Neutrophil Gelatinase-Associated Lipocalin as a Biomarker of Renal Impairment in Patients With Multiple Myeloma

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Abstract

This study assessed whether neutrophil gelatinase-associated lipocalin (NGAL) can be used as a biomarker of renal impairment in patients with multiple myeloma. Plasma NGAL levels significantly correlated with the degree of renal impairment and also correlated with markers of myeloma burden.

Background: Renal impairment (RI) is a common complication of multiple myeloma (MM) and is associated with an unfavorable prognosis. Several new markers of kidney damage have been introduced in recent years. The aim of this study was to assess whether neutrophil gelatinase-associated lipocalin (NGAL) can be used as a biomarker of RI in patients with MM. **Patients and Methods:** A total of 199 samples from patients with MM were studied. Plasma NGAL, serum creatinine (sCr), cystatin C (Cys-C), serum free light chain (sFLC), and myeloma protein (M protein) concentrations were measured, and estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease formula. **Results:** Plasma NGAL levels significantly correlated with the degree of RI as defined by the Kidney Disease Improving Global Outcomes chronic kidney disease stage and with sCr and Cys-C concentrations and eGFR. Moreover, the plasma NGAL level was predictive of the myeloma burden and showed a significant correlation with sFLC, dFLC (difference between involved and uninvolved FLC), and M-protein concentrations. **Conclusion:** Plasma NGAL is not only a sensitive biomarker of renal function but may also be a useful marker that reflects tumor burden in patients with MM.

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Introduction

Multiple myeloma (MM) is a clonal B-cell disease of slowly proliferating plasma cells that is accompanied by myeloma protein (M protein) production and lytic bone lesions. Renal impairment (RI) is a common complication of MM.[1,2] RI, defined as a serum creatinine (sCr) level > 2 mg/dL, is one of the diagnostic criteria of symptomatic MM, and it constitutes 1 of the 4 "CRAB" features (calcium level elevation, renal impairment, anemia, and bone lesions) that indicate myeloma-related organ dysfunction.[3] Although RI is considered a less frequent problem nowadays because of early myeloma diagnosis and effective therapy, mild RI, defined as an estimated glomerular filtration rate (eGFR) < 90 mL/min/1.73 m^2, is known to be present in at least 25% to 50% of patients with MM.[4] The main cause of RI in patients with MM is renal tubular injury associated with light chain cast nephropathy.[5] This condition is characterized by distinctive tubular casts composed of monoclonal light chains and Tamm-Horsfall proteins that obstruct the distal tubule and the thick ascending loop of Henle.[6] Moreover, the monoclonal light chain burden stimulates the local production of proinflammatory cytokines (interleukin-6, macrophage chemoattractant protein-1, and tumor necrosis factor α), which results in tubulointerstitial inflammation that progresses rapidly to fibrosis.[7,8]

Several studies have confirmed that RI at the time of diagnosis is associated with poor survival,[9-12] and even with the introduction of

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novel agents such as thalidomide and bortezomib, the reversibility of RI is associated with improved survival.[10,13] Therefore, the rapid diagnosis of RI in patients with MM followed by the prompt institution of disease-specific treatment is critical for improving patient outcomes.

The standard assessment of renal function in patients with MM includes the measurement of sCr concentration, creatinine clearance (CrCl), and eGFR, as calculated using the Modification of Diet in Renal Disease (MDRD) equation. However, sCr is primarily a marker of glomerular filtration and, if GFR is not significantly reduced, sCr is an insensitive and unreliable marker in the diagnosis of renal tubular injury.[14] Cystatin C (Cys-C) has also been evaluated as a marker of kidney disease in patients with MM in a few studies, but the results have been inconsistent.[15-17] Therefore, there has been a lack of early biomarkers of structural kidney injury.

One of the most promising and earliest biomarkers of kidney injury is neutrophil gelatinase-associated lipocalin (NGAL) (lipocalin 2 [LCN2]). NGAL, a member of the lipocalin family, is a 25-kDa protein expressed at low levels in several human tissues, including the kidney. NGAL is one of the earliest, most robustly expressed proteins in the kidney after ischemic or nephrotoxic injury.[18] The advantage of NGAL over conventional biomarkers of RI such as sCr and serum urea nitrogen is that the levels often increase long before any changes in sCr or urea nitrogen or urine output occur. Therefore, NGAL may also be an early, sensitive marker of RI in patients with MM.

The aim of this study was to evaluate correlations between plasma levels of NGAL in patients with MM and RI to determine if NGAL is a feasible early marker of kidney damage in patients with MM.

Patients and Methods

Study Participants

From November 2012 to January 2013, aliquots of left-over plasma and serum samples from 199 symptomatic patients with MM attending Seoul St. Mary's Hospital for diagnosis or monitoring of MM were collected and used in this cross-sectional study. This included 14 samples from patients with MM at initial diagnosis and 185 samples from follow-up visits. Responses to MM treatment, at the time the plasma NGAL levels were measured, were classified as defined by the International Myeloma Working Group uniform criteria for MM.[19] The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and was approved by the Institutional Review Board of the Seoul St. Mary's Hospital (KC12DESE0775).

Laboratory Methods

NGAL levels in plasma samples were determined using the Alere Triage NGAL test on a Triage MeterPro (Alere; San Diego, CA). A cutoff value of 153 pg/mL was used to separate normal values from higher values, based on the manufacturer's recommendation. sCr levels were measured on a Hitachi 7600 analyzer (Hitachi, Tokyo, Japan) using a compensated Jaffe Kinetic assay (Roche Diagnostics Ltd, United Kingdom). The eGFR (mL/min/1.73 m^2) was calculated using the isotope dilution mass spectroscopy-traceable 4-variable MDRD study equation.[20] Serum Cys-C levels were measured on the Hitachi 7600 analyzer using a latex particle—enhanced turbidimetric immunoassay (HBI Co, Anyang, Korea). Serum free light chains (FLCs) were measured using the Hitachi 7600 analyzer and Freelite reagents (The Binding Site Ltd, Birmingham, United Kingdom). Serum protein electrophoresis and immunotyping were performed using the Capillarys 2 automated system (Sebia; Norcross, GA) for capillary electrophoresis. Serum total protein levels were determined using Biuret reagent (Sekisui Medical Co Ltd, Tokyo, Japan) on the Hitachi 7600 analyzer, and M-protein concentrations were calculated by multiplying the levels of serum total protein by the percentage of paraprotein measured using the Capillarys 2 system. Involved FLC (iFLC) is defined as the actual value of serum immunoglobulin κ FLCs in patients with monoclonal κ plasma cells or of serum immunoglobulin λ FLCs in patients with clonal λ plasma cells; dFLC is defined as the difference between involved and uninvolved FLCs.[21]

Statistical Analyses

Independent-samples *t* tests or Mann-Whitney *U* tests were used to compare continuous variables between patients with high plasma NGAL levels and those with normal plasma NGAL levels. Categorical variables were compared using the χ^2 test. Associations between variables were assessed using Pearson correlation analysis, and FLC levels and the ratios were log-transformed for statistical analysis. Multivariate stepwise regression analysis was used to assess predictors of plasma NGAL levels. To measure the sensitivity and specificity of NGAL at different cutoff values, a receiver operating characteristic (ROC) curve was generated, and the area under the curve (AUC) was calculated. MedCalc version 12.1.4 (MedCalc Software, Mariakerke, Belgium) was used for all statistical analyses; $P < .05$ was considered statistically significant.

Results

Patient Characteristics

The characteristics of the 199 patients included in this study are shown in Table 1. The male-to-female ratio was 1:1.1. The mean age was 60.6 years (range, 35-84 years); 14 samples (7%) represented MM at initial diagnosis, and 185 samples (93%) represented patients with MM who had been previously administered MM treatments (which included a transplant in 41.2%). Among the 185 follow-up patients with MM, 84 samples (42%) were at complete response (CR) when NGAL levels were measured, 14 (7%) were at very good partial response (VGPR), 26 (13%) were at partial response (PR), and 61 (31%) were at progressive disease (PD). Additionally, there was no evidence of infections during the preceding 2-week period when the plasma NGAL levels were measured.

Plasma NGAL Levels in Patients With MM

Plasma NGAL levels in the 199 patients with MM varied between 15 and 1300 ng/mL, with a median value of 92 ng/mL (95% CI, 79.6-102.0 ng/mL); 48 patients (24%) had values above the manufacturer's recommended cutoff point of 153 ng/mL. Age and sCr, Cys-C, iFLC, and M-protein concentrations were significantly higher, and hemoglobin and hematocrit values and eGFRs were significantly lower in patients with MM with plasma NGAL levels above the cutoff value of 153 ng/mL than in those with normal plasma NGAL levels (Table 2).

Patients with International Staging System (ISS) stage III myeloma had increased median plasma NGAL (123 ng/mL; range, 32-1110