Comparison Of Immunonephelometric And Immunoturbidimetric Methods for Measuring Beta 2-Microglobulin: Fit for Purpose in Routine Clinical Laboratories

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Abstract

Serum free beta 2-microglobulin (b2M) level has been regarded as an independent biomarker in several cancers, and has traditionally been analyzed using methods such as Immunonephelometry on specialized analyzers. It is now possible to perform this test on clinical chemistry analyzers, using immunoturbidimetry. The aim of this study was to compare these two methods, for measuring serum b2M level. Forty three samples which were randomly chosen from sera of patients with various malignant conditions were analyzed for serum b2M level, both by immunonephelometric method in Beckman Immage 800 (Beckman Coulter Inc., CA, USA) nephelometer, and by immunoturbidimetric method in Beckman UniCel DXC 800 Synchron (Beckman Coulter Inc., CA, USA) auto-analyzer. Method comparison demonstrated good agreement between the immunonephelometric and immunoturbidimetric b2M testing, in which we found good correlation (r=0.973) and high accuracy (slope=1.009). As a conclusion, Beckman UniCel DXC 800 Synchron immunoturbidimetric b2M assay is suitable for routine use, and correlates well with representative immunonephelometric assays on the Beckman Immage 800 analyzer. The ability to perform specific protein analyses such as b2M on an integrated clinical chemistry/immunoassay system, can allow consolidation of testing on a single platform and results in improved laboratory operations efficiency. It is thought that immunoturbidimetric method can be used safely in the routine screening of serum b2M level, for different types of malignancies.

Keywords: Beta 2-Microglobulin; Immunonephelometry; Immunoturbidimetry; Method Comparison

Introduction

Beta 2-microglobulin (b2M) is a nonglycosylated protein with a molecular mass of 11,800 Da. It forms the beta chain of the human leukocyte antigen (HLA) class I molecule through noncovalent linkage on cell surfaces, and has a 7-stranded beta-pleated structure. Because it is noncovalently associated with the alpha chain of MHC class I molecules and has no direct attachment to the cell membrane, b2M on the cell surface can exchange with free soluble b2M. Free b2M is found in body fluids (including serum, urine, and synovial fluid), as a result of shedding from cell surfaces or intracellular release [1-3]. Approximately, 100-300 mg of b2M is synthesized on a daily basis, i.e. 2-4 mg per kg of body weight [4].

The determination of b2M in serum or plasma is an aid in the clinical assessment of activation of the cellular immune system and a tumor marker [5]. Recent studies demonstrated that b2M is extensively involved in the functional regulation of immunity, survival, proliferation, apoptosis and even metastasis in cancer cells [5-9]. b2M protein is present at low levels in serum, urine and other body fluids under normal physiological conditions, and is almost exclusively catabolized within the kidney. Many studies have demonstrated that serum or urine b2M concentration is increased in a variety of abnormal cell growth diseases, including breast cancer, prostate cancer, lung cancer, renal cancer, gastrointestinal and nasopharyngeal cancers, multiple myeloma and especially lymphocytic malignancies such as non-Hodgkin’s lymphoma and multiple myeloma [10-16]. In these malignancies, the b2M test serves as an independent and significant prognostic factor [17] and it is a helpful indicator for disease development and therapy estimation. Additionally, elevated serum concentrations of b2M are seen with dysfunction of renal glomeruli, while disorders affecting epithelial cells of the proximal tubules will cause an enhanced excretion of this protein in the urine [18-20]. In all these clinical conditions, using sensitive methods are essential to analyze b2M.

Specific proteins have traditionally been analyzed using methodologies, such as Immunonephelometry on specialized analyzers. Immunonephelometry is a well recognized and accepted methodology, but it requires a specialized analyzer that performs a limited menu of tests. A variety of immunoturbidimetric assays are now available, and can be adapted to general purpose clinical chemistry analyzers that are “Open Systems” [21]. Immunoturbidimetric assays have been improved considerably over time. These improvements include advances in antibody purification techniques, enhancements in instrument design and function and production of new reference materials based on global standardization initiatives [22-26]. Additional practical advantages of immunoturbidimetric protein assays include random access analysis instead of batch testing, relatively rapid turn around time, high volume testing capability, cost reduction through consolidation of testing on a single platform, elimination of stand alone methodology, but it requires a specialized analyzer that performs a limited menu of tests. A variety of immunoturbidimetric assays are now available, and can be adapted to general purpose clinical chemistry analyzers that are “Open Systems” [21]. Immunoturbidimetric assays have been improved considerably over time. These improvements include advances in antibody purification techniques, enhancements in instrument design and function and production of new reference materials based on global standardization initiatives [22-26]. Additional practical advantages of immunoturbidimetric protein assays include random access analysis instead of batch testing, relatively rapid turn around time, high volume testing capability, cost reduction through consolidation of testing on a single platform, elimination of stand alone
specialized analyzers, and time and effort required to maintain them [21]. Collectively, these characteristics of immunoturbidimetric assays have resulted in more reliable tests and reduced laboratory variances to help clinicians.

b2M test has become a high volume test in clinical laboratories due to its increasing clinical use. Although it has multiple clinical uses, it is measured by only a few automated instruments. Clinical laboratories are under increasing pressure to select the best method, to provide large volumes of high quality analyses in shorter turn-around-times and at the lowest possible cost. Thus, this study was designed to make a method comparison for serum b2M levels, by using immunonephelometric and immunoturbidimetric method in Beckman Immage 800 nephelometer and immunoturbidimetric method in Beckman UniCel DXC 800 Synchron auto-analyzer.

Materials and methods
Collection of specimens
Randomly chosen serum samples of 43 patients with various malignant conditions, which were referred to the Central Laboratory of Cukurova University Balcali Hospital, (Adana, Turkey) from different clinics in 2011 were included in this study. Serum samples were stored at 70°C before being assayed. Patient ages ranged between 9-77.

Immuonassays
Serum b2M level of all samples were analyzed concurrently, both by immunonephelometric method in Beckman Immage 800 (Beckman Coulter Inc., CA, USA) nephelometer and by immunoturbidimetric method in Beckman UniCel DXC 800 Synchron (Beckman Coulter Inc., CA, USA) auto-analyzer. Additionally, total of 43 specimens were analyzed two times by both methods. Sentinel reagents were used to perform b2M testing on Beckman Immage 800 nephelometer (Ref:A09445 Sentinel Diagnostics, Milan, Italy) and Beckman UniCel DXC 800 Synchron auto-analyzer (Ref:11505H, Sentinel Diagnostics, Milan, Italy).

The Beckman Immage 800 performs non-competitive rate nephelometric assays, in which a polyclonal rabbit anti-human b2M is covalently coupled to polystyrene particles of uniform size. A reaction between these immunoparticles and b2M molecules in the patient specimen, results in the formation of agglutinates that produce a change in absorbance, with the magnitude of the change being proportional to the quantity of b2M contained in the sample.

Turbidimetric b2M kit is a latex immunoassay, developed to measure b2M levels in serum and urine samples by immunoturbidimetric method. When an antigen-antibody reaction occurs between b2M in a sample and an anti-b2M antibody which has been adsorbed to latex particles, agglutination results. This agglutination is detected as a change in absorbance, with the magnitude of the change being proportional to the quantity of b2M contained in the sample.

One analyst performed all the analyses, employing the same batches of reagent, quality control material and calibrators. Control material was included in each analytical series to guarantee that analytical imprecision was within the acceptable limits, according to our internal quality control protocol. In addition, external quality assurance program (Bio-Rad Program, California USA) was applied to b2M test, during the period of the study.

Linearity and detection limit
Linearity studies were performed using blends of samples with different b2M concentrations, that fell within the lower and upper limits of the calibration range. The resultant pools (consisting of 12 points) were assayed in duplicate, using the assay’s normal sample volume settings. The observed mean concentration was plotted vs. the expected concentration, and data were examined by linear regression and visual inspection of the bias plots.

The lower limit of detection was defined as the lowest protein concentration corresponding to the mean absorbance value, plus 3 SDs of 10 repeated measurements of the sample diluent [24].

Data analysis
All results were examined by Bland-Altman analysis and then evaluated by MedCalc software. Correlation relations of both methods were analysed by Pearson correlation analysis, and regression equivalences were analysed by Passing-Bablock regression analysis. SPSS-13.00 (Statistical Package for Social Sciences, Chicago, USA) was used for the whole statistical analyses.

Results
Results of b2M levels of 43 patients which were measured both in Beckman Immage 800 and Beckman UniCel DXC 800, were analysed by Bland-Altman analysis and the plots of difference against mean for both methods is given in figure 1.

In the graphics, differences were seen to be randomly collected around zero, and 95% of it were between ±1, 96sd and no relation was found between the differences and means. Mean of the differences and standard deviation were found as 0.22 and 0.607, respectively. It has been designated that the result of b2M measured by the immunoturbidimetric method, could be either 1.41 mg/L higher or 0.97 mg/L lower than the results attained by the immunonephelometric method, and results of two methods were concordant.

According to the results of two methods, the mean and standard deviation attained by the Beckman UniCel DXC 800 and Beckman Immage 800 devices were 2.74±1.84 mg/L and 2.96±2.22 mg/L.

Figure 1: Bland-Altman plot of b2M by the turbidimetry and nephelometry. The X axis indicates the arithmetic mean between the values of b2M by turbidimetry and nephelometry in each patient; the Y axis indicates the difference between the values of b2M by turbidimetry and nephelometry in each patient.
respectively. There was a significant (at the p=0.01 level (r=0.973)), and acceptable relation between two analyses performed by correlation and regression analyses. The regression equivalence between two methods was y=1.176x-0.263, and regression standard error was 0.52. Scatter-plots of the results obtained by both methods are given in Figure 2.

**Discussion**

**b2M** is found to act similarly as a prototypical oncogenic factor, capable of stimulating growth and progression of various cancers and plays a key regulatory role in stimulating cancer metastasis [17]. Free b2M level in serum has been regarded as an independent biomarker in several cancers and has been used to stage diseases, and serial measurements of this protein are used for monitoring response to therapy [27]. Although b2M has multiple clinical uses, it is measured by only a few automated instruments. This study was designed to compare immunonephelometric and immunoturbidimetric methods for analyzing serum b2M levels, to ensure that the performance of immunoturbidimetric assay could be acceptable for clinical needs and fit for purpose.

There was good correlation (r=0.973) and high accuracy (slope=1.009) between Beckman UniCel DXC 800 and Beckman Immage 800 assays. This is perhaps because both methods share the same physical principle (scattering) to measure the antigen–antibody complexes. Note however, the Beckman UniCel DXC 800 assay relies on the principle of turbidimetry, which is dependent on scattering and then absorption. Additionally, the assays were found to be linear over the calibration range and the analytical sensitivity consistent with previously described findings for serum proteins [24]. High correlation coefficient indicates that specimens used to compare the two methods cover the range of the assay. The method comparison results were analysed using Passing–Bablok approach, where the results did not depend on the assignment of the methods specific to X or Y. Since Passing–Bablok plot is insensitive to outliers, the scaled median absolute deviation was calculated, which is similar to the standard error of estimate (0.52).

There are many studies in the literature which compared the results of different specific proteins, such as CRP, IgA, IgG, IgM, C3, C4, haptoglobin, transferrin, apolipoproteins, RF etc. held by nephelometric and turbidimetric methods, and results of two methods were found to be correlated with each other [21,28-32]. Correia et al. [28] found that CRP levels measured by either methods were correlated with each other, in both unstable angina patients and in patients with non-ST elevation acute myocardial infarction. In Dominici et al. [30] study, the two systems were found to perform substantially equally, both in hsCRP and in CRP measurement; but in the hsCRP assay, the precision of nephelometry (CV% in the interval 3.0-5.8) was lower than that of turbidimetry (CV% in the interval 1.8-2.3). The classification of results by the two methods into three predefined relative risk classes gave 18% rate of discordance, in any case by one class only. In their study, the two methods proved reliable and comparable in the measurement of hsCRP, and they mention that precision should be improved. Denham et al. [29] demonstrated that by their performance characteristics, the Architect ci8200 immunoturbidimetric specific protein assays are suitable for routine use, and correlate well with representative immunonephelometric assays on the Beckman Immage analyzer. In a study of Ledue et al. [25], in which they evaluated the method performance characteristics of 14 serum protein analytes (alfa 1-antitrypsin, alfa 2-macroglobulin, albumin, apolipoproteins AI and B, C3, C4, haptoglobin, IgA, IgG, IgM, orosomucoid, transferrin and transthyretin) in Roche Cobas C501, they found an excellent correlation between immunonephelometric and immunoturbidimetric assays.

Over the past several decades, numerous commercial auto-analyzers for the measurement of serum proteins have been introduced. Presently, 40% of laboratories that participate in the College of American Pathologists (CAP) proficiency program use a dedicated nephelometer (source: CAP’s 2009 Diagnostic Immunology Survey C). There are, however, a growing number of laboratories who see the adaptation of chemistry analyzers for protein measurement based on immunoturbidimetry, as a logical extension. Our study showed that results of the immunoturbidimetric and immunonephelometric assays for measuring serum b2M levels were highly correlated. It is thought that immunoturbidimetric method of Beckman UniCel DXC 800, can be used safely in the routine screening of serum b2M levels for different types of malignancies. Additionally, using this method can bring many advantages such as rapid turn around time, high volume testing capability and cost reduction for routine clinical laboratories.

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**Figure 2:** Scatter-plots for the method comparison study between the immunonephelometric and immunoturbidimetric methods. The solid line indicates the line of identity (x=y).
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