Cystatin-C is an independent prognostic factor for survival in multiple myeloma and is reduced by bortezomib administration

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ABSTRACT

Background

Renal impairment is a common complication of multiple myeloma. Cystatin-C is considered an accurate marker of glomerular filtration rate in several renal disorders. Microarray analysis has revealed that cystatin-C is one of the most highly up-regulated genes in multiple myeloma. The aim of this study was to evaluate the serum levels of cystatin-C in myeloma patients, explore possible correlations with clinical data, including survival, and assess the effect of bortezomib on cystatin-C in relapsed multiple myeloma.

Design and Methods

We measured serum cystatin-C in 157 newly diagnosed, previously untreated myeloma patients, in 28 patients with relapsed disease pre- and post-bortezomib therapy and in 52 healthy controls, using a latex particle-enhanced nephelometric immunoassay.

Results

In newly diagnosed patients, cystatin-C was elevated and showed strong correlations with advanced ISS stage, extensive bone disease, high β_2-microglobulin, high serum creatinine, and low creatinine clearance. Multivariate analysis revealed that only cystatin-C and lactate dehydrogenase had an independent prognostic impact on patients' survival. The combination of cystatin-C and lactate dehydrogenase revealed three prognostic groups of patients: a high-risk group (both elevated cystatin-C and lactate dehydrogenase) with a median survival of 24 months, an intermediate-risk group (elevated cystatin-C or elevated lactate dehydrogenase) with a median survival of 48 months and a low-risk group (both low cystatin-C and lactate dehydrogenase) in which median survival has not yet been reached ($p<0.001$). Cystatin-C could also identify a subset of ISS-II patients with worse outcome. Relapsed patients had higher cystatin-C levels even compared to newly diagnosed patients. Treatment with bortezomib produced a significant reduction of cystatin-C, mainly in responders.

Conclusions

Serum cystatin-C is not only a sensitive marker of renal impairment but also reflects tumor burden and is of prognostic value in myeloma. Its reduction after treatment with bortezomib reflects bortezomib's anti-myeloma activity and possibly bortezomib's direct effect on renal function.

Key words: multiple myeloma, renal impairment, cystatin-C, bortezomib, survival.

Citation: Terpos E, Katodritou E, Tsiftsakis E, Kastritis E, Christoulas D, Pouli A, Michalis E, Verrou E, Anargyrou K, Tsionos K, Dimopoulos MA, and Zervas K, on behalf of the Greek Myeloma Study Group, Greece. Cystatin-C is an independent prognostic factor for survival in multiple myeloma and is reduced by bortezomib administration. Haematologica 2009; 94:372-379. doi:10.3324/haematol.2008.000638

Manuscript received September 12, 2008. Revised version arrived October 31, 2008. Manuscript accepted November 6, 2008.

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Introduction

Renal impairment is a common complication of multiple myeloma (MM) and correlates with poor survival.[1-3] Standard assessment of kidney function in MM patients includes serum creatinine and creatinine clearance (CrCl). Both measurements probably underestimate the prevalence of renal dysfunction. This may be due to enhanced creatinine excretion that gives false normal results of both Cr and CrCl in several cases.[4] Furthermore, serum creatinine is related to muscle mass of the patients and cachexia is a frequent problem in myeloma. Glomerular filtration rate (GFR) estimation is the best method for evaluating renal function, but the 51Cr-labeled EDTA clearance is time-consuming, expensive, and not readily available in many hospitals.[5] Cystatin-C (Cys-C) is a cysteine protease inhibitor, which is produced by nearly all nucleated cells and excreted into the bloodstream.[6] Cys-C is freely filtered by the glomerulus and is reabsorbed and metabolized by the proximal tubule; therefore, it is considered an accurate endogenous marker of GFR in various types of kidney diseases.[7,8] In several renal disorders Cys-C was found to be superior to serum Cr in terms of diagnostic sensitivity for reduced GFR.[9-12] It seems that the use of an equation which includes serum Cys-C level in combination with serum Cr, age, gender, and race provides the most accurate estimate of GFR in chronic kidney disease.[13] In one myeloma study, Cys-C was also found to very accurately reflect GFR.[14] However, concerns have been expressed that Cys-C may be affected by malignant progression.[15,16] Microarray analysis has revealed that Cys-C is one of the most highly up-regulated genes in MM.[17] Thus, our hypothesis was that Cys-C serum levels may not only reflect GFR but also myeloma burden and thus may serve as an important prognostic factor.

Bortezomib is a top class proteasome inhibitor with anti-myeloma activity, which is very effective in MM patients with renal dysfunction.[18-20] The aim of this study was to evaluate the serum levels of Cys-C in both newly diagnosed and relapsed patients with MM, explore possible correlations with clinical data, including survival, and assess the effect of bortezomib on Cys-C levels in relapsed MM.

Design and Methods

Patients

One hundred and fifty-seven newly diagnosed, symptomatic MM patients from five major Greek centers (Theagenion Cancer Center of Thessaloniki, Department of Clinical Therapeutics of the University of Athens School of Medicine, General Air Force Hospital of Athens, "G. Gennimatas" General Hospital of Athens and "St Savvas" Cancer Hospital of Athens), who were diagnosed between 1995 and 2007, were studied. All patients had serum samples collected before initiation of treatment and stored at -70°C. A grading of bone involvement in 3 groups according to the radiographical evaluation of the skeleton was made. Group A included patients with no lytic lesions but with osteoporosis; group B included patients with osteolytic lesions in 1-3 areas of the skeleton, and group C included patients with lytic lesions in more than 3 areas of the skeleton and/or a pathological fracture due to MM.

Twenty-eight myeloma patients who had relapsed after previous therapies were also evaluated prospectively. Single agent bortezomib was administered at a dose of 1.3 mg/m^2 on days 1, 4, 8, and 11 of a 3-week cycle for 4 cycles. Responders, according to European Bone Marrow Transplantation Group (EBMT) criteria,[21] could continue for 4 more cycles, while in non-responders dexamethasone at a dose of 12 mg/m^2 on days 1-4 of each cycle was added. Serum for the measurement of Cys-C was obtained on day 1 of cycle 1 and on day 21 of cycles 4 and 8, and remained frozen at -70°C till the day of measurement.

Finally, 52 healthy controls of similar age (median age: 67.5 years, range: 35-80 years) and gender (29M/23F) were also studied in order to be used as an internal validated control group. Each control was examined to ensure that there was no evidence of renal disease after CrCl measurements and that no medication had been received that could alter the normal renal function during the previous six months.

The study was approved by the Ethics Committees of the participating institutions.

Measurement of cystatin-C

Serum cystatin-C was measured on the Behring Nephelometer-II analyzer using a latex particle-enhanced nephelometric immunoassay (Dade Behring, Liederbach, Germany), as previously described.[22] The assay range is 0.195 to 7.330 mg/L; the reference range for healthy persons, according to the manufacturer, ranges from 0.53 to 0.95 mg/L. The intra-assay coefficient of variation ranges from 2.0 to 2.8%, and the inter-assay coefficient of variation ranges from 2.3 to 3.1%.

Measurement of other biochemical parameters

Serum urea, creatinine, uric acid, sodium, potassium, calcium, phosphates, magnesium, protein and albumin were measured using the Bayer-Advia 1650 Clinical Chemistry System (Tarrytown, NY, USA). β2-microglobulin was determined by particle enhanced immunonephelometry using the Dade-Behring BN Prospec nephelometer (Dade Behring, Liederbach, Germany). In 24 h urine samples measurements of creatinine, uric acid, electrolytes, protein, and beta2-microglobulin were assessed as previously described for serum determinations. CrCl values were available for all patients at the time of diagnosis. CrCl had been calculated as the ratio of urine creatinine (mg/dL) x 24 h urine volume (L) over plasma Cr (mg/dL). All CrCl results were adjusted for body surface area (1.73 m^2).

Statistical analysis

Differences between patients and controls as well as between different patient subsets were evaluated using the Mann-Whitney test. Differences between baseline, 4- and 8-cycle values of the studied parameters post-

bortezomib were evaluated using the Wilcoxon signed rank test. The Spearman Rank correlation test was employed to examine relationships between various parameters and clinical patient characteristics. Survival probabilities were calculated by the Kaplan-Meier method and comparisons made using the log-rank test to identify potential prognostic factors. Variables found to be statistically significant at the $p<0.05$ level were entered into a multivariate model using Cox regression analysis to identify the most statistically significant model. All p values are two sided, the level of significance is <0.05 and confidence intervals refer to 95% boundaries.

Results

Newly diagnosed multiple myeloma

The characteristics of 157 newly-diagnosed patients are shown in Table 1. Serum Cys-C was increased in MM patients compared to healthy controls [median (range): 1.01 mg/L (0.24-5.61 mg/L) *vs.* 0.72 mg/L (0.47-0.95 mg/L), $p<0.0001$]. Ninety patients (57.3%) had higher Cys-C levels than the upper normal limit of 0.95 mg/L, while only 37 (23.5%) had elevated serum Cr (>1.4 mg/dL, which was the upper normal limit in our laboratory for men and >1.2 mg/dL for women), and 21 (13.3%) had Cr level of ≥2 mg/dL. In terms of CrCl, 97 patients (61.7%) had values lower than 80 mL/min/1.73 m^2, which is considered the lower normal limit in our laboratory, and 24 (15.2%) had CrCl values below 30 mL/min/1.73 m^2. No control subject had a CrCl value below 85 mL/min/1.73 m^2 (median: 107 mL/min/1.73 m^2, range: 85-121 mL/min/1.73 m^2).

Patients with ISS stage III had increased median Cys-C (1.91 mg/L; range: 0.35-5.61 mg/L) compared to stage I (0.84 mg/L, range: 0.32-1.67 mg/L; $p<0.0001$) and stage II patients (0.95 mg/L, range: 0.24-2.17; $p<0.0001$; Figure 1), while there was no statistical difference between ISS stage I and II patients in terms of Cys-C serum levels. More precisely, in ISS stage III only 3/50 (6%) patients had normal Cys-C, compared to 37/56 (66%) patients who had ISS stage I and 26/51 (50%) patients who had ISS stage II and normal Cys-C levels. On the contrary, normal creatinine levels were observed in the vast majority of patients with ISS stage I (53/56, 94%) and ISS stage II (45/51, 88%) and in 25/50 (50%) patients with ISS stage III disease.

Patients who had advanced lytic lesions in the skeletal survey (group C; n=57) had higher values of Cys-C (median: 1.22 mg/L, range: 0.32-5.42 mg/L) compared to patients of groups A (median: 0.86 mg/L, range: 0.34-5.61 mg/L; $p=0.01$) and B (median: 1.05 mg/L, range: 0.24-3.15 mg/L; $p<0.01$).

Patients who had >50% of plasma cell infiltration in the bone marrow trephines (n=61) had increased levels of Cys-C (median: 1.04 mg/L, range: 0.32-5.61 mg/L) compared with all others (median: 0.90 mg/L, range: 0.24-5.42 mg/L; $p=0.036$).

Cys-C showed strong correlations with serum β2-microglobulin (r=0.648, $p<0.0001$; Figure 2A), creatinine (r=0.705, $p<0.0001$), CrCl (r=-0.549, $p<0.0001$; Figure 2B) and urea (r=0.471, $p<0.0001$), and weaker correlations with albumin (r=-0.241, $p=0.002$), hemoglobin (r=-0.333, $p<0.0001$), free-light chain ratio (r=0.312, $p<0.001$), LDH (r=0.177, $p=0.027$) and ferritin (r=0.277, $p=0.001$). Unfortunately, we had cytogenetic results (with FISH analysis) in only 38 patients: of those 12 had del13, 3 had t(4;14), and one had del17p, t(14;16) and t(11;14). In this cohort we found no correlation between high cystatin-C levels with any of the cytogenetic abnormalities.

Table 1. Clinical characteristics of multiple myeloma patients.

Parameter	Newly-diagnosed MM	Relapsed MM
N. of patients	157	28
Gender (M/F)	87/70	17/11
Age median (range)	68 (36-94)	68 (34-80)
Type of MM: IgG/IgA/BJ/IgD/NS	94-38-21-3-1	16-7-3-1-1
Stage at diagnosis (ISS)	Stage I: 56 (35%) Stage II: 51 (32%) Stage III: 50 (32%)	9 (32%) 9 (32%) 10 (35%)
Bone disease status		
Group A/B/C	51 (32%)/49 (31%)/57 (36%)	
Median number of lines of previous treatment (range)		2 (1-4)
Parameters at baseline		
Hb <10 g/dL	52 (33%)	10 (35%)
Creatinine > upper normal limit	37 (23%)	6 (21%)
Creatinine >2 mg/dL	21 (13%)	4 (14%)
Albumin <3.5 g/dL	76 (48%)	12 (42%)
Serum β_2-microglobulin >3 mg/L	98 (62%)	15 (53%)
Serum β2-microglobulin >6 mg/L	48 (30%)	7 (25%)
Urinary β2-microglobulin >0 mg/L	12 (7%)	3 (10%)
CRP >10 mg/L	31 (20%)	6 (21%)
LDH > 240 U/L	47 (29%)	5 (17%)
Urine specific gravity (mean±SD)	1005.2±4.6	1007.1±3.2

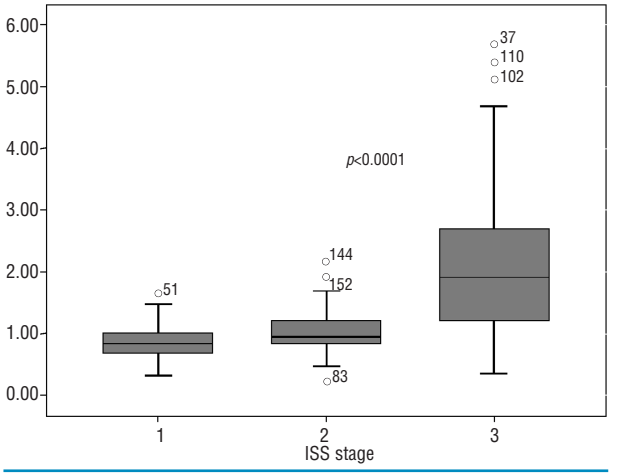

Figure 1. Cystatin-C serum levels and ISS. Myeloma patients with ISS stage III disease had increased serum levels of Cys-C compared to patients who had ISS stage I and II disease.

The median survival of all patients was 48 months. The median follow-up of the patients was 20 months (range: 7-113 months). The median number of lines of received therapies was 3 (range: 1-7). Only 27 patients had been diagnosed before 2000 and of those 15 had never been exposed to novel agents (*i.e.* thalidomide, bortezomib or lenalidomide). The vast majority of patients (137/157, 87%) had received conventional chemotherapy (either MP or VAD) as first-line anti-myeloma therapy, while of the other 20 patients, 7 had received PAD, 8 TD and 5 MDT participating in respective clinical trials. Thirty-four patients (21%) had received both bortezomib and an IMiD (thalidomide or lenalidomide) during the course of their disease. Thirty-three out of 67 (49%) patients who were aged below or equal to 65 years had been given high-dose therapy (200 mg/m^2 of melphalan) with autologous stem cell support (ASCT). The treatment given to the patients had no impact on survival in this cohort of patients, *i.e.* patients who received novel agents during the course of their disease *vs.* all others, or patients who were given ASCT *vs.* all others had no difference in terms of overall survival. This was mainly due to the different treatment schedules given to the patients, the different follow-up period and the low numbers of patients in different regimens.

The univariate analysis showed that Cys-C (as a dichotomous variable: $p<0.001$), serum β2-microglobulin (either as a continuous: $p<0.001$, or a dichotomous variable: $p=0.003$), LDH (either as a dichotomous or a continuous variable: $p<0.001$), ISS ($p=0.001$), hemoglobin (continuous variable: $p=0.01$), CrCl (continuous variable: $p=0.002$), bone disease ($p<0.001$) and albumin (continuous variable: $p<0.001$) predicted for survival (Table 2A), while gender, age, myeloma subtype, plasma cell infiltration, urinary β2-microglobulin, paraprotein level, CrCl as a dichotomous variable (<80 mL/min *vs.* 80 mL/min), Cys-C as a continuous variable ($p=0.066$), and calcium did not produce a prognostic significance in this cohort of patients. In particular, the median survival of patients with normal Cys-C levels (≤0.95 mg/L) has not yet been reached, while in patients with high Cys-C (>0.95 mg/L) the median survival was 27 months (95% CI 16.9-37.0; $p<0.0001$, Figure 3A). Furthermore, Cys-C levels could identify a subset of patients with poor prognosis within ISS stage II: patients with elevated Cys-C (n=25) had a median survival of 37 months, while the median survival of patients with normal Cys-C levels (n=26) has not yet been reached ($p=0.021$; Figure 3B).

The multivariate analysis revealed that only Cys-C and LDH as dichotomous variables had independent

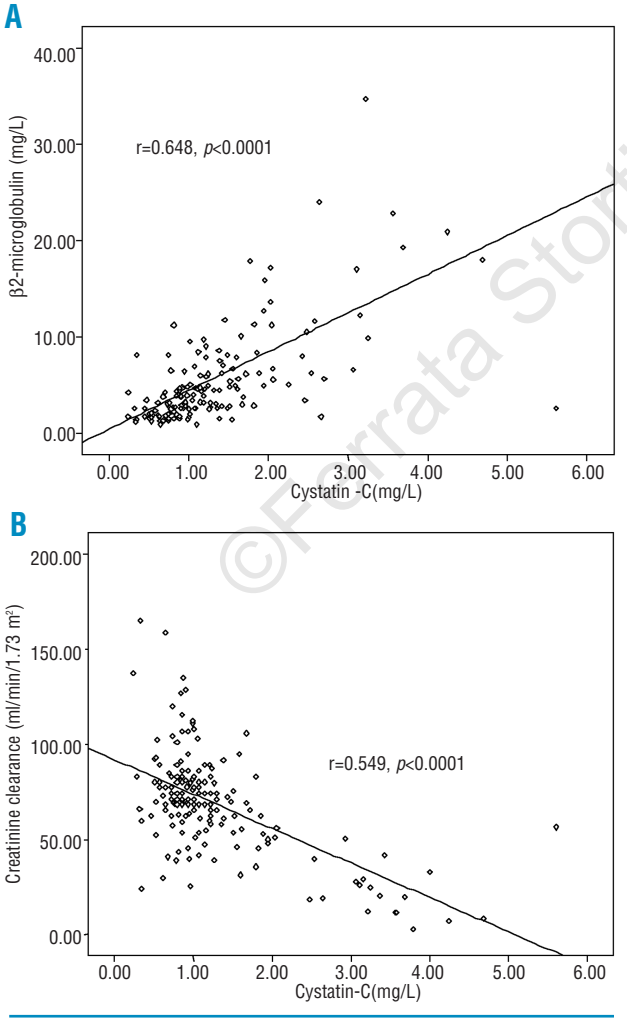

Figure 2. Correlations between Cys-C, β2-microglobulin and creatinine clearance. Strong correlations of serum Cys-C levels with both serum β2-microglobulin (A; positive correlation) and CrCl (B; negative correlation).

Table 2A. Univariate analysis: statistically significant variables.

Variable	Hazard ratio (*p* value)	95% CI
Cys-C (>0.95 mg/L)	0.321 (<0.001)	0.148-0.697
ISS stage	1.7 (0.001)	1.23-2.36
β2-microglobulin (continuous variable)	1.062 (<0.001)	1.021-1.103
β2-microglobulin (≥3.5 mg/L)	0.42 (0.013)	0.212-0.831
β2-microglobulin (≥5.5 mg/L)	0.391 (0.003)	0.208-0.733
LDH (>240 U/L)	0.275 (0.002)	0.12-0.622
LDH (continuous variable)	1.003 (<0.001)	1.002-1.005
Hemoglobin (continuous variable)	0.782 (0.01)	0.634-0.964
Creatinine clearance (continuous variable)	0.986 (0.002)	0.974-0.999
Bone disease (A+B *vs.* C)	0.33 (<0.001)	0.166-0.656
Albumin (continuous variable)	0.529 (<0.001)	0.304-0.922

Table 2B. Multivariate analysis (Hazards model).

Variable	Hazard ratio (*p* value)	95% CI
Cystatin-C (>0.95 mg/L)	0.322 (0.038)	0.110-0.940
LDH (>240 U/L)	0.151 (0.011)	0.035-0.648

prognostic value for survival, even though we included in the multivariate model β2-microglobulin as a dichotomous variable (at a cut-off point of either 3.5 mg/L or 5.5 mg/L) or as a continuous variable (Table 2B). Patients with both high levels of Cys-C and LDH (high risk group; n=46) had a median survival of 24 months (95% CI 18.2-29.7); patients who had increased one of the two parameters (either Cys-C or LDH; intermediate risk group; n=65) had a median survival of 48 months (95% CI 18.9-77.0), while the median survival of patients who had normal values of both LDH and Cys-C (low risk group, n=46) has not yet been reached ($p<0.001$; Figure 3C).

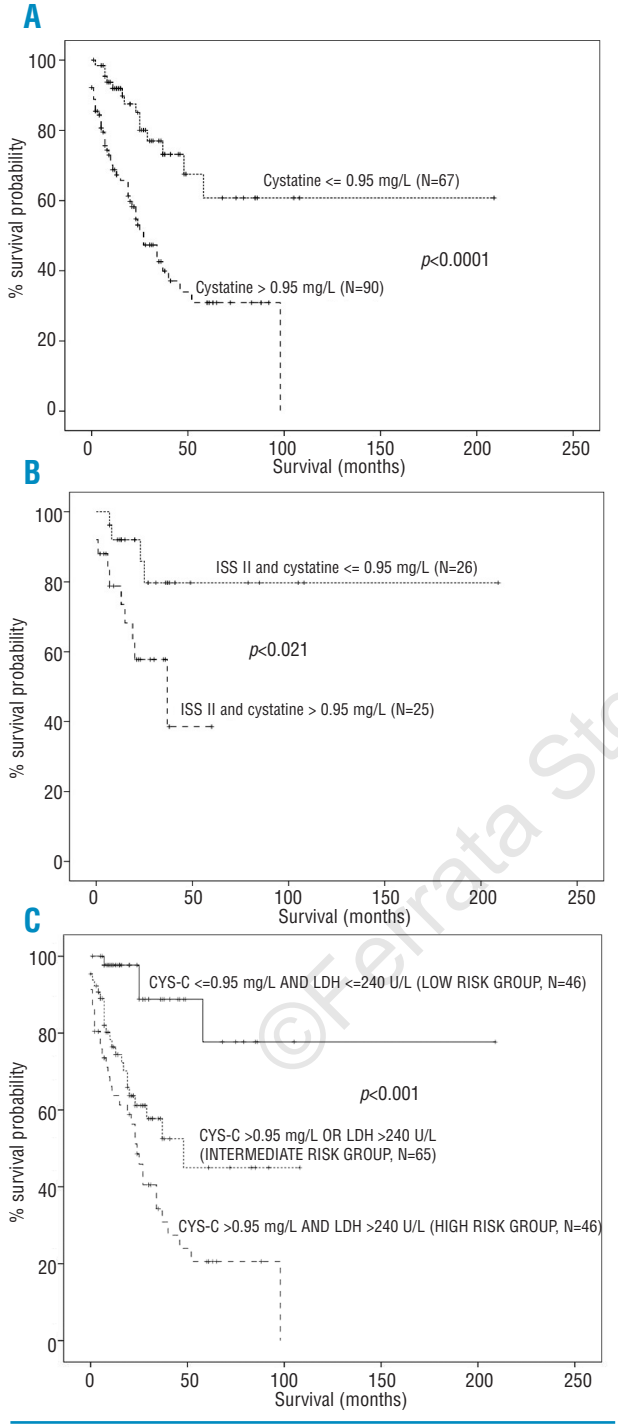

Figure 3. Cystatin-C and survival. Probability of survival of all newly-diagnosed myeloma patients (A) and patients with ISS stage II disease (B) according to Cys-C levels (high *vs.* normal values). Poor survival for patients who had high serum levels of both Cys-C and LDH (high-risk group) compared with those who had either high serum Cys-C or high LDH (intermediate risk group) and those who had normal levels of both Cys-C and LDH ($p<0.001$) (C).

In order to demonstrate that Cys-C correlates with tumor load and disease stage independently of the renal function (ISS stage III patients have more renal insufficiency than the others) we analyzed Cys-C >0.95 mg/L (dichotomous variable), Cys-C (continuous variable), CrCl (continuous variable) and ISS by using Cox's regression analysis. Only Cys-C >0.95 mg/L showed statistical significance ($p=0.02$; Hazards Ratio=0.236; 95% CI 0.07-0.8). These results confirm that when correcting for creatinine clearance, elevated Cys-C (>0.95 mg/L) remains a strong, independent predictor for survival.

Relapsed multiple myeloma

Twenty-eight patients were studied prospectively. All patients had previously received steroids. Eighteen had received the combination of melphalan plus prednisolone and 10 patients had been given the VAD regimen as initial therapy. Sixteen patients had previously received thalidomide and 26 patients were receiving zoledronic acid, at the standard dose of 4 mg, iv, monthly, at the time of relapse.

All but 2 patients completed 4 cycles of therapy. These 2 patients died due to sepsis during the 2nd and 3rd cycle of therapy. The objective response rate (CR+PR) after 4 cycles of therapy was 57%: 2 patients achieved CR (7%) and 14 PR (50%; 3 of them had a vgPR, *i.e.* paraprotein detectable only by immunofixation). Sixteen patients continued on bortezomib alone for 4 more cycles, while 3 patients who were rated as stable disease continued for 4 more cycles with the addition of dexamethasone. There was no change in response rate in the 16 responders after 8 cycles of therapy, while 1/3 patients with SD achieved a PR after 8 cycles of therapy.

Patients with relapsed myeloma had higher median Cys-C levels (1.36 mg/L, range: 0.73-6.82 mg/L) compared to controls ($p<0.0001$) and newly-diagnosed patients ($p<0.01$). At the time of relapse, only 3 patients (10.8%) had normal Cys-C compared to 22 patients (78.5%) who had normal Cr values. Bortezomib produced a significant reduction of Cys-C levels (median: 1.07 mg/L, range: 0.54-3.13 mg/L; $p<0.01$, Figure 4A) after 4 cycles of therapy in all patients. Responders had a greater reduction than non-responders ($p=0.01$; Figure 4B). Six patients who had elevated creatinine levels before bortezomib responded to bortezomib and normalized serum Cr. However there was no difference in CrCl before and after 4 cycles of bortezomib monotherapy in any patients (median values: 67 mL/min/1.73m^2 pre-bortezomib *vs.* 71 mL/min/1.73m^2 post-bortezomib; $p=0.134$). Nineteen patients who completed the 8 cycles of bortezomib monotherapy continued to experience a further reduction of Cys-C levels (median: 0.82 mg/dL, range: 0.58-1.64; $p=0.02$ compared with their values after 4 cycles of therapy).

Discussion

To the best of our knowledge this is the first study which showed that serum Cys-C correlates with advanced disease and is an independent prognostic factor for survival in myeloma patients, while it is reduced after treatment with bortezomib. Cys-C is a 122-amino acid, 13 kDa protein, which is a member of a family of potent, non-covalent, competitive inhibitors of mammalian lysosomal cysteine proteinases.[6] It has multiple biological functions, including controlling extracellular proteolysis via inhibition of cysteine peptidases,[23] modulation of the immune system,[24] and exertion of antibacterial and antiviral activities. It is considered to be a housekeeping gene since it is transcribed at a relatively constant level and is expressed in all nucleated cells.[12] Cys-C is considered a sensitive endogenous marker of GFR and accurately reflects renal insufficiency in various types of kidney disease.[7-13,25,26] Renal impairment is a common complication in MM.[1-3] Its prevalence seems to be underestimated by the measurement of serum Cr only.[12] To-date, there is only one study in 39 myeloma patients at different stages of the disease (most of the patients had already received therapy at the time of the study) evaluating Cys-C. In this study, Cys-C had a very good correlation with GFR, evaluated by the gold standard procedure, and was more sensitive than serum Cr in detecting moderate reductions in GFR; however, in all patients there was no superiority of Cys-C against serum Cr in detecting GFR reduction.[14] Moreover, the authors found a strong correlation between Cys-C and serum creatinine and β2-microglobulin; this observation was confirmed in our study and in another study by Finney *et al.*[27] In our study, we showed that newly-diagnosed patients had increased levels of Cys-C compared to controls. Furthermore, the majority of them (57%) had increased serum Cys-C versus only 22% who had increased serum creatinine. This finding in addition to the strong correlation between Cys-C and CrCl observed in our study suggests that Cys-C is a sensitive marker of renal impairment in myeloma patients. Nevertheless, we found no difference between the proportion of patients with reduced CrCl and those with increased Cys-C levels. We should also emphasize that the measurement of Cys-C is faster and easier compared to that of CrCl performed in 24 h urine collection, which is imprecise and inconvenient.[28] Moreover, there are several possible advantages of Cys-C as an endogenous marker, including the constant rate of production, lack of effect of age, gender or muscle mass on Cys-C generation, free filtration at the glomerulus because of its small size and basic pH, complete reabsorption and catabolism by the proximal tubule cells, lack of renal tubular secretion, lack of re-absorption back into the bloodstream, and absence of known problems with analytical interference.[12] Furthermore, a recent study by Stevens *et al.* suggests that the measurement of Cys-C in combination with serum Cr, age, sex, and race provides the most accurate estimate of GFR in chronic kidney disease.[13]

We observed that almost all patients with ISS stage III and patients with advanced lytic bone disease had elevated values of Cys-C, while patients with relapsed disease had higher values of Cys-C even compared to newly-diagnosed patients. Furthermore, Cys-C had an independent prognostic value for survival, excluding from our multivariate model β2-microglobulin, which is considered to date as one of the strongest predictors for survival in MM.[29] β2-microglobulin reflects both tumor burden and renal insufficiency in MM. Then why is Cys-C able to exclude it from our multivariate model? It is well-known that renal dysfunction correlates with poor survival.[30,31] Cys-C is reabsorbed and metabolized in the tubule; thus it is influenced not only by glomerular filtration, but also by tubular function and due to these characteristics may reflect the renal impairment better than other parameters. Thus the sensitive reflection of renal impairment by Cys-C levels may be at least partially responsible for its strong predictive value. Moreover, a gene expression profiling study by de Vos *et al.* showed that Cys-C is one of the most highly up-

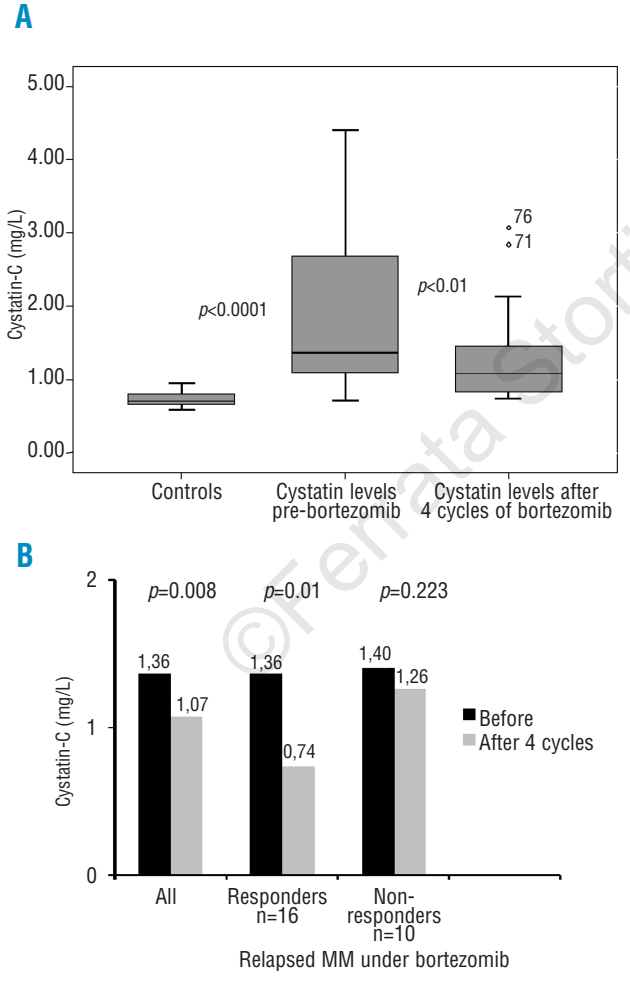

Figure 4. Single agent bortezomib and cystatin-C levels. Bortezomib produced a dramatic reduction of Cys-C after 4 cycles of therapy (A). Patients who responded to bortezomib showed a significant reduction of Cys-C [median (range): from 1.36 mg/L (0.73-6.82 mg/L) to 0.74 mg/L (0.54-1.42 mg/L); *p*=0.01] while patients who did not respond to bortezomib could not reduce their Cys-C levels significantly [from 1.40 mg/L (0.74-5.67 mg/L) to 1.26 mg/L (0.71-3.13); *p*=NS] (B).

regulated genes in MM (almost 50-fold up-regulated).[17] It seems that the over expression of Cys-C by myeloma cells is important for the development of lytic bone disease as it inhibits legumain which is a human endopeptidase and a negative regulator of osteoclast function.[32] The strong correlation found in our study between extensive bone disease and Cys-C levels further supports the above pre-clinical observation. The overexpression of Cys-C gene by myeloma cells indicates that its levels in the serum may also reflect tumor burden. This hypothesis is further strengthened by the strong correlation of Cys-C with advanced disease and survival. Thus it seems that Cys-C reflects both tumor load and renal function in myeloma patients and therefore has such a significant predictive value. Furthermore, the assessment of Cys-C permitted the identification of a subset of ISS stage II patients who had a worse survival and may benefit from more aggressive treatment modalities. For all these reasons, Cys-C excluded β2-microglobulin from our multivariate model and it seems to be a valuable predictive marker for myeloma patients. However, this has to be confirmed by other studies before the routine use of this marker for myeloma patients, taking into consideration that its measurement is more expensive than that of β2-microglobulin and serum creatinine. Nevertheless, we have to stress that Cys-C in combination with LDH produced a prognostic model that separates myeloma patients into 3 groups (high-, intermediate- and low-risk groups), which can be easily used by clinicians. The validation of this system prospectively may provide a useful tool for the prognosis of myeloma patients.

The reduction of Cys-C by bortezomib in responders further supports the notion that Cys-C also reflects myeloma burden. However, even in non-responders there was a reduction in median levels of Cys-C, which did not reach statistical significance. The reduction of Cystatin-C was observed even in the 3 patients who received dexamethasone combination after 4 cycles of bortezomib monotherapy. Steroids have been shown to increase the levels of Cys-C.[33] Thus this reduction as well as the normalization of serum Cr in 6/6 patients who had high Cr values pre-bortezomib may be explained by the positive effect of bortezomib on renal function of myeloma patients; an effect which has been recently described.[18-20,34,35]

One aspect of Cys-C measurement is that Cys-C can be affected by other pathological conditions, such as thyroid dysfunction,[36] or the use of glucocorticoids that has been associated with higher concentrations of Cys-C.[37,38] These effects suggest that the generation of Cys-C may increase in settings of increased metabolic rate, perhaps as a result of increased cell turnover. In our cohort population, no patient had received any steroid therapy before sampling for Cys-C measurement. Furthermore, because Cys-C is produced by all nucleated cells, is freely filtered across the glomerular membrane, and the filtration rate appears to be unaffected by severe illness,[39,40] it acts as a very sensitive endogenous marker of GFR.

In conclusion, our study provides evidence that Cys-C is a sensitive marker of renal insufficiency in myeloma patients, correlates with advanced ISS stage and advanced bone disease, and has an independent prognostic significance for survival. Importantly, its combination with LDH produces a prognostic model which separates newly-diagnosed myeloma patients into 3 risk groups: high-, intermediate- and low-risk groups. Therefore, its measurement provides important information for both tumor load and renal function in myeloma patients. If our data are confirmed by other studies, routine assessment of cystatin-C in myeloma may be recommended, while its combination with LDH will be a useful tool for the prognosis of myeloma patients.

Authorship and Disclosures

ET: conception and design of the study; analysis and interpretation of data; measurement of Cystatin-C; administration of therapy and follow-up of the patients; drafting the manuscript; final approval of the version to be published. EK: statistical analysis and interpretation of data; administration of therapy and follow-up of the patients; final approval of the version to be published. ET: measurement of Cystatin-C and performance of all laboratory parameters; final approval of the version to be published: EK, DC, AP, EM, EV, KA, KT: interpretation of data; administration of therapy and follow-up of the patients; final approval of the version to be published: M-AD and KZ: conception and design of the study; administration of therapy and follow-up of the patients; final approval of the version to be published.

ET received financial support from Janssen-Cilag; AD received financial support from Ortho Biotech. All other authors declare that they have no conflicts of interest.

This paper has been presented as a poster in the American Society of Hematology Annual Meeting 2007 (Blood 2007;110:444a, abstract No1484).

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