

ORIGINAL**Assessment of renal function in Japanese children with malignancies using serum cystatin C**Nami Nakamura, MD,^{1,2} Hiroyoshi Watanabe, MD, PhD,¹ Kazumi Okamura, MD, PhD,¹ and Shoji Kagami, MD, PhD¹¹Department of Pediatrics, the University of Tokushima Graduate School, Tokushima, Japan, ²Department of Pediatrics, The Tsurugi Town Handa Hospital, Tokushima, Japan

Abstract : Several factors besides renal function influence serum cystatin C (CysC) levels. The present study evaluates the value of serum CysC and the equation for CysC based estimated glomerular filtration rate (CysC-eGFR) for Japanese children with malignancies. We collected information at 36 time points from 13 patients aged ≤ 17 years with malignancies. We assessed tumor activity, cell recovery phase after chemotherapy, neutropenia phase, inflammation response and medication with granulocyte-colony stimulating factor, steroid, and levothyroxine as risk factors associated with serum CysC levels. Although no 24-h creatinine clearance (CCr) data collected at 36 time points indicated renal dysfunction, serum CysC levels were above and below the reference values at four and five time points, respectively. The frequency of elevated serum CysC levels was higher in patients without therapy or with stable or progressive disease than among those with a complete or partial response ($p = 0.0046$). The correlation coefficient between CCr and CysC-eGFR was 0.355 ($p = 0.054$), but this improved to 0.663 ($p = 0.0010$) when restricted to patients with a complete or partial response. Levels of serum CysC might become elevated regardless of renal function, and CysC-eGFR might become unpredictable during the active phase of tumors. *J. Med. Invest.* 65 : 231-235, August, 2018

Keywords : cystatin C, estimated glomerular filtration rate, cancer, chemotherapy

INTRODUCTION

The accurate assessment of renal function is essential for implementing safe chemotherapy among patients with malignancies (1-3). Glomerular filtration rate (GFR) in the Japanese clinical setting has commonly been estimated as 24-h creatinine clearance (CCr), although the gold standard is inulin clearance (CIn). Equations for estimated GFR based on creatinine (Cr-eGFR) and cystatin C (CysC-eGFR) were established for Japanese children during 2013 (4) and 2014 (5), respectively. The Cr-eGFR has been recommended for pediatric patients with chronic kidney disease (CKD), but CysC-eGFR should be used for patients with abnormal muscle mass because muscle mass affects serum creatinine levels. In pediatric patients with malignancies and lower muscle mass than healthy children, Cr-eGFR can overestimate their renal function and result in an overdose of chemotherapeutic agents (6). On the other hand, thyroid dysfunction (7), human immunodeficiency virus (HIV) infection (8), and medication with steroids or immunosuppressive agents can affect serum cystatin C (CysC) values (9-11). Therefore, CysC-eGFR is not recommended for such patients. CysC is associated with the pathology of metastatic and invasive cancer (12-15). We aimed to define the applicability of CysC-eGFR by investigating factors other than renal function that affect serum CysC levels in pediatric patients with malignancies.

MATERIALS AND METHODS

The study population comprised 13 patients aged up to 17 years

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with malignancies who underwent chemotherapy and simultaneous measurements of their CCr and serum CysC values at our hospital between January 2013 and February 2015. Information about the patients retrospectively collected from their clinical records included age, weight, height, body surface area (BSA), serum creatinine, urinary creatinine, urine volume, serum CysC, white blood cell count (WBC), absolute neutrophil count (ANC), serum C reactive protein (CRP), free-thyroxine (fT4), disease diagnosis, tumor activity (without therapy, complete [CR] or partial [PR] response, stable [SD] or progressive [PD] disease), duration between the day of starting chemotherapy and the test day, medication with granulocyte-colony stimulating factor (G-CSF), steroid, and levothyroxine on the test day, and steroid dose. We classified the solid tumor activity according to the response evaluation criteria in solid tumors (RECIST) guidelines (16). Table 1 defines the criteria for CR, PR, SD and PD. Patients with complete remission of hematological malignancies such as acute leukemia and lymphoma after induction chemotherapy were classified as having achieved CR. Complete remission was defined as $< 5\%$ blasts in bone marrow and the absence of extramedullary disease in patients with acute leukemia, and the absence of residual tumor and extranodal disease in patients with lymphoma. The Ethics Committee at Tokushima University Hospital approved the study (Approval number : 2398).

Urine was collected for two or three consecutive days. Results were excluded when failure to complete urine collection was documented in the clinical record. Both serum and urinary creatinine values were measured in-house using an automatic analyzer (Hitachi Ltd., Tokyo, Japan) with enzymatic assays (Shino-Test Corporation, Kanagawa, Japan). Levels of serum CysC were measured using a colloidal gold immunoassay (Alfreda Pharma Corporation, Osaka, Japan) at SRL Inc. (Tokyo, Japan), which was calibrated to the standardized value to ERM-DA472/IFCC. The reference serum CysC values were those published in the Chronic Kidney Disease (CKD) guidelines 2012 (17) for patients aged ≤ 16

years (Table 2), and values established by SRL Inc. for patients aged 17 years (male and female, 0.63 – 0.95 and 0.56 – 0.87 mg/L, respectively).

Table 1. Definitions for tumor activity classification

	Definition
Complete response	Disappearance of all target lesions and normalization of tumor marker level
Partial response	At least a 30% decrease compared with baseline sum of longest diameter of target lesions, and/or tumor marker level maintained above normal limit
Stable disease	Neither sufficient shrinkage to qualify as partial response nor sufficient increase to qualify as progressive disease, compared with smallest sum of longest diameter of target lesions since treatment started
Progressive disease	At least a 20% increase compared with smallest sum of longest diameter of target lesions since treatment started or emergence of one or more new lesions

Table 2. Reference serum cystatin C values

Age	Sex	2.5 th percentile (mg/L)	50 th percentile (mg/L)	97.5 th percentile (mg/L)
3 – 5 months		0.88	1.06	1.26
6 – 11 months		0.72	0.98	1.25
12 – 17 months		0.72	0.91	1.14
18 – 23 months		0.71	0.85	1.04
2 – 11 years		0.61	0.78	0.95
12 – 14 years	Male	0.71	0.86	1.04
	Female	0.61	0.74	0.91
15 – 16 years	Male	0.53	0.75	0.92
	Female	0.46	0.61	0.85

The CysC-eGFR was calculated using the formula $104.1 \times 1/\text{CysC (mg/L)} - 7.80 \text{ (mL/min/1.73 m}^2\text{)}$, which was determined by measuring CIn in pediatric CKD patients between the ages of 1 month and 18 years (5). Correlations between CCr and CysC-eGFR were analyzed.

We focused on factors that might influence serum CysC levels, and divided the patients into paired groups according to tumor activity (without therapy or with SD or PD vs. with CR or PR); cell recovery phase after chemotherapy (duration between the day on which chemotherapy was started and the test day > 14 vs. ≤ 14 days); neutropenic phase ($\text{ANC} \geq 1000$ vs. < 1000/ μL); inflammation response ($\text{CRP} \geq 0.3$ vs. < 0.3 mg/dL); and medication with or without G-CSF, steroid, and levothyroxine. We compared the frequency of serum CysC above or below reference values between each pair of groups.

Normal distribution was assessed using Kolmogorov-Smirnov tests. Descriptive statistics for continuous variables are presented as mean ± standard deviation (SD), except for age, which is presented as median and range. This is because the distribution of age was not normal. Categorical variables are presented as frequencies (%) with 95% confidence intervals (CI). Differences in mean values

between the two groups were analyzed using *t* tests or Welch tests, followed by *F* tests. Differences in frequencies between groups were analyzed using Fisher exact tests. Correlations were analyzed using Pearson correlation coefficients. Values of $p < 0.05$ were considered to indicate statistically significant differences. All data were statistically analyzed using EZR (Saitama Medical Center, Jichi Medical University).

RESULTS

We collected information from seven male and six female patients at 19 and 17 time points, respectively. Patients were diagnosed with intracranial germ cell tumors ($n = 3$); malignant peripheral nerve sheath tumor with neurofibromatosis type 1 ($n = 2$); and acute lymphoblastic leukemia (ALL), non-Hodgkin lymphoma (NHL), testicular germ cell tumor, rhabdomyosarcoma, osteosarcoma, Wilms tumor, neuroblastoma, and malignant glioblastoma ($n = 1$ each).

The median age at the time of the test was 4 (range; 1 – 17) years. The mean CCr was $140.9 \pm 28.6 \text{ mL/min/1.73 m}^2$. CCr data collected at only one time point was below the reference value of $90 \text{ mL/min/1.73 m}^2$ ($89.1 \text{ mL/min/1.73 m}^2$; 2.8%; 95% CI, 0.1% – 14.5%) and CCr data at all other time points were above the reference values. With respect to serum CysC, values at four time points (11.1%; 95% CI, 3.1% – 26.1%) and five time points (13.9%; 95% CI 4.7% – 29.5%) were above and below the reference values, respectively. The correlation coefficient between CCr and CysC-eGFR was 0.405 ($p = 0.014$).

Values for serum CysC were above the reference values in four patients at four time points, and below the reference value at five of six time points in a female patient who was undergoing chemotherapy for intermediate risk Stage 3 neuroblastoma diagnosed by biopsy before starting therapy. The tumor did not completely disappear after eight cycles of chemotherapy. The residual tumor comprised an area of calcification and some ganglioneuroma or ganglioneuroblastoma at the end of the chemotherapy treatment. We excluded the data from this patient from further analysis as we considered that unique personal factors influenced her serum CysC levels.

We analyzed risk factors associated with elevated serum CysC levels other than renal function among data derived at 30 time points from 12 patients (male, 19 time points; female, 11 time points). The median age at the time of the test was 8.5 (range; 1 – 17) years. The mean CCr was $137.6 \pm 27.5 \text{ mL/min/1.73 m}^2$. Information about levothyroxine medication was obtained at five time points from a patient with pituitary hypothyroidism whose fT4 levels were normalized by hormone replacement therapy. Information about steroid medication was obtained at seven time points from three patients. Information about medication with therapeutic doses of prednisolone was obtained at one time point each from one patient with ALL and from another with NHL. Information about medication with maintenance doses of hydrocortisone as hormone replacement therapy for a patient with pituitary adrenocortical insufficiency was obtained at five time points.

Values for serum CysC at four time points were above the reference values (13.3%, 95% CI, 3.8% – 30.7%). The correlation coefficient between CCr and CysC-eGFR was 0.355 ($p = 0.0544$). Mean CCr did not significantly differ between groups with elevated and normal serum CysC values (127.0 ± 19.5 vs. $139.2 \pm 28.5 \text{ mL/min/1.73 m}^2$; $p = 0.415$). Therefore, we considered that factors other than renal function influenced serum CysC in group with elevated serum CysC values.

Table 3 shows the results of comparisons of the frequency of elevated serum CysC levels between groups divided according to restrictive risk factors. The frequency of elevated serum CysC was

Table 3. Comparison of frequency of elevated serum cystatin C values

Risk factors	Elevated CysC	Normal CysC	P
Tumor activity			
Without therapy or SD or PD	4	5	0.0046
With CR or PR	0	21	
Cell recovery phase after chemotherapy (N=20)			
> 14 days	0	13	1
≤ 14 days	0	7	
Neutropenic phase			
ANC ≥ 1000/μL	4	14	0.13
ANC < 1000/μL	0	12	
Inflammation response			
CRP ≥ 0.3 mg/dL	1	2	0.36
CRP < 0.3 mg/dL	3	24	
G-CSF medication			
With	0	7	0.548
Without	4	19	
Steroid medication			
With	1	6	1
Without	3	20	
Levothyroxine medication			
With	1	4	0.538
Without	3	22	

CysC cystatin C, SD stable disease, PD progressive disease, CR complete response, PR partial response, ANC absolute neutrophil count, CRP serum C reactive protein, G-CSF granulocyte-colony stimulating factor. Comparisons were analyzed using Fisher exact test

statistically higher in a group without therapy or with SD or PD than in a group that achieved CR or PR (44.9% vs. 0.0%; $p = 0.0046$). The correlation between CCr and CysC-eGFR in a group without therapy or with SD or PD was expressed by the equation ; $CysC-eGFR = -0.1639 \times CCr + 135.29$, with a correlation coefficient of -0.345 ($p = 0.3633$). The correlation between CCr and CysC-eGFR in a group with CR or PR was expressed by the equation ; $CysC-eGFR = 0.3745 \times CCr + 73.096$, with a correlation coefficient of 0.663 ($p = 0.0011$) (Figure 1).

DISCUSSION

Cystatin C is a 13-kDa low-molecular-weight protein comprising 120 amino acids. Almost all human nuclear cells produce CysC at a constant rate and release it into the bloodstream. Serum CysC levels have served as an endogenous GFR marker, like serum creatinine, because it is reabsorbed and catabolized by kidney tubules and is not returned to blood after free infiltration of the glomeruli (18, 19).

Because serum CysC values are not influenced by muscle mass, unlike creatinine (20), CysC-eGFR rather than Cr-eGFR is recommended for assessments of renal function among patients with abnormal muscle mass than healthy matched controls. We reported that Cr-eGFR can overestimate the renal function of Japanese pediatric patients undergoing chemotherapy as they have less muscle mass than healthy children (6). Several reports suggest that CysC is more appropriate than creatinine for evaluating renal function in pediatric patients with malignancies (21, 22).

However, with thyroid dysfunction (7), HIV (8), and medication with steroids or immunosuppressive agents can alter serum CysC

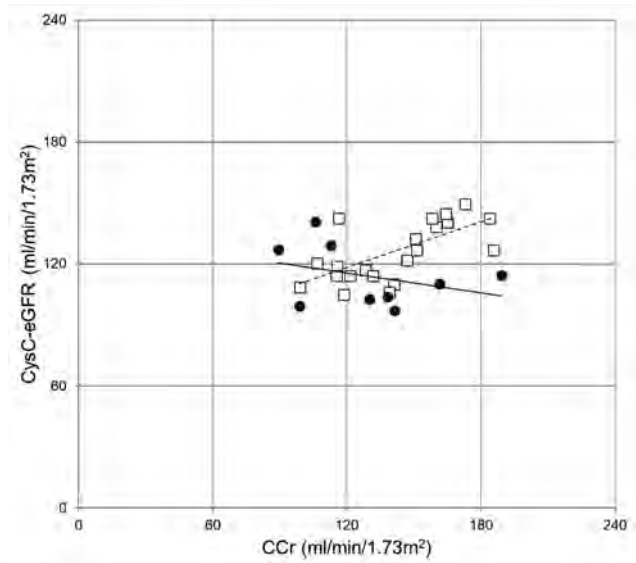


Figure 1. Scatter plot of CCr and CysC-eGFR in a group without therapy or with SD or PD (circles) and a group with CR or PR (squares). Solid and broken line shows relationships between CCr and CysC-eGFR in a group without therapy or with SD or PD and a group with CR or PR, respectively.

levels (9-11). Moreover, CysC is an inhibitor of cysteine proteases, such as cathepsin, which might be associated with the metastasis and invasion of tumor cells (23). Studies of patients with several types of malignant tumors have found elevated serum CysC levels regardless of renal function (12-15).

We found a low correlation coefficient between CCr and CysC-eGFR in pediatric patients with malignancies. Although no CCr data collected at 36 time points indicated deteriorated renal function, serum CysC levels were above and below the reference values at four and five time points, respectively. Therefore, we considered that factors other than renal function influence serum CysC. Serum CysC values were below the reference at five of six time points from a female patient with intermediate risk Stage 3 neuroblastoma. We considered that this was caused by factors specific to this patient. Tumor cells generally tend to quickly grow and divide, and therefore chemotherapeutic agents can inhibit cell division and induce tumor regression. However, the histological response of intermediate risk Stage 3 neuroblastoma cells to chemotherapy sometimes indicated maturation (24). Her neuroblastoma cells had also differentiated into ganglioneuroma or ganglioneuroblastoma after chemotherapy. The ability of tumor cells to differentiate might affect serum CysC levels. Further studies should evaluate serum CysC levels in patients with malignancies that undergo maturation after chemotherapy. In the present study, we excluded her findings from further analysis and investigated analyzed factors that might be associated with elevated serum CysC levels other than the deterioration of renal function in the clinical setting of malignancy based on the remaining data derived from 30 time points.

We compared the frequency of elevated serum CysC levels among the risk factors of tumor activity, cell recovery phase after chemotherapy, neutropenic phase, inflammation response, and medication with G-CSF, steroid, or levothyroxine. Only tumor activity was statistically associated with elevated serum CysC values. Levels of serum CysC were elevated in data collected at four of nine time points before treatment or at the stage of SD or PD. On the other hand, data collected at all 21 time points at the CR or PR

stage revealed normal serum CysC levels, suggesting that serum CysC can become elevated during active tumor-cell proliferation. Moreover, the correlation coefficient between CCr and CysC-eGFR improved to 0.663 ($p = 0.0011$) when data from only 21 time points obtained at the time of CR or PR were analyzed.

The relationship between steroidal therapy and serum CysC has been discussed (9-11). The present study found that steroid medication was not a risk factor for influencing serum CysC levels. Various doses of steroids are often administered for various reasons in the clinical setting of malignancy, such as tumor reduction, decompression of intracranial pressure, and hormone replacement for hypopituitarism. The influence of steroids on serum CysC levels should be separately assessed in a future study.

The present study has some limitations. Firstly, we adopted CCr as the measurement of the GFR. Generally, the CCr estimates higher than the true GFR compared with the CIn, because of tubular creatinine secretion. If a more reliable measurement of GFR, such as CIn, had been adopted in the present study, the relationship between measured GFR and CysC-eGFR might have been clearer. Secondly, the equation of CysC-eGFR for Japanese children was developed based on CIn in pediatric patients with CKD. No findings in the present study indicated obvious renal dysfunction, and the mean CCr was 140.9 ± 28.6 mL/min/1.73 m². However, the median maximum CIn collected from CKD patients to develop the equation of CysC-eGFR for Japanese children was 71.0 (interquartile range ; 52.9 – 97.2) mL/min/1.73 m². The CysC-eGFR equation for Japanese children has not yet been validated among children in general. Sharma AP *et al.* (25) concluded that the diagnostic accuracy of several CysC-eGFR equations for Europeans and Canadian children, such as the equations of Bökenkamp, Filler, Grub, and Zapitelli, varied at different GFR. The equation of CysC-eGFR for Japanese children might also have been inaccurate due to variations in GFR.

CONCLUSIONS

Levels of serum CysC can fluctuate regardless of renal function. In pediatric patients with malignancies, serum CysC levels might become elevated, and CysC-eGFR might become unpredictable during the active phase of tumors.

CONFLICT OF INTEREST-DISCLOSURE

The authors have declared that no conflict of interest exists.

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