L. It is concluded that there was a significant difference in the intercept and a constant error between both methods.

The residual plot (Figure 6b) showed that most of the datapoints were distributed along the regression line and within the 95% CI of the mean difference. It was observed that there were a few outliers with large positive difference of AlbNEPH (around [4 – 7] g/L) at lower AlbBCG.

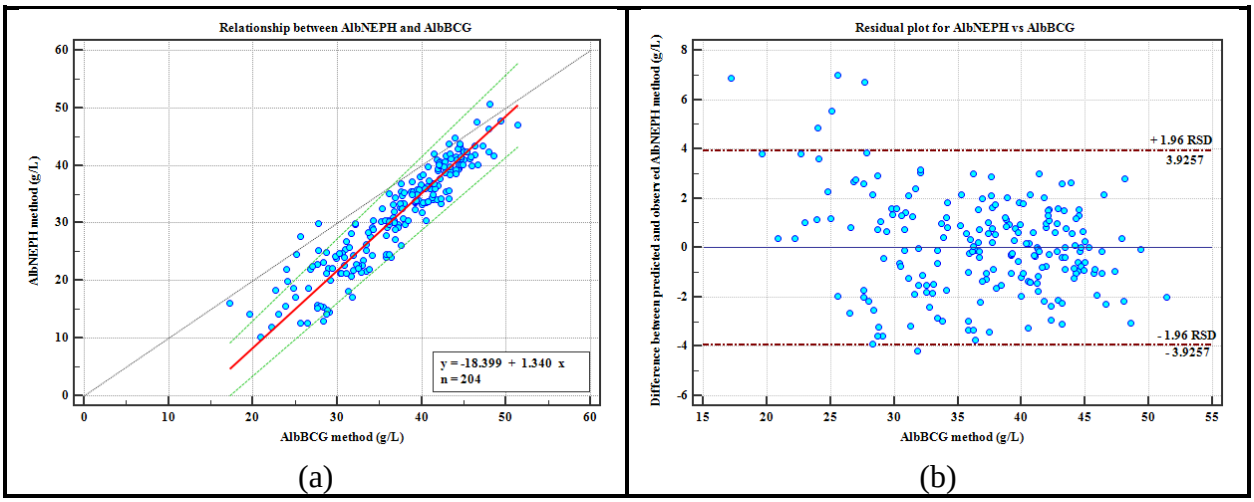


Figure 6: (a) Scatterplot showed AlbNEPH plotted against AlbBCG. An increase in AlbBCG was followed by an increase in AlbNEPH. (b) Residual plot showed the difference of predicted and observed values of AlbNEPH plotted against AlbBCG.

3.2.3 Bland-Altman (Difference) plot – [not relevant in this study]

Bland-Altman plot is another method comparison study which is used to assess the mean differences of two measurement methods and evaluate the agreement between both methods. It is constructed based on scatterplot-XY where the y-axis is composed of the difference between two measurements while the x-axis consists of the mean of both measurements. If there is no significant difference between both measurements, it means that there is no systematic error in both measurements. The systematic error can be assessed from the intervals, the 95% upper and lower limits of agreement, which can be calculated from the mean and standard deviation of both measurements. Sample results are plotted and evaluated whether the datapoints lie within or beyond the 95% limits of agreement. There is agreement between both methods if most of the datapoints are within the limits of agreement.

Systematic error was observed, see figure 7, from this analysis with the mean difference ± SD (5.7 ± 3.5 g/L) which indicated that the mean of AlbNEPH was 5.7 g/L less than that of AlbBCG. Based on 95% limits of agreement or mean difference ± 1.96*SD, the 95% lower limits of agreement (5.7 – 1.96*3.5) was -1.2 g/L while the 95% upper limits of agreement (5.7 + 1.96*3.5) was 12.6 g/L. It was observed that a few datapoints lied beyond the 95% upper and lower limits of agreement. However, most of the datapoints lied within the 95% limits of agreement, therefore, both methods agreed with each other. The regression line indicated that there was a descending trend in the relationship between the difference and mean of both methods which could be regarded as proportional error.

It is important to note that, one of the assumptions for Bland-Altman analysis is that the data should be normally distributed. It is only after the analysis, the author realized that the data is not normally distributed, see Appendix 4a-c. Briefly, histogram plotted suggested that the curve was slightly skewed to the right with the mean > median (5.7025 > 5.2500), Shapiro-Wilk test rejected the normality and the box and whisker plot did not appear to be symmetrical. Therefore, Bland-Altman plot is not adequate and relevant for this study.

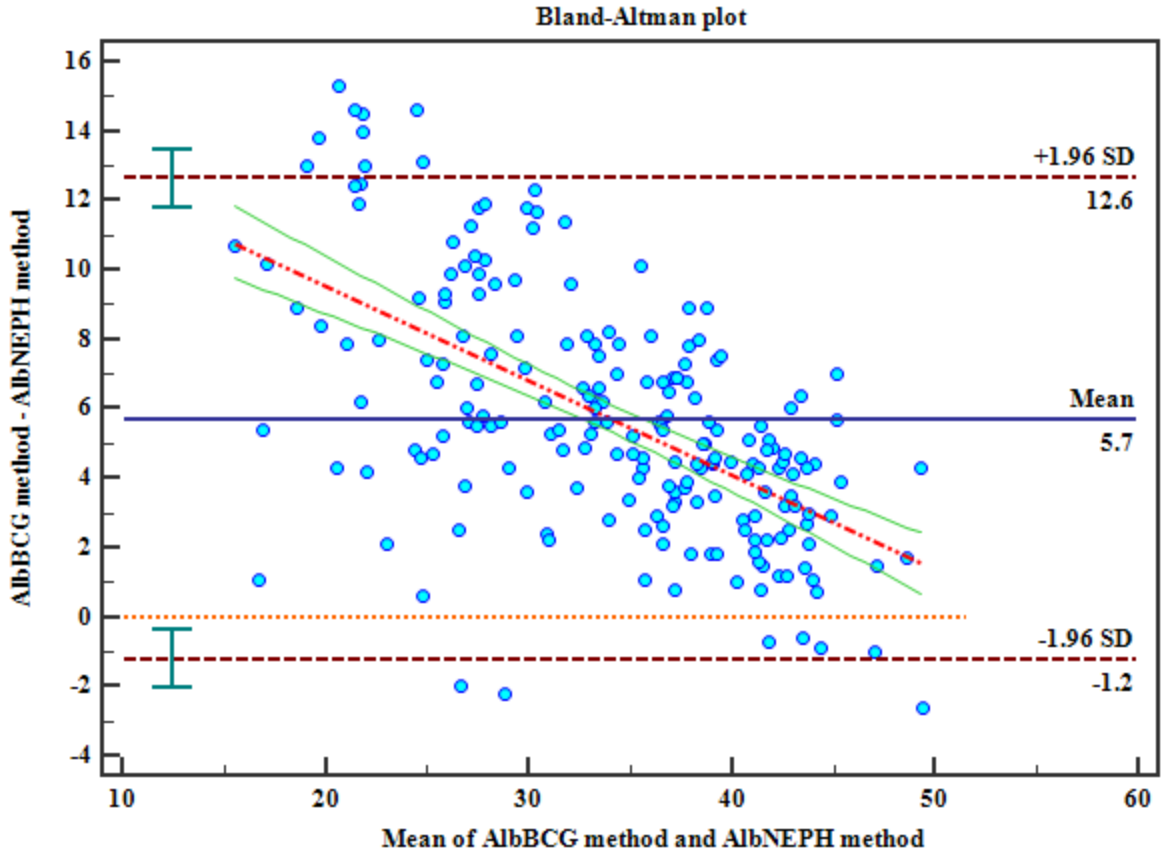


Figure 7: Bland-Altman plot showed the difference of AlbBCG and AlbNEPH against the mean of both methods.

3.3 Multiple Linear Regression

In this study, the data was analyzed by using multiple linear regression in order to determine the best model which could potentially be making a better prediction. It involves two or more independent variables in which the analysis explains and predicts the variation in the dependent variables. The analysis will either produce a better or poor regression model. Since it is difficult to see whether the variables are correlated with each other by only analyzing from the multiple regression, it is recommended to perform some pre-testing of the variables before testing the variables in the multiple linear regression. There are several pre-processing steps, namely scatterplots, correlation, and simple linear regression to test the variables as well as to select the best variables before applying them in multiple linear regression.

Independent variables in this study, which were age, gender, creatinine concentration and mean of AlbBCG and AlbNEPH were used to make predictions for difference of AlbBCG and AlbNEPH or known as dependent variable. Correlation studies and scatterplots were conducted to observe relationship between the independent and dependent variables. In addition, it was also necessary to study the correlations and test the relationships between the independent variables. The reason being was that some of the independent variables, but not all, were better at predicting the dependent variable. So, those that exhibited good correlation with the dependent variable were selected to be included in the multiple linear regression analysis. There was a total of 10 relationships that needed to be considered, which included 4 different relationships between the independent and dependent variables as well as 6 other relationships between the independent variables. The 6 relationships between the independent variables were used to test the potential risk of multicollinearity and check whether these independent variables were correlated with each other.

Dependent variable to independent variable scatterplots were generated, see figure 8. Based on visual examination on the scatterplots, only one appeared to show linear relationship, that is, the difference of AlbBCG and AlbNEPH against mean of AlbBCG and AlbNEPH. On the other hand, age, gender, and creatinine concentration did not show any linear relationship to the difference of AlbBCG and AlbNEPH.

Independent variable to independent variable scatterplots were generated, see figure 9. Based on the scatterplots, there appeared to be no linear relationship between the independent variables i.e.

there were no multicollinearity. Since none of the independent variables were correlated, it was possible to include them in the multiple linear regression analysis. However, from the scatterplots in figure 8, it was observed that only the mean of AlbBCG and AlbNEPH was actually showing correlation with the difference of AlbBCG and AlbNEPH. Therefore, it was obvious that among the 4 independent variables, only the mean of AlbBCG and AlbNEPH was to be included into the multiple regression.

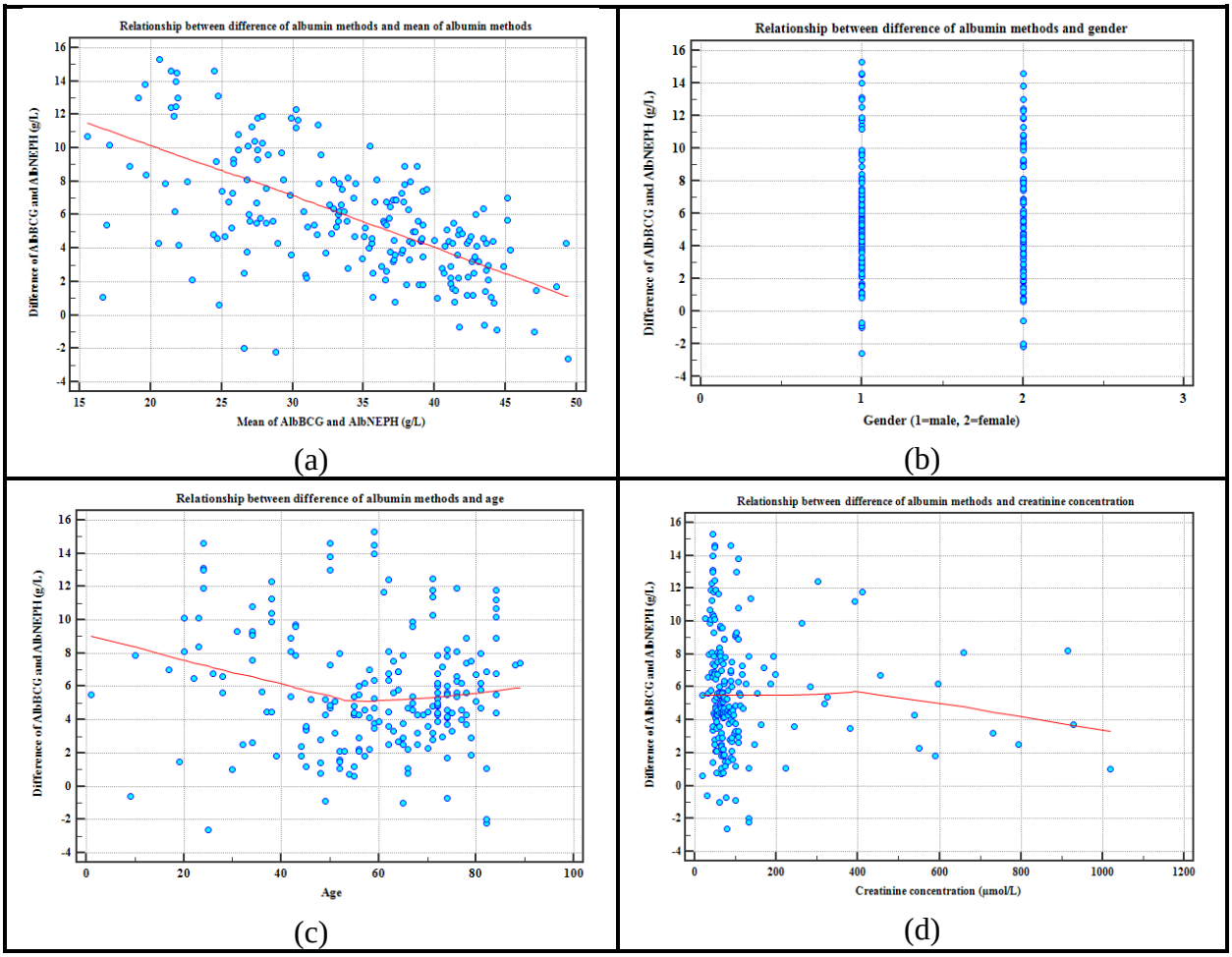


Figure 8: (a) Scatterplot showed the relationship between difference of albumin methods and mean of albumin methods. (b) Scatterplot showed the relationship between difference of albumin methods and gender. (c) Scatterplot showed the relationship between difference of albumin methods and age. (d) Scatterplot showed the relationship between difference of albumin methods and creatinine concentration. Only scatterplot in (a) showed linear relationship between the variables.

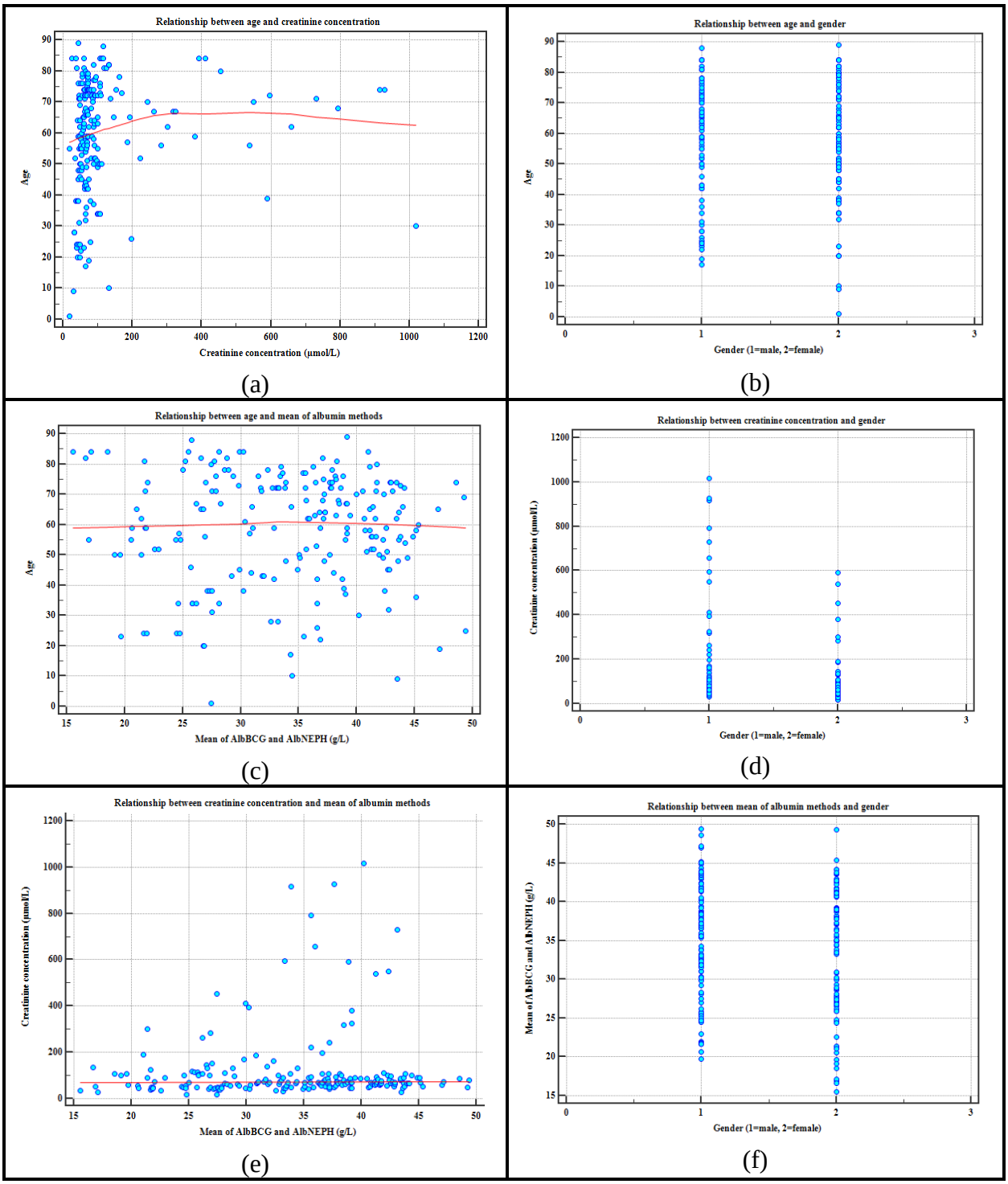


Figure 9: (a) Scatterplot showed the relationship between age and creatinine concentration. (b) Scatterplot showed the relationship between age and gender. (c) Scatterplot showed the relationship between age and mean of albumin methods. (d) Scatterplot showed the relationship between creatinine concentration and gender. (e) Scatterplot showed the relationship between creatinine concentration and mean of albumin methods. (f) Scatterplot showed the relationship between mean of albumin methods and gender. None of the variables (a-f) indicated linear relationship.

Correlation study was then performed to confirm if the statements made from the rough visualization of the scatterplots were valid. Based on the correlation between independent and dependent variables, it was confirmed that the mean of AlbBCG and AlbNEPH had a linear correlation ($r = -0.5922$) with the difference of AlbBCG and AlbNEPH and a p-value of < 0.0001. Based on the correlation between independent variables, it was confirmed that none of the independent variables had a linear correlation with each other or even statistically significant. Detailed analysis results can be found in Appendix 5 and 6.

From the scatterplots in figure 8 and 9 together with correlation analysis, it was confirmed that only the mean of AlbBCG and AlbNEPH and the difference of AlbBCG and AlbNEPH appeared to be correlated. In addition, there were no correlations between the independent variables i.e. no multicollinearity existed. Yet again, those that were not correlated with dependent variable could not be included in the regression. The only ones to be included in the multiple regression analysis were those showing good linear correlations.

Simple linear regression is an important step before conducting the multiple regression analysis. Four simple linear regression based on the relationships between independent and dependent variables were studied:

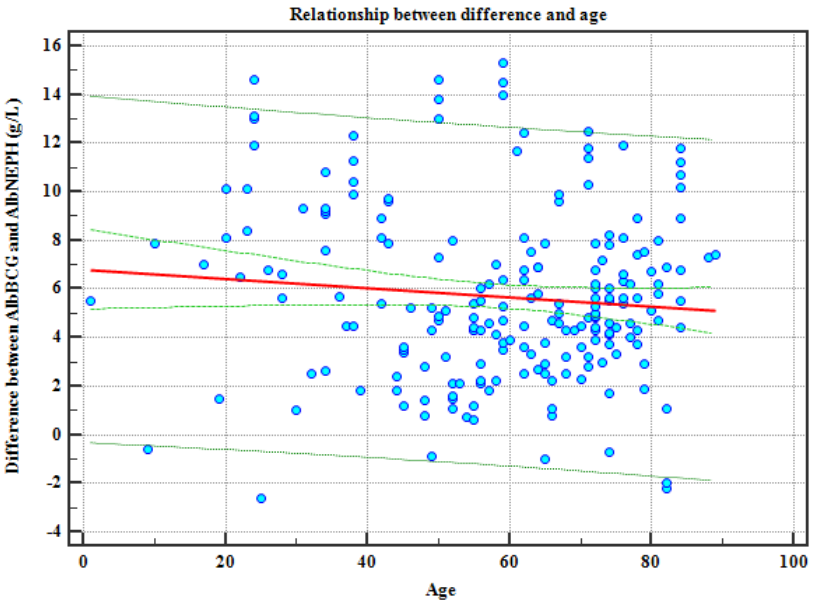
1. Difference of AlbBCG and AlbNEPH versus mean of AlbBCG and AlbNEPH
2. Difference of AlbBCG and AlbNEPH versus age
3. Difference of AlbBCG and AlbNEPH versus gender
4. Difference of AlbBCG and AlbNEPH versus creatinine concentration

The scatterplots of the simple linear regression are shown in figure 10 and the detailed of the analysis can be found in Appendix 7. It was observed that the only one with a good correlation was between the difference of AlbBCG and AlbNEPH and mean of AlbBCG and AlbNEPH with a regression model as follows:

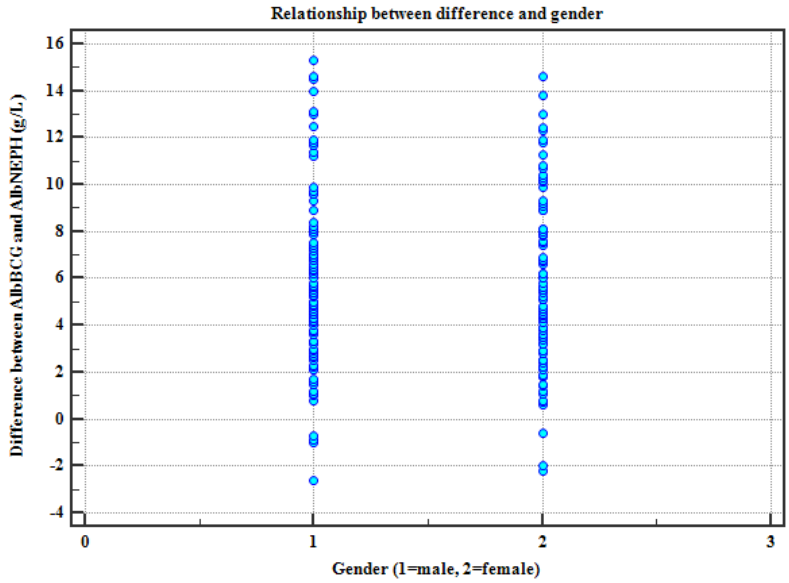
$$Difference = -0.274 * mean + 15.030 \qquad \text{Equation 3}$$

The regression model predicted that each 1 g/L increase in the mean of AlbBCG and AlbNEPH was associated with a 0.274 g/L decrease in the difference of AlbBCG and AlbNEPH with a p-value of <0.0001. This means that when the average albumin level increased, the difference of both albumin measurement methods would decrease. $R^2 = 0.3507$ indicated that the mean of

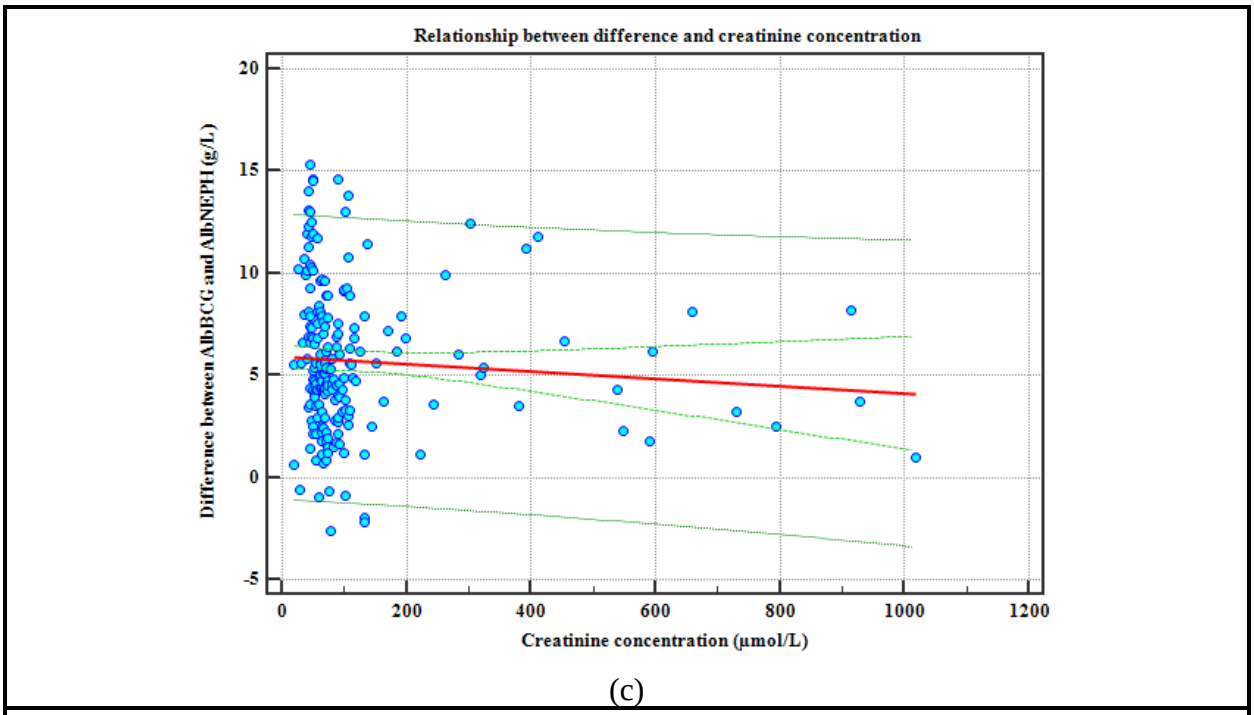
AlbBCG and AlbNEPH explained 35% of the variation in the difference of AlbBCG and AlbNEPH. Large F-ratio (F-ratio =109.10) with low p-value ($p < 0.0001$) showed that the overall model was significant.



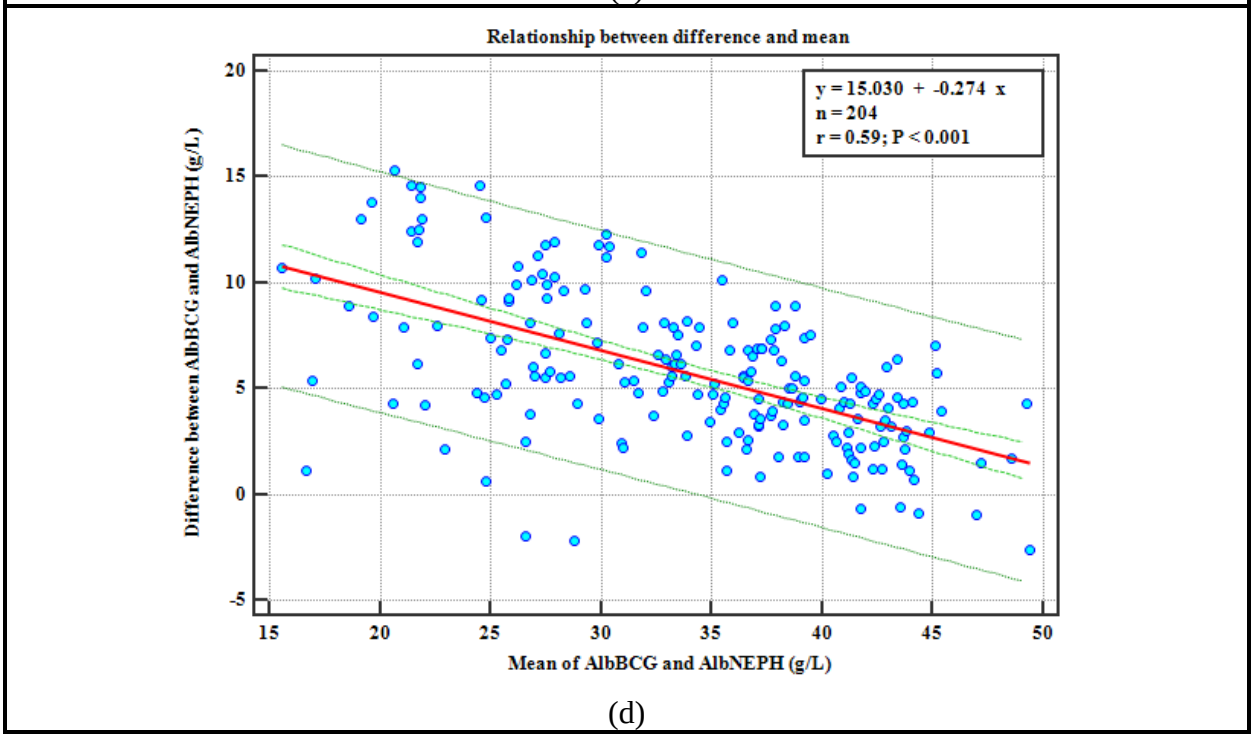
(a)



(b)



(c)



(d)

Figure 10: (a) Scatterplot showed the relationship between difference of albumin methods and age. (b) Scatterplot showed the relationship between difference of albumin methods and gender. (c) Scatterplot showed the relationship between difference of albumin methods and creatinine concentration. (d) Scatterplot showed the relationship between difference of albumin methods and mean of albumin methods. Only scatterplot in (d) showed linear relationship between the variables.

Simple linear regressions based on two x-variable were analyzed and the detailed analysis results can be found in Appendix 8. It was observed that none of the regression analysis using two x-

variables predicted better model than the previous regression analysis using only one x-variable. The analyses which included age, gender and creatinine level as the x-variables shows lower F-value with high p-value, this means that the overall model was not significant. In addition, large standard error with literally zero R^2 were observed. This confirmed that there was absolutely no linear relationship between these independent variables and the dependent variable. The analyses which included mean of albumin measurement methods as one of the x-variable particularly showed larger F-value with low p-value which was statistically significant. Besides, the standard error was lower with R^2 at around 0.3. This confirmed that there was somewhat linear relationship between the variables. With all these analyses, the age, gender, and creatinine concentration were not the variables that contributed to the difference of albumin measurement methods, but the mean of albumin measurement methods appeared to be the best predictor for the model.

Even though the regression based on 2 x-variable clearly did not show any correlation between the dependent and independent variables, it was still possible to present a rough visualization of the variables by generating scatterplots with 2 x-variables where one of them was plotted as bubble size.

The first scatterplot was difference of albumin methods and age and gender, where the genders were plotted as binary bubble size i.e. male = small bubble and female = large bubble, see figure 11. It was observed that most of the datapoints were distributed around the age of 40 to 80 years for both genders and with a variation of the difference which was around 0 g/L to 8 g/L.

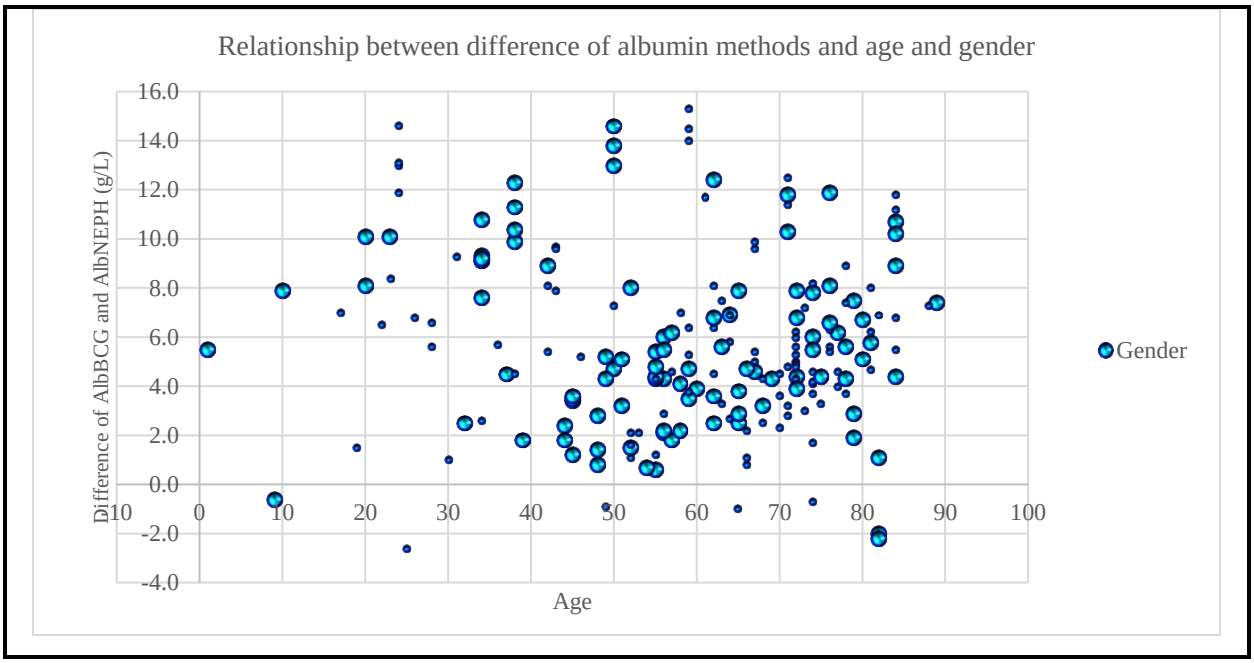


Figure 11: Scatterplot showed the relationship between difference of albumin methods and age and gender. Small bubble = male, large bubble = female.

The second scatterplot was difference of albumin methods and age and creatinine concentration, where the creatinine concentration was plotted as adjustable bubble size i.e. the higher the creatinine concentration, the bigger the bubble size, see figure 12. It was observed that a few samples at the age of around 20 to 60 years with normal creatinine levels showed larger difference of albumin methods. This means that a large difference which occurred at lower albumin concentration did not necessarily increase creatinine concentration.

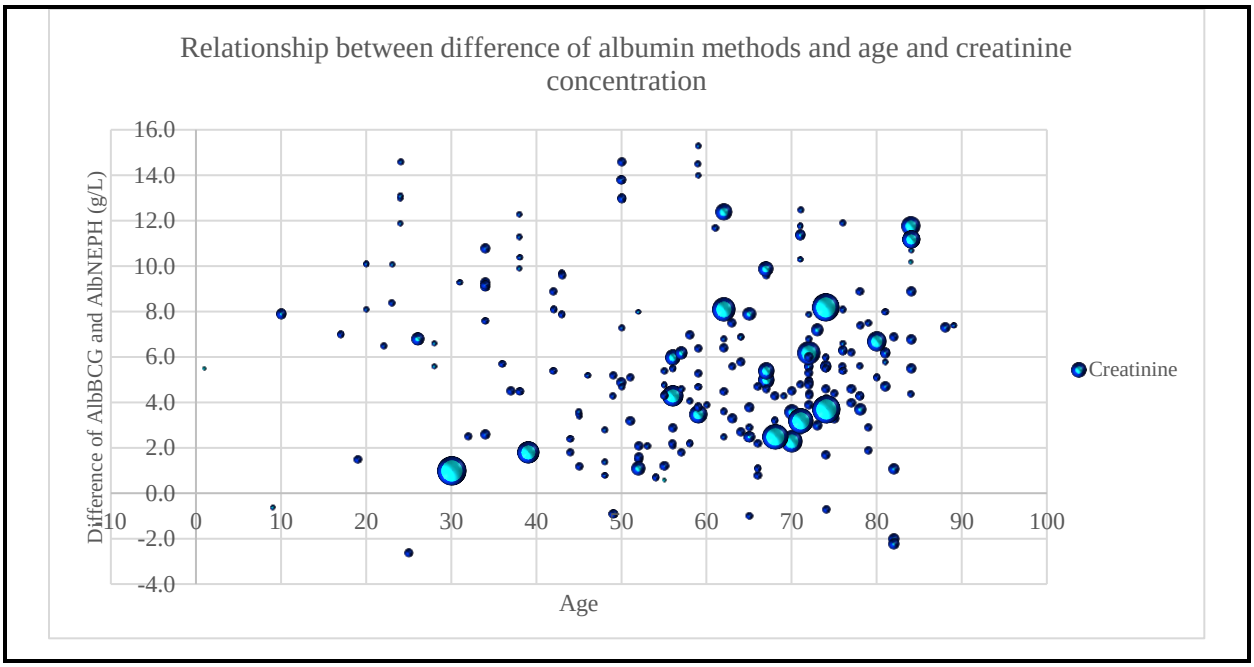


Figure 12: Scatterplot showed the relationship between difference of albumin methods and age and creatinine concentration.

The third scatterplot was difference of albumin methods and age and mean of albumin method, where the mean of albumin method was plotted as adjustable bubble, see figure 13. It was difficult to observe and identify the relationship between the variables. It was roughly observed that a higher difference of albumin methods was seen in lower mean of albumin methods.

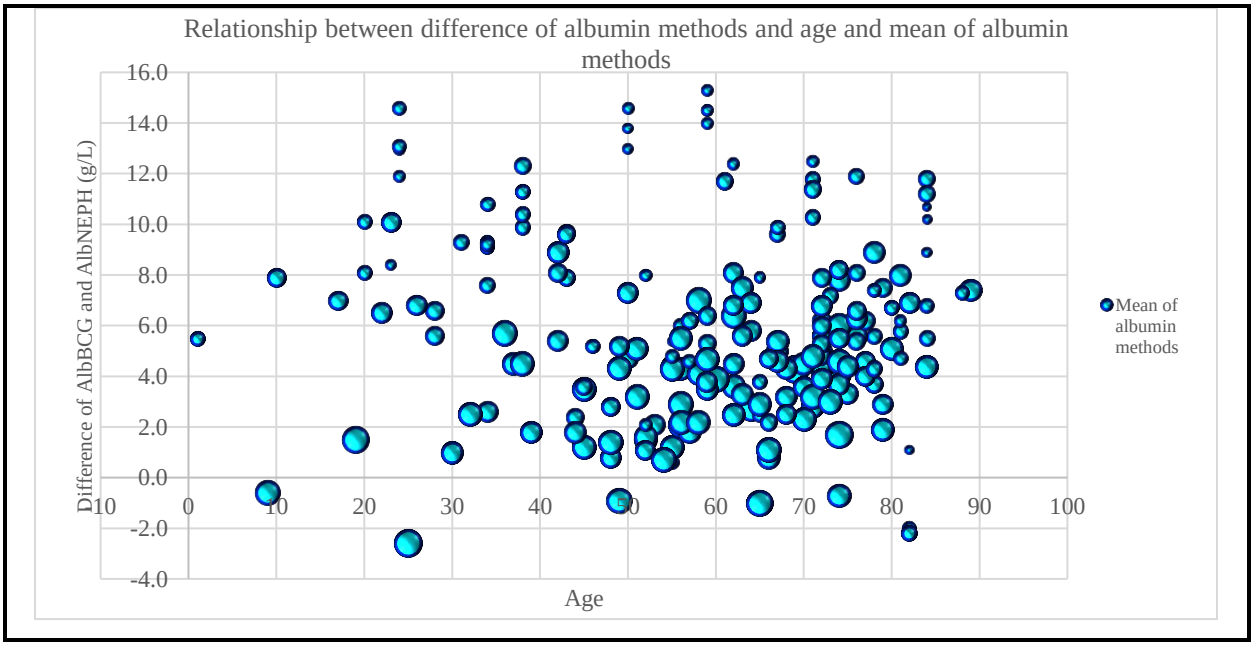


Figure 13: Scatterplot showed the relationship between difference of albumin methods and age and mean of albumin methods.

The fourth scatterplot was difference of albumin methods and gender and creatinine concentration, where the creatinine concentration was plotted as adjustable bubble, see figure 14. It was observed that males tended to have higher creatinine levels but did not necessarily show low albumin concentrations or increase in the difference of albumin methods.

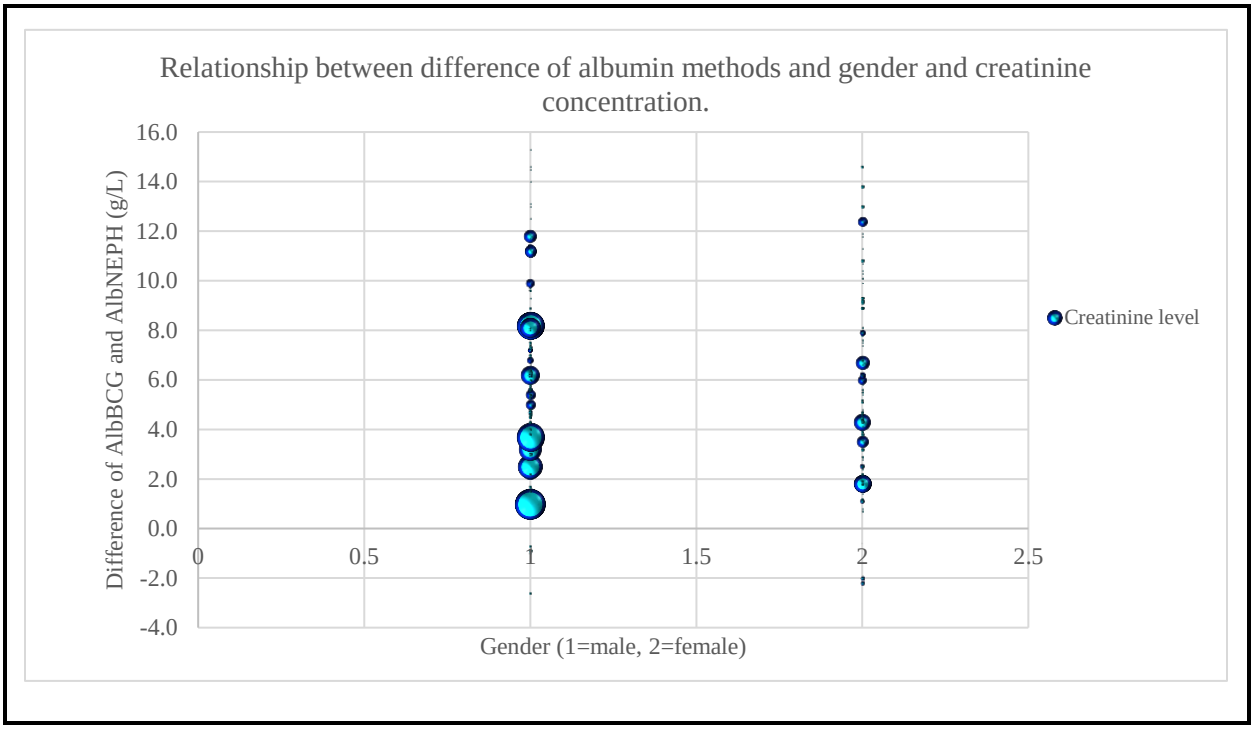


Figure 14: Scatterplots showed the relationship between difference of albumin methods and creatinine level and gender.

The fifth scatterplot was difference of albumin methods and mean of albumin methods and creatinine concentration, where the creatinine concentration was plotted as adjustable bubble, see figure 15. There was a descending trend in the relationship between the difference and mean of albumin methods. Higher differences were observed when the mean was less than 30 g/L.

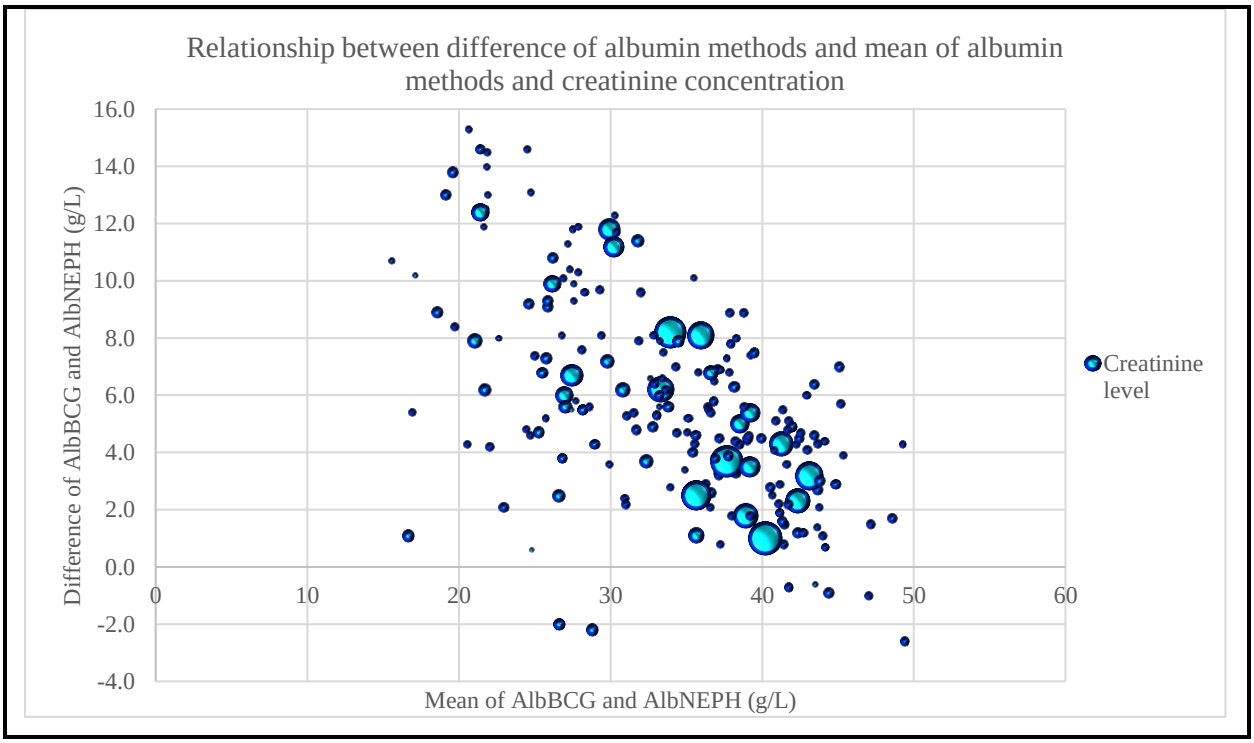


Figure 15: Scatterplot showed the relationship between difference of albumin methods and mean of albumin methods and creatinine concentration.

The sixth scatterplot was difference of albumin methods and mean of albumin methods and gender, where the genders were plotted as binary bubble size i.e. male = small bubble and female = large bubble, see figure 16. It was observed that there was descending trend between the difference and the mean of albumin methods. Male and female are quite evenly distributed.

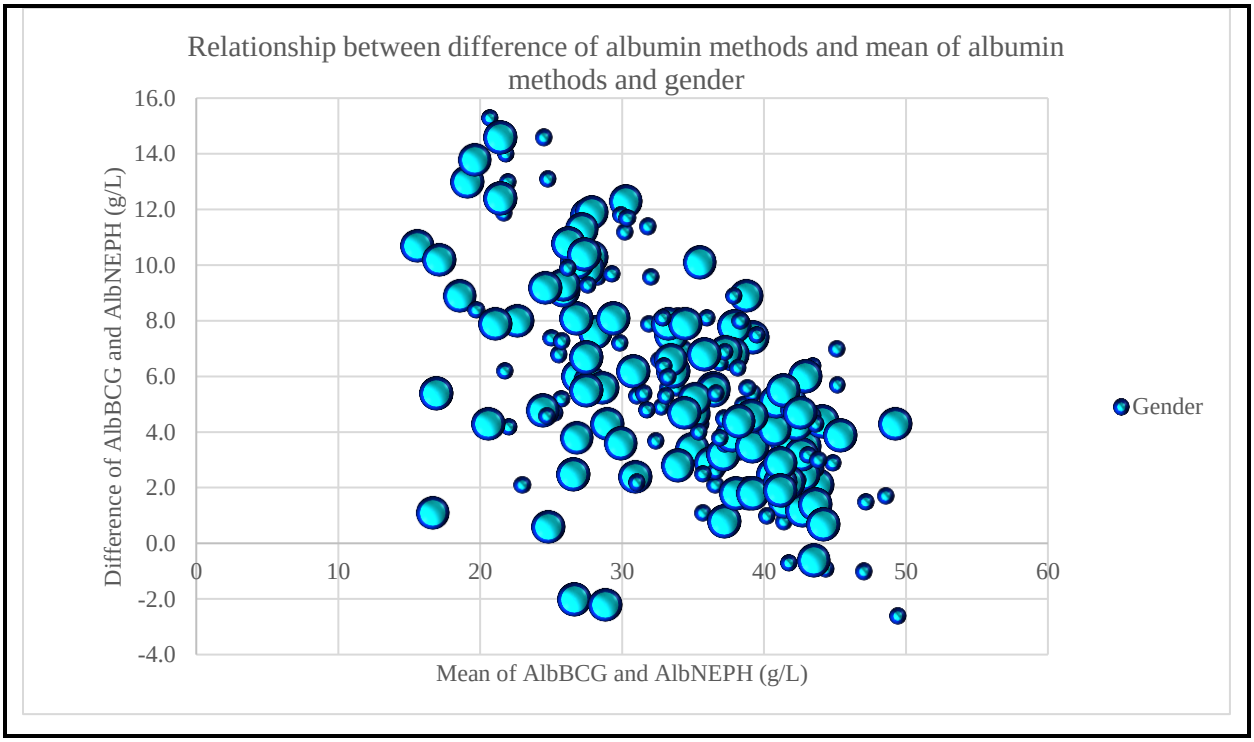


Figure 16: Scatterplot showed the relationship between difference of albumin methods and mean of albumin methods and gender. Small bubble = male, large bubble = female

Multiple linear regression analysis was used to study the relationship between the difference of AlbBCG and AlbNEPH and all the independent variables. Two regression analysis were generated where one used the 'Enter' method i.e. the regression did not eliminate any non-significant variables. Another analysis used 'Backward' method in which the regression automatically removed any non-significant variables. The multiple regression using 'Enter' method showed that all the variables were included into the analysis. It did not produce a model that was better than the one using 'Backward' method. The reason was that too many independent variables produced too much variance which were unnecessary on the model which in turn caused overfitting in the model. The 'Backward' method analysis produced the same result as the one in the simple linear regression analysis with one x-variable. Detailed results for 'Enter' and 'Backward' methods can be found in Appendix 9 and 10.

A model option, table 3, is produced to summarize the overall values in the regression analysis. According to the summary table, the best model option (highlighted) was determined, and it was from the simple linear regression with one x-variable. Besides, this was also tested the same in the

'Backward' method of multiple regression. The best model generated from these analyses was thus "*Difference = -0.274*mean + 15.030 from equation 3*" with the highest F-value, low standard error of the regression and high R^2.

Table 3: Summary of the model option showed statistical values for each of the generated regression model based on different x-variables.

Types of regression		F-value	p-value	St. error	R^2	X-variables			
						Age	Gender	Creatinine	Mean Alb. method
Simple linear regression (1 x-variable)	*Diff. vs age*	1.97	0.16	3.53	0.01	X			
	Diff. vs creatinine	1.29	0.26	3.53	0.01			X	
	Diff. vs gender	0.13	0.72	3.54	0.001		X		
	*Diff. vs mean**	109.1	<0.0001	2.86	0.35				X
Simple linear regression (2 x-variable)	*Diff. vs age & creatinine*	1.46	0.24	3.53	0.01	X		X	
	Diff. vs age & gender	1.09	0.34	3.54	0.01	X	X		
	Diff. vs age & mean	56.49	<0.0001	2.84	0.36	X			X
	Diff. vs creatinine & gender	0.81	0.45	3.54	0.01		X	X	
	Diff. vs creatinine & mean	54.61	<0.0001	2.86	0.35			X	X
	Diff. vs gender & mean	55.76	<0.0001	2.85	0.36		X		X
Multiple linear regression	*Diff. vs age, gender, creatinine & mean*	29.08	<0.0001	2.84	0.37	X	X	X	X

* the acceptable model

4.0 Discussion

Accuracy testing of AlbBCG against AlbNEPH showed some convincing results throughout the various analysis. The analysis indicated that the difference between the mean of both methods was statistically significant. Based on this finding, subsequent analyses were conducted. Passing-Bablok regression provided an overview of how both methods behaved without having any influence on each other. This regression produced slightly higher correlation coefficient than the least square method regression, thus yielding better regression model for prediction. The use of variables interchangeably between x- and y-axis was to estimate their regression model. One of the models (Equation 1) could be used to assess the accuracy of AlbBCG based on AlbNEPH while the other model (Equation 2) could possibly be used to estimate AlbNEPH based on AlbBCG. Correlation between AlbBCG and AlbNEPH, $r = 0.944$ ($p<0.0001$) appeared to be strong which was quite similar to the other research of similar background (6). Since the correlation did not give any more detail other than the strength of the methods' relationship, analysis involving difference between both methods were conducted.

Bland-Altman analysis that involved the difference of AlbBCG and AlbNEPH was utilized to find the mean difference which was essentially useful in determining how much AlbBCG actually deviated from AlbNEPH. The analysis by Bland-Altman showed systematic error and random error. The same applied to Passing-Bablok regression analysis which confirmed result quality from Bland-Altman analysis. Based on the observed mean difference ([5.7 +_3.5] g/L), it was certain that there was differences to some degree between both AlbBCG and AlbNEPH methods even though the methods were in agreement with each other. However, there were some limitations in the Bland-Altman analysis. The result obtained from this analysis could possibly be not reliable due to two factors. One of the factors is small sample size which produces lower mean difference and leads to the reduction of the limits of agreement (34). Another important factor is the data distribution. It is based on the assumption that only normally distributed data can be analyzed with Bland-Altman plot (35). Based on the investigation, the data was found to be not normally distributed. Therefore, Bland-Altman analysis was not suitable in this study and that the results provided were invalid.

Multiple regression analysis showed that there was an influence of the mean albumin measurement towards the difference of the measurement methods. It was also possible to predict the difference

of AlbBCG and AlbNEPH based on the model. For instance, a mean albumin concentration of 50 g/L would have a difference of 1.33 g/L, while a mean albumin concentration of 20 g/L would have a difference of 9.55 g/L. This clearly showed that at lower albumin concentration, the difference of albumin measurement methods tended to be higher. Higher mean albumin concentration contributed to lower difference of albumin measurement methods, whilst lower mean albumin contributed to higher difference of albumin measurement methods. Besides, the number of samples with low albumin concentration were analysed based on the albumin measurement method used, where 83 samples measured by AlbNEPH were less than 30 g/L while only 37 samples measured by AlbBCG were less than 30 g/L. Such difference could be linked to the overestimation of AlbBCG at lower albumin concentration. It was found out that the creatinine concentration was not correlated to the difference of the albumin methods. This was also supported by a research which also claimed to have the similar outcome (36). The mean creatinine level based on the AlbNEPH results of less than 30 g/L was 90.8µmol/L, which is within the normal range. This suggests that people in the sample with lower than 30 g/L albumin concentration tended to have normal creatinine level. From the regression analysis, there were no linear relationship between the difference of albumin methods with age and gender; similar findings were also concluded by Zhang et al. (18).

Variation in the results between the albumin measurement methods were identified as systematic errors. The variation occurred possibly due to the different analysis methodology applied in each of the instrument. Systematic errors consist of proportional and constant error. These errors were identified from interpretations of the regression models and Bland-Altman plot. Constant error was identified from the y-intercept of the regression. In Bland-Altman analysis, AlbBCG produced positive mean difference which led to constant error. Proportional error was identified from the slope of the regression. In the Bland-Altman analysis, the difference of AlbBCG and AlbNEPH decreased as the mean of both methods increased which was where proportional error occurred.

One of the reasons behind such difference could be linked to interference. Lower albumin levels tend to have increased globulin levels which could possibly interfere with the binding of bromocresol green dye. This can lead to increased dye-binding which is actually not albumin-bound but in fact globulin-bound (11). Other interfering molecules like bilirubin as well as hemolyzed and lipemic serum could be the possible causes of the erroneous results if present at

higher concentrations (27,30). These are probably due to the preanalytical factors, namely techniques used during phlebotomy. Pressure applied from the prolonged use of tourniquet leads to an increase risk of hemolysis of the blood sample. Lipemic serum is due to highly concentrated triglycerides or fat molecules in the blood sample.

Limitations of this study can be traced back to the study design where a lack of other biochemistry tests affects data analysis. Additional tests such as urine albumin and creatinine should be included. By comparing the serum and urine albumin results, one could expect high urine albumin concentration and a low serum albumin which is due to albumin leakage especially in chronic kidney disease patients (33). The reason for this additional urine albumin test is to evaluate if the serum albumin levels are overestimated by BCG method. Besides, urine and serum creatinine could be used in the calculation of estimated glomerular filtration rate (eGFR) which is highly regarded for its clinical significance and it is expected to have positive correlation with albumin (18). Another potential biochemistry component to be tested is total protein (3). Total protein level might be useful to find the levels of globulin where globulin = total protein – albumin. Total protein level can help to identify high globulin concentration resulted from AlbNEPH that could possibly be the interference component in AlbBCG. Study design which involves non normally distributed data or skewed distribution may lead to inaccurate analysis especially in the analysis of measurement error. The 204 samples tested in this study is randomly selected from the laboratory based in the hospital contain higher number of less healthy patients. Samples are therefore suggested to be selected based on evenly distributed number categorized by some of the important characteristics such as health condition and age.

Albumin level is clinically relevant and important especially for chronic kidney disease patients and should have their albumin levels tested periodically. In fact, these patients are also the most vulnerable towards developing hypoalbuminemia. This means that inaccurate result reporting leads to serious consequences especially those who are actually hypoalbuminemia and in need of treatment (37). Other clinical significance of albumin is that it is useful to adjust calcium levels. If a low albumin concentration being overestimated by BCG method and calcium is not adjusted based on this result, the patient could be at risk of undertreatment (38). In summary, the use of different albumin measurement methods should be evaluated in order to improve the quality and accuracy of the results produced.

5.0 Conclusion

In this study, accuracy of the bromocresol green method in plasma albumin is studied. This was done by analysing 204 patient blood sample from St. Olav hospital. The following conclusions are drawn from the study:

1. There was significant difference between albumin concentration results measured by bromocresol green (BCG) method and by immunonephelometric (NEPH) method. Based on regression analyses, the difference between the methods was identified as systematic error.
2. Correlation of AlbBCG and AlbNEPH method was considerably high which suggested that an increase in AlbNEPH was also accompanied by an increase in AlbBCG. The model is AlbBCG = 0.746*AlbNEPH + 13.732.
3. There was no correlation between the difference of AlbBCG and AlbNEPH and the independent variables i.e. age, gender, and creatinine concentration. Only the mean of AlbBCG and AlbNEPH showed a certain degree of linear relationship with the difference of the methods. It is concluded that the difference of the albumin methods changed accordingly with the mean of albumin methods i.e. bigger difference at lower albumin concentration.

Possible cause of the difference can be linked to interference. Interfering particles especially globulin molecules which bind onto BCG molecules, tend to overestimate albumin results.

Developing better study design is suggested for future work. Larger sample size and more parameters, such as the measurement of serum globulin should be included in order to assist in the identification of overestimated albumin concentration.

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Appendix

Appendix 1: Student Paired Sample T-test data analysis between AlbBCG and AlbNEPH.

Hypothesis	H_0: $\mu_{AlbBCG} = \mu_{AlbNEPH}$ H_1: $\mu_{AlbBCG} \neq \mu_{AlbNEPH}$
Sample 1	AlbBCG_method
Sample 2	AlbNEPH_method

	Sample 1	Sample 2
Sample size	204	204
Arithmetic mean	36.9441	31.2417
95% CI for the mean	36.0110 to 37.8772	30.0244 to 32.4589
Variance	45.6883	77.7506
Standard deviation	6.7593	8.8176
Standard error of the mean	0.4732	0.6174

Mean difference	-5.7025
Standard deviation of differences	3.5365
Standard error of mean difference	0.2476
95% CI of difference	-6.1907 to -5.2142
Test statistic t	-23.030
Observed t	-10.8988; -14.2187
Degrees of Freedom (DF)	203
Two-tailed probability	P < 0.0001
H_0 is rejected; There is a significant difference between AlbBCG and AlbNEPH method.	

Appendix 2: Passing and Bablok regression: AlbBCG (y-variable) against AlbNEPH (x-variable)

Variable X	AlbNEPH_method
Variable Y	AlbBCG_method

Sample size	204

	Variable X	Variable Y
Lowest value	10.2000	17.2000
Highest value	50.7000	51.4000
Arithmetic mean	31.2417	36.9441
Median	33.2000	37.7000
Standard deviation	8.8176	6.7593
Standard error of the mean	0.6174	0.4732

Regression Equation

y = 13.732195 + 0.746341 x	
Systematic differences	
Intercept A	13.7322
95% CI	12.0537 to 15.2207
Proportional differences	
Slope B	0.7463
95% CI	0.7036 to 0.7919
Random differences	
Residual Standard Deviation (RSD)	2.0029
± 1.96 RSD Interval	-3.9257 to 3.9257
Linear model validity	
Cusum test for linearity	No significant deviation from linearity (P=0.99)

Spearman rank correlation coefficient

Correlation coefficient	0.944
Significance level	P<0.0001
95% CI	0.927 to 0.957

Appendix 3: Passing and Bablok regression: AlbNEPH (y-variable) against AlbBCG (x-variable).

Variable X	AlbBCG_method
Variable Y	AlbNEPH_method

Sample size	204

	Variable X	Variable Y
Lowest value	17.2000	10.2000
Highest value	51.4000	50.7000
Arithmetic mean	36.9441	31.2417
Median	37.7000	33.2000
Standard deviation	6.7593	8.8176
Standard error of the mean	0.4732	0.6174

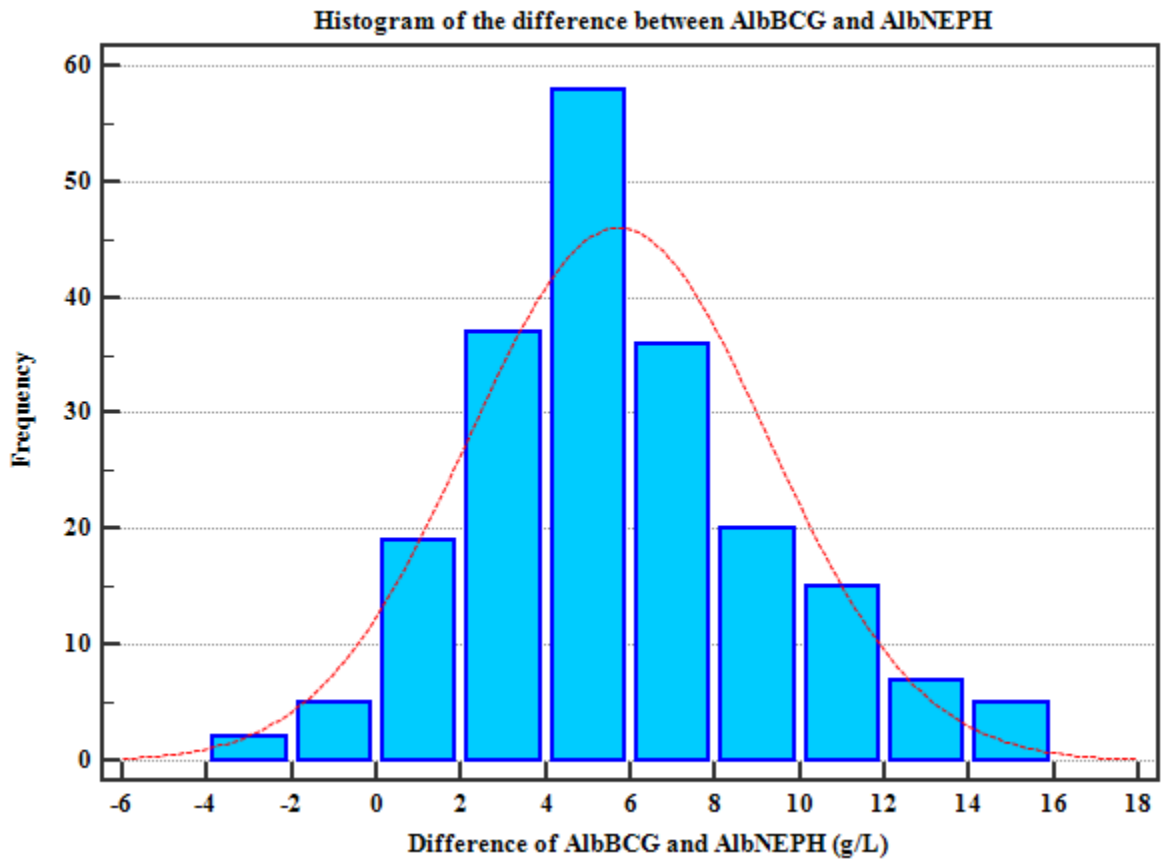
Regression Equation

y = -18.399346 + 1.339869 x	
Systematic differences	
Intercept A	-18.3993
95% CI	-21.6328 to -15.2203
Proportional differences	
Slope B	1.3399
95% CI	1.2627 to 1.4213
Random differences	
Residual Standard Deviation (RSD)	2.0029
± 1.96 RSD Interval	-3.9257 to 3.9257
Linear model validity	
Cusum test for linearity	No significant deviation from linearity (P=0.99)

Spearman rank correlation coefficient

Correlation coefficient	0.944
Significance level	P<0.0001
95% CI	0.927 to 0.957

Appendix 4a: Histogram shows the distribution of the difference between AlbBCG and AlbNEPH



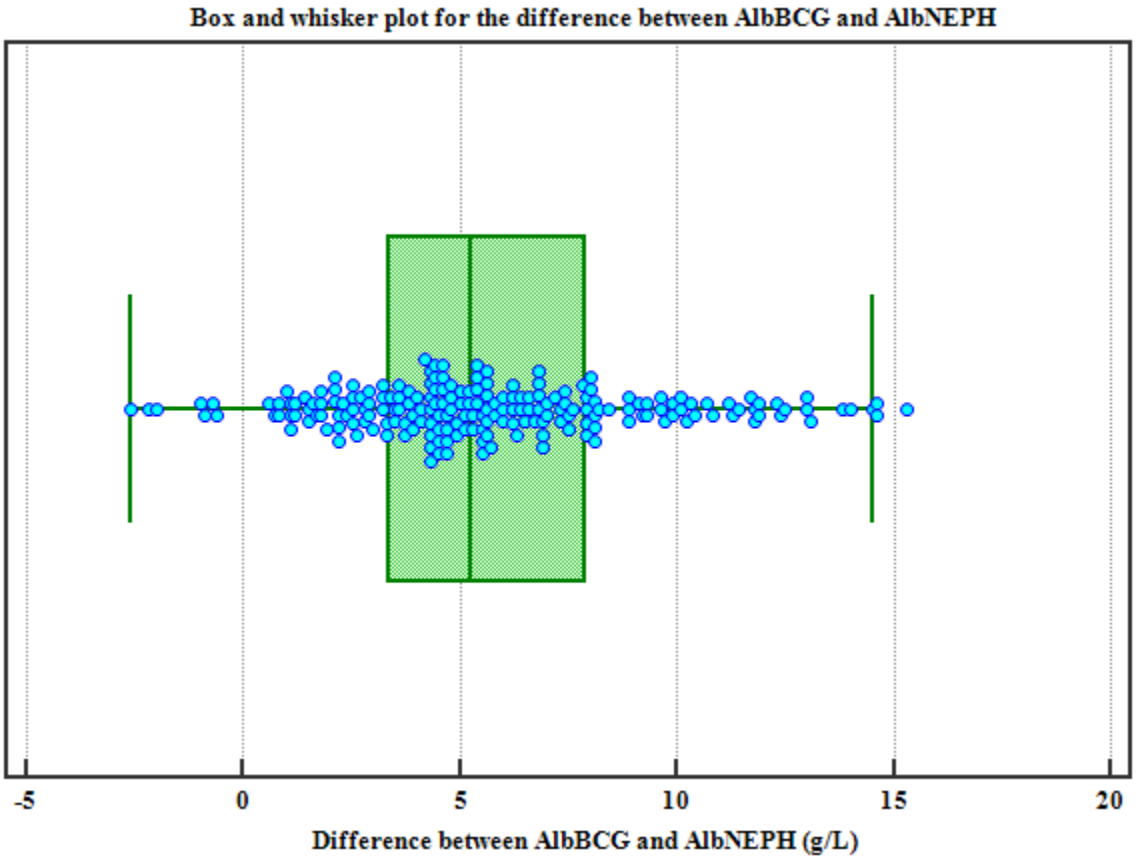
Appendix 4b: Summary statistics for the difference between AlbBCG and AlbNEPH.

Variable	Difference__AlbBCG_AlbNEPH_

Sample size	204
Lowest value	-2.6000
Highest value	15.3000
Arithmetic mean	5.7025
95% CI for the Arithmetic mean	5.2142 to 6.1907
Median	5.2500
95% CI for the median	4.7000 to 5.6000
Variance	12.5070
Standard deviation	3.5365
Relative standard deviation	0.6202 (62.02%)
Standard error of the mean	0.2476
Coefficient of Skewness	0.4694 (P=0.0072)
Coefficient of Kurtosis	0.08220 (P=0.6967)
Shapiro-Wilk test for Normal distribution	W=0.9773 reject Normality (P=0.0022)

Percentiles		95% Confidence interval
2.5	-0.7800	-2.2946 to 0.8000
5	0.8000	-0.8838 to 1.2369
10	1.5900	1.0918 to 2.1646
25	3.3500	2.6637 to 3.9839
75	7.8500	6.9000 to 8.5817
90	10.8400	9.7707 to 11.9330
95	12.4300	11.6446 to 13.9838
97.5	13.8800	12.4638 to 14.7656

Appendix 4c: Box and whiskers plot shows the difference between AlbBCG and AlbNEPH.



Appendix 5: Dependent variable to independent variable correlation study.

Variable Y	Difference__AlbBCG_AlbNEPH_
Variable X	Mean_of_AlbBCG_and_AlbNEPH
Sample size	204
Correlation coefficient r	-0.5922
Significance level	P<0.0001
95% Confidence interval for r	-0.6747 to -0.4951

(a)

Variable Y	Difference__AlbBCG_AlbNEPH_
Variable X	Gender
Sample size	204
Correlation coefficient r	-0.02486
Significance level	P=0.7241
95% Confidence interval for r	-0.1617 to 0.1129

(b)

Variable Y	Difference__AlbBCG_AlbNEPH_
Variable X	Age
Sample size	204
Correlation coefficient r	-0.09823
Significance level	P=0.1622
95% Confidence interval for r	-0.2325 to 0.03968

(c)

Variable Y	Difference__AlbBCG_AlbNEPH_
Variable X	Creatinine_concentration
Sample size	204
Correlation coefficient r	-0.07955
Significance level	P=0.2581
95% Confidence interval for r	-0.2146 to 0.05847

(d)

Appendix 6: Independent variable to independent variable correlation study.

Variable Y	Age
Variable X	Creatinine_concentration

Sample size	204
Correlation coefficient r	0.1223
Significance level	P=0.0814
95% Confidence interval for r	-0.01534 to 0.2554

(a)

Variable Y	Age
Variable X	Gender

Sample size	204
Correlation coefficient r	-0.07835
Significance level	P=0.2653
95% Confidence interval for r	-0.2134 to 0.05966

(b)

Variable Y	Age
Variable X	Mean_of_AlbBCG_and_AlbNEPH

Sample size	204
Correlation coefficient r	0.004456
Significance level	P=0.9496
95% Confidence interval for r	-0.1330 to 0.1417

(c)

Variable Y	Creatinine_concentration
Variable X	Gender

Sample size	204
Correlation coefficient r	-0.1894
Significance level	P=0.0067
95% Confidence interval for r	-0.3185 to -0.05338

(d)

Variable Y	Creatinine_concentration
Variable X	Mean_of_AlbBCG_and_AlbNEPH

Sample size	204
Correlation coefficient r	0.07223
Significance level	P=0.3046
95% Confidence interval for r	-0.06580 to 0.2075

(e)

Variable Y	Mean_of_AlbBCG_and_AlbNEPH
Variable X	Gender

Sample size	204
Correlation coefficient r	-0.08969
Significance level	P=0.2021
95% Confidence interval for r	-0.2243 to 0.04828

(f)

Appendix 7: Simple linear regression (one x-variable).

Dependent Y	Difference__AlbBCG_AlbNEPH_
Independent X	Age

Least squares regression

Sample size	204
Coefficient of determination R^2	0.009649
Residual standard deviation	3.5281

Regression Equation

y = 6.8207 + -0.01903 x

Parameter	Coefficient	Std. Error	95% CI	t	P
Intercept	6.8207	0.8345	5.1752 to 8.4662	8.1733	<0.0001
Slope	-0.01903	0.01357	-0.04578 to 0.007718	-1.4029	0.1622

Analysis of Variance

Source	DF	Sum of Squares	Mean Square
Regression	1	24.4985	24.4985
Residual	202	2514.4303	12.4477

F-ratio	1.9681
Significance level	P=0.1622

(a)

Dependent Y	Difference__AlbBCG_AlbNEPH_
Independent X	Creatinine_concentration

Least squares regression

Sample size	204
Coefficient of determination R^2	0.006328
Residual standard deviation	3.5340

Regression Equation

y = 5.9146 + -0.001773 x

Parameter	Coefficient	Std. Error	95% CI	t	P
Intercept	5.9146	0.3102	5.3030 to 6.5263	19.0671	<0.0001
Slope	-0.001773	0.001563	-0.004856 to 0.001310	-1.1342	0.2581

Analysis of Variance

Source	DF	Sum of Squares	Mean Square
Regression	1	16.0651	16.0651
Residual	202	2522.8637	12.4894

F-ratio	1.2863
Significance level	P=0.2581

(b)

Dependent Y	Difference__AlbBCG_AlbNEPH_
Independent X	Gender

Least squares regression

Sample size	204
Coefficient of determination R^2	0.0006182
Residual standard deviation	3.5442

Regression Equation

y = 5.9639 + -0.1755 x

Parameter	Coefficient	Std. Error	95% CI	t	P
Intercept	5.9639	0.7802	4.4255 to 7.5023	7.6440	<0.0001
Slope	-0.1755	0.4964	-1.1542 to 0.8033	-0.3535	0.7241

Analysis of Variance

Source	DF	Sum of Squares	Mean Square
Regression	1	1.5695	1.5695
Residual	202	2537.3593	12.5612

F-ratio	0.1250
Significance level	P=0.7241

(c)

Dependent Y	Difference__AlbBCG_AlbNEPH_
Independent X	Mean_of_AlbBCG_and_AlbNEPH

Least squares regression

Sample size	204
Coefficient of determination R^2	0.3507
Residual standard deviation	2.8568

Regression Equation

y = 15.0304 + -0.2736 x

Parameter	Coefficient	Std. Error	95% CI	t	P
Intercept	15.0304	0.9152	13.2259 to 16.8349	16.4238	<0.0001
Slope	-0.2736	0.02619	-0.3253 to -0.2220	-10.4452	<0.0001

Analysis of Variance

Source	DF	Sum of Squares	Mean Square
Regression	1	890.3950	890.3950
Residual	202	1648.5338	8.1611

F-ratio	109.1029
Significance level	P<0.0001

(d)

Appendix 8: Simple linear regression (2 x-variable)

Dependent Y	Difference__AlbBCG_AlbNEPH_

Least squares multiple regression

Method	Enter

Sample size	204
Coefficient of determination R^2	0.01428
R^2-adjusted	0.004471
Multiple correlation coefficient	0.1195
Residual standard deviation	3.5286

Regression Equation

Independent variables	Coefficient	Std. Error	t	P	$r_{partial}$	$r_{semipartial}$	VIF
(Constant)	6.9081						
Age	-0.01741	0.01367	-1.273	0.2044	-0.08946	0.08917	1.015
Creatinine_concentration	-0.001528	0.001573	-0.972	0.3324	-0.06837	0.06804	1.015

Analysis of Variance

Source	DF	Sum of Squares	Mean Square
Regression	2	36.2536	18.1268
Residual	201	2502.6751	12.4511

F-ratio	1.4558
Significance level	P=0.2357

Zero order and simple correlation coefficients

Variable	Difference__AlbBCG_AlbNEPH_	Age
Age	-0.09823	
Creatinine_concentration	-0.07955	0.1223

(a)

Dependent Y	Difference__AlbBCG_AlbNEPH_

Least squares multiple regression

Method	Enter

Sample size	204
Coefficient of determination R^2	0.01072
R^2-adjusted	0.0008722
Multiple correlation coefficient	0.1035
Residual standard deviation	3.5350

Regression Equation

Independent variables	Coefficient	Std. Error	t	P	$r_{partial}$	$r_{semipartial}$	VIF
(Constant)	7.1945						
Age	-0.01953	0.01364	-1.432	0.1536	-0.1005	0.1005	1.006
Gender	-0.2312	0.4966	-0.4656	0.6420	-0.03282	0.03266	1.006

Analysis of Variance

Source	DF	Sum of Squares	Mean Square
Regression	2	27.2068	13.6034
Residual	201	2511.7220	12.4961

F-ratio	1.0886
Significance level	P=0.3387

Zero order and simple correlation coefficients

Variable	Difference__AlbBCG_AlbNEPH_	Age
Age	-0.09823	
Gender	-0.02486	-0.07835

(b)

Dependent Y	Difference__AlbBCG_AlbNEPH_

Least squares multiple regression

Method	Enter

Sample size	204
Coefficient of determination R^2	0.3598
R^2-adjusted	0.3535
Multiple correlation coefficient	0.5999
Residual standard deviation	2.8436

Regression Equation

Independent variables	Coefficient	Std. Error	t	P	$r_{partial}$	$r_{semipartial}$	VIF
(Constant)	16.1119						
Age	-0.018532	0.01093	-1.694	0.0918	-0.1186	0.09559	1.000
Mean_of_AlbBCG_and_AlbNEPH	-0.2734	0.02607	-10.486	<0.0001	-0.5946	0.5918	1.000

Analysis of Variance

Source	DF	Sum of Squares	Mean Square
Regression	2	913.5952	456.7976
Residual	201	1625.3335	8.0862

F-ratio	56.4908
Significance level	P<0.0001

Zero order and simple correlation coefficients

Variable	Difference__AlbBCG_AlbNEPH_	Age
Age	-0.09823	
Mean_of_AlbBCG_and_AlbNEPH	-0.5922	0.004456

(c)

Dependent Y	Difference__AlbBCG_AlbNEPH_

Least squares multiple regression

Method	Enter

Sample size	204
Coefficient of determination R^2	0.007981
R^2-adjusted	-0.001890
Multiple correlation coefficient	0.08934
Residual standard deviation	3.5399

Regression Equation

Independent variables	Coefficient	Std. Error	t	P	$r_{partial}$	$r_{semipartial}$	VIF
(Constant)	6.3711						
Creatinine_concentration	-0.001948	0.001595	-1.221	0.2234	-0.08583	0.08581	1.037
Gender	-0.2922	0.5049	-0.579	0.5634	-0.04079	0.04066	1.037

Analysis of Variance

Source	DF	Sum of Squares	Mean Square
Regression	2	20.2630	10.1315
Residual	201	2518.6657	12.5307

F-ratio	0.8085
Significance level	P=0.4470

Zero order and simple correlation coefficients

Variable	Difference__AlbBCG_AlbNEPH_	Creatinine_concentration
Creatinine_concentration	-0.07955	
Gender	-0.02486	-0.1894

(d)

Dependent Y	Difference__AlbBCG_AlbNEPH_

Least squares multiple regression

Method	Enter

Sample size	204
Coefficient of determination R^2	0.3521
R^2-adjusted	0.3456
Multiple correlation coefficient	0.5933
Residual standard deviation	2.8609

Regression Equation

Independent variables	Coefficient	Std. Error	t	P	$r_{partial}$	$r_{semipartial}$	VIF
(Constant)	15.0869						
Creatinine_concentration	-0.0008241	0.001269	-0.649	0.5168	-0.04576	0.03687	1.005

Dependent Y	Difference__AlbBCG_AlbNEPH_

Least squares multiple regression

Method	Enter

Sample size	204
Coefficient of determination R^2	0.3568
R^2-adjusted	0.3504
Multiple correlation coefficient	0.5973
Residual standard deviation	2.8503

Regression Equation

Independent variables	Coefficient	Std. Error	t	P	$r_{partial}$	$r_{semipartial}$	VIF
(Constant)	15.9681						
Gender	-0.5547	0.4008	-1.384	0.1679	-0.09716	0.07829	1.008

Mean_of_AlbBCG_and_AlbNEPH	-0.2724	0.02630	-10.356	<0.001	-0.5899	0.5880	1.005

Analysis of Variance

Source	DF	Sum of Squares	Mean Square
Regression	2	893.8465	446.9232
Residual	201	1645.0823	8.1845

F-ratio	54.6061
Significance level	P<0.0001

Zero order and simple correlation coefficients

Variable	Difference__AlbBCG_AlbNEPH_	Creatinine_concentration
Creatinine_concentration	-0.07955	
Mean_of_AlbBCG_and_AlbNEPH	-0.5922	0.07223

(e)

Mean_of_AlbBCG_and_AlbNEPH	-0.2769	0.02624	-10.551	<0.001	-0.5970	0.5968	1.008

Analysis of Variance

Source	DF	Sum of Squares	Mean Square
Regression	2	905.9573	452.9787
Residual	201	1632.9715	8.1242

F-ratio	55.7565
Significance level	P<0.0001

Zero order and simple correlation coefficients

Variable	Difference__AlbBCG_AlbNEPH_	Gender
Gender	-0.02486	
Mean_of_AlbBCG_and_AlbNEPH	-0.5922	-0.08969

(f)

Appendix 9: Multiple regression with the inclusion of all the variables.

Dependent Y	Difference__AlbBCG_AlbNEPH_

Least squares multiple regression

Method	Enter

Sample size	204
Coefficient of determination R^2	0.3689
R^2-adjusted	0.3562
Multiple correlation coefficient	0.6074
Residual standard deviation	2.8375

Regression Equation

Independent variables	Coefficient	Std. Error	t	P	$r_{partial}$	$r_{semipartial}$
(Constant)	17.3236					
Age	-0.01894	0.01101	-1.720	0.0869	-0.1211	0.09688
Gender	-0.6640	0.4066	-1.633	0.1041	-0.1150	0.09195
Creatinine_concentration	-0.0009424	0.001288	-0.732	0.4653	-0.05179	0.04119
Mean_of_AlbBCG_and_AlbNEPH	-0.2759	0.02617	-10.544	<0.0001	-0.5987	0.5938

Analysis of Variance

Source	DF	Sum of Squares	Mean Square
Regression	4	936.6977	234.1744
Residual	199	1602.2311	8.0514

F-ratio	29.0849
Significance level	P<0.0001

Zero order and simple correlation coefficients

Variable	Difference__AlbBCG_AlbNEPH_	Age	Gender	Creatinine_concentration
Age	-0.09823			
Gender	-0.02486	-0.07835		
Creatinine_concentration	-0.07955	0.1223	-0.1894	
Mean_of_AlbBCG_and_AlbNEPH	-0.5922	0.004456	-0.08969	0.07223

Residuals

Shapiro-Wilk test for Normal distribution	W=0.9821 reject Normality (P=0.0106)

Appendix 10: Multiple regression with the elimination of non-significant variable.

Dependent Y	Difference__AlbBCG_AlbNEPH_

Least squares multiple regression

Method	Backward
Enter variable if P<	0.05
Remove variable if P>	0.051

Sample size	204
Coefficient of determination R^2	0.3507
R^2-adjusted	0.3475
Multiple correlation coefficient	0.5922
Residual standard deviation	2.8568

Regression Equation

Independent variables	Coefficient	Std. Error	t	P	$r_{partial}$	$r_{semipartial}$
(Constant)	15.0304					
Mean_of_AlbBCG_and_AlbNEPH	-0.2736	0.02619	-10.445	<0.0001	-0.5922	0.5922

Variables not included in the model
Age
Gender
Creatinine_concentration

Analysis of Variance

Source	DF	Sum of Squares	Mean Square
Regression	1	890.3950	890.3950
Residual	202	1648.5338	8.1611

F-ratio	109.1029
Significance level	P<0.0001

Zero order and simple correlation coefficients

Variable	Difference__AlbBCG_AlbNEPH_	Age	Gender	Creatinine_concentration
Age	-0.09823			
Gender	-0.02486	-0.07835		
Creatinine_concentration	-0.07955	0.1223	-0.1894	
Mean_of_AlbBCG_and_AlbNEPH	-0.5922	0.004456	-0.08969	0.07223

Residuals

Shapiro-Wilk test for Normal distribution	W=0.9808 reject Normality (P=0.0069)

Appendix 11: Raw data

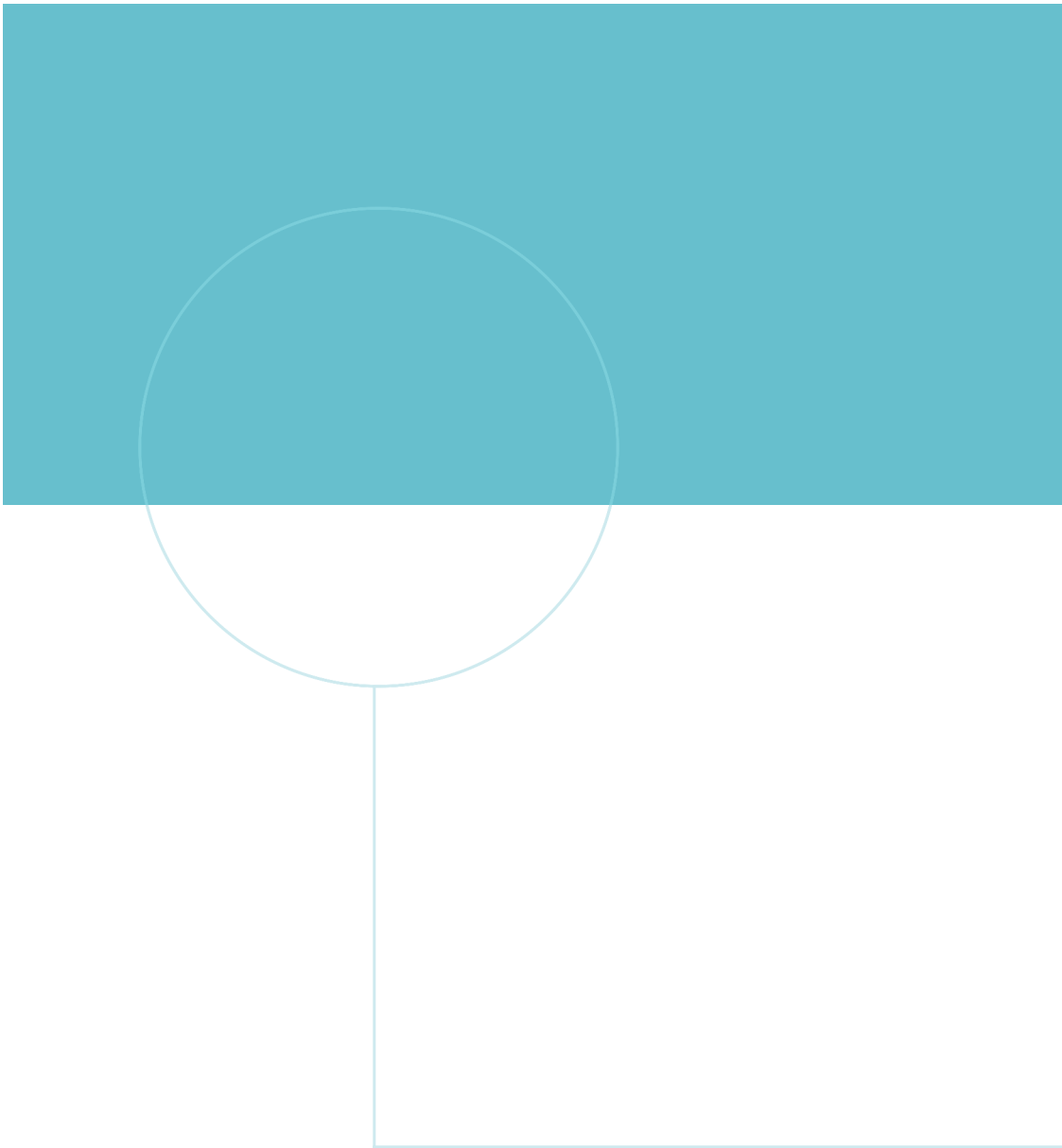
SERIAL NUMBER	GENDER	YEAR	AGE	CREATININE - ADVIA CHEMISTRY	ALBUMIN (BCG) - ADVIA CHEMISTRY	ALBUMIN (IMMUNOASSAY) - ATELLICA NEPH
1	M	1954	66	70.3	41.8	41.0
2	M	1953	67	62.1	33.1	23.5
3	F	1975	45	43.2	36.6	33.2
4	M	1995	25	78.8	48.1	50.7
5	M	1955	65	60.1	46.5	47.5
6	M	1996	24	39.1	27.6	15.7
7	F	1949	71	46.5	33.0	22.7
8	M	1977	43	63.3	35.8	27.9
9	F	1970	50	101.0	25.6	12.6
10	F	1986	34	65.1	31.9	24.3
11	M	1967	53	54.3	37.6	35.5
12	F	2000	20	42.0	30.8	22.7
13	F	1968	52	35.8	26.6	18.6
14	F	1982	38	37.0	32.5	22.6
15	F	1964	56	282.3	29.9	23.9
16	M	1946	74	76.3	41.4	42.1
17	M	1971	49	100.4	43.9	44.8
18	M	1936	84	111.3	30.9	25.4
19	F	1938	82	133.3	17.2	16.1
20	F	1939	81	39.6	30.6	24.8
21	F	1968	52	81.9	42.2	40.7
22	M	1938	82	87.8	40.5	33.6
23	M	1948	72	62.5	41.1	36.1
24	M	1965	55	99.1	42.9	41.7
25	M	1970	50	112.3	44.4	39.5
26	M	1946	74	69.6	45.0	40.9
27	M	1946	74	152.1	29.8	24.2
28	F	1936	84	61.2	43.2	38.8
29	M	1954	66	64.3	44.5	43.4
30	F	1975	45	53.4	44.6	41.1
31	F	1986	34	99.3	30.4	21.3
32	F	1936	84	107.7	23.0	14.1
33	F	1975	45	74.2	43.3	42.1
34	F	1964	56	50.6	44.8	42.7
35	F	1941	79	67.7	37.7	34.8
36	M	1992	28	33.3	35.9	29.3
37	F	1964	56	538.9	43.4	39.1
38	M	1956	64	90.0	45.0	42.3
39	M	1984	36	68.0	48.0	42.3
40	M	1949	71	85.5	41.9	39.1

41	F	1948	72	64.2	46.3	41.9
42	M	1968	52	92.0	42.1	40.5
43	F	1978	42	72.1	43.2	34.3
44	M	1948	72	68.5	37.7	33.4
45	M	1974	46	49.9	28.3	23.1
46	M	1986	34	106.4	37.9	35.3
47	M	1968	52	89.6	24.0	21.9
48	F	1940	80	65.6	44.3	39.2
49	F	1931	89	45.0	42.9	35.5
50	M	1970	50	48.3	41.3	34.0
51	M	1946	74	72.1	24.1	19.9
52	M	1961	59	49.3	29.1	14.6
53	F	1955	65	145.2	27.8	25.3
54	F	1965	55	54.0	22.7	18.4
55	F	1936	84	36.2	20.9	10.2
56	M	1996	24	44.1	28.4	15.4
57	M	1997	23	59.7	23.9	15.5
58	M	1939	81	119.2	27.6	22.9
59	F	1949	71	47.5	33.4	21.6
60	M	1992	28	31.4	36.0	30.4
61	M	1968	52	222.9	36.2	35.1
62	F	1942	78	55.3	31.4	25.8
63	F	1970	50	105.8	26.5	12.7
64	M	1945	75	107.6	38.8	35.5
65	M	1943	77	92.8	37.9	33.3
66	F	1976	44	65.1	32.1	29.7
67	M	1948	72	108.7	36.6	31.0
68	M	1977	43	62.6	34.1	24.4
69	F	2000	20	48.8	31.9	21.8
70	F	1986	34	103.2	30.5	21.2
71	M	1949	71	48.0	28.0	15.5
72	F	1982	38	42.6	36.4	24.1
73	F	1970	50	51.0	37.4	32.7
74	M	1936	84	115.5	28.9	22.1
75	M	1936	84	411.4	35.8	24.0
76	F	1958	62	59.6	43.4	39.8
77	F	1988	32	63.9	44.0	41.5
78	F	1946	74	73.4	41.8	34.0
79	M	2003	17	65.1	37.8	30.8
80	F	1960	60	52.7	47.3	43.4
81	F	1981	39	590.3	39.8	38.0
82	M	1942	78	71.9	42.3	33.4
83	M	1948	72	595.9	36.4	30.2
84	M	1957	63	100.4	39.9	36.6
85	F	1958	62	49.6	41.9	39.4

86	F	1951	69	49.7	51.4	47.1
87	F	1972	48	54.0	37.6	36.8
88	M	1956	64	81.1	39.7	33.9
89	F	1965	55	18.6	25.1	24.5
90	M	1949	71	136.9	37.5	26.1
91	F	1986	34	100.0	29.2	20.0
92	F	1938	82	131.5	25.6	27.6
93	F	1972	48	47.7	35.3	32.5
94	F	2019	1	18.2	30.2	24.7
95	M	1936	84	392.9	35.8	24.6
96	F	1955	65	100.4	28.7	24.9
97	M	1996	24	49.0	31.8	17.2
98	M	1939	81	124.6	24.8	18.6
99	F	1965	55	52.8	19.6	14.2
100	M	1961	59	44.3	28.3	13.0
101	F	1944	76	50.5	33.8	21.9
102	F	1982	38	42.4	32.8	21.5
103	F	1952	68	64.2	38.7	35.5
104	F	1963	57	185.2	33.9	27.7
105	M	1950	70	549.2	43.5	41.2
106	M	1990	30	1018.4	40.7	39.7
107	M	1948	72	99.5	35.2	30.3
108	M	1953	67	318.8	41.0	36.0
109	M	1953	67	324.4	41.9	36.5
110	F	1938	82	132.7	27.7	29.9
111	M	2001	19	73.3	47.9	46.4
112	M	1964	56	90.9	46.3	43.4
113	F	1976	44	64.3	38.9	37.1
114	M	1946	74	915.3	38.0	29.8
115	M	1944	76	68.1	41.6	36.0
116	M	1950	70	86.8	42.2	37.7
117	F	1943	77	70.8	36.7	30.5
118	M	1952	68	793.5	36.9	34.4
119	M	1948	72	83.8	34.1	29.3
120	F	1963	57	70.0	40.1	38.3
121	M	1950	70	242.8	39.0	35.4
122	F	1969	51	97.9	44.2	41.0
123	M	1958	62	658.1	40.0	31.9
124	M	1961	59	71.4	33.7	28.4
125	M	1946	74	80.9	45.7	41.1
126	F	1964	56	70.6	42.8	40.6
127	M	1948	72	77.9	35.7	30.4
128	F	1962	58	63.9	42.2	40.0
129	F	1965	55	45.5	41.2	36.8
130	F	1941	79	56.1	37.2	29.7

131	M	1942	78	162.0	34.2	30.5
132	M	1949	71	730.9	44.7	41.5
133	F	1971	49	55.8	44.4	40.1
134	F	1983	37	87.9	41.3	36.8
135	F	1948	72	45.9	37.2	29.3
136	M	1965	55	66.3	45.8	41.5
137	F	1972	48	44.5	44.3	42.9
138	M	1958	62	88.0	46.6	40.2
139	F	1955	65	57.3	42.6	39.7
140	M	1994	26	198.1	40.0	33.2
141	M	1946	74	928.1	39.5	35.8
142	F	1940	80	454.4	30.8	24.1
143	F	1948	72	92.0	39.7	35.8
144	F	1961	59	379.5	40.9	37.4
145	F	1941	79	72.3	42.1	40.2
146	M	1962	58	89.0	48.6	41.6
147	M	1947	73	106.9	45.3	42.3
148	F	1946	74	61.3	45.9	39.9
149	F	1966	54	65.2	44.5	43.8
150	F	1962	58	51.2	42.8	38.7
151	F	2011	9	29.2	43.2	43.8
152	M	1939	81	60.3	42.3	34.3
153	F	1969	51	68.6	43.4	38.3
154	M	1989	31	45.9	32.2	22.9
155	M	1947	73	170.5	33.4	26.2
156	F	1965	55	50.2	26.8	22.0
157	F	1948	72	56.3	41.2	34.4
158	M	1961	59	43.1	28.8	14.8
159	F	1936	84	26.0	22.2	12.0
160	M	1996	24	43.4	31.3	18.2
161	F	1955	65	191.7	25.0	17.1
162	F	1958	62	301.5	27.6	15.2
163	F	1970	50	88.8	28.7	14.1
164	M	1943	77	88.7	37.4	33.4
165	M	1952	68	81.0	40.6	36.3
166	M	1942	78	69.4	28.7	21.3
167	M	1977	43	67.6	36.8	27.2
168	F	1942	78	95.8	31.1	26.8
169	M	1944	76	72.0	34.2	28.8
170	F	1956	64	48.3	40.7	33.8
171	F	1986	34	107.3	31.6	20.8
172	F	1982	38	44.6	32.5	22.1
173	F	1957	63	61.3	39.2	33.6
174	M	1959	61	57.7	36.2	24.5
175	F	1946	74	62.0	39.2	33.7

176	M	1957	63	89.4	43.2	35.7
177	M	1998	22	52.1	40.1	33.6
178	F	2010	10	131.6	38.4	30.5
179	F	1953	67	70.6	41.4	36.8
180	M	1944	76	107.6	41.3	35.0
181	F	1944	76	56.6	33.4	25.3
182	F	1944	76	44.9	36.7	30.1
183	M	1958	62	73.8	39.4	34.9
184	M	1949	71	62.2	44.1	39.3
185	M	1956	64	41.4	40.7	33.8
186	M	1978	42	62.2	36.9	28.8
187	F	1971	49	68.1	37.7	32.5
188	F	1945	75	69.3	40.4	36.0
189	M	1932	88	115.4	29.4	22.1
190	M	1963	57	54.9	27.0	22.4
191	M	1982	38	79.2	44.7	40.2
192	F	1964	56	60.7	44.1	38.6
193	F	1954	66	72.5	36.7	32.0
194	F	1958	62	49.5	39.2	32.4
195	F	1961	59	64.7	44.9	40.2
196	M	1961	59	72.2	36.1	29.7
197	M	1948	72	91.3	36.2	30.2
198	M	1953	67	261.9	31.1	21.2
199	M	1946	74	87.4	49.4	47.7
200	F	1997	23	39.9	40.5	30.4
201	F	1975	45	43.8	31.7	28.1
202	M	1954	66	70.3	32.1	29.9
203	M	1961	59	84.6	38.8	35.0
204	M	1978	42	70.4	39.3	33.9



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