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Differential Effect of Corticosteroids on Serum Cystatin C in Thrombocytopenic Purpura and Leukemia

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Abstract The aim of our study was to evaluate the influence of steroid therapy on serum cystatin C (cysC) concentrations in patients with acute lymphoblastic leukemia (ALL) and idiopthiás thrombocytopenias purpura (ITP). We studied 17 patients with ITP (girls: boys=5:12, mean age: 7.6 yrs, range between 1 to 17 years) and 18 patients with ALL (girls: boys=6:12, mean age: 6.3 yrs, range between 2 to 17 years). CysC and white blood cell count (WBC) in both group of patients were determined before and after 300 mg/m^2 cumulative dose of steroid therapy. Corticosteroids increased the level of cysC in both groups of patients, however significant increase was found only in ITP patients between pre- and posttreatment values (0,96±0,27 mg/L vs. 1,16±0,3 mg/L, p=0,02). Pretreatment cysC concentrations were within the reference range in patients with ITP but not with ALL and were significantly higher in ALL patients, than in ITP patients (1,23±1,12 mg/L vs. 0,96±0,27 mg/L, p=0,02). Pretreatment WBC of ALL patients were significantly higher than of ITP patients (22,58 G/L, min. 3,5 G/L, max. 102,1 G/L vs. 7,46 G/L, min. 4,8 G/L, max. 12,3 G/L, p=0.03). We have found significant correlation between pretreatment cysC and WBC values in ALL patients (p=0.04). Although the concentration of cysC may be slightly and reversibly influenced by corticosteroid treatment, cysC is sensitive to detect early and moderate deteoriantation of GFR in children with cancer.

Keywords Acute lymphoblastic leukemia · Coricosteroid therapy · Cystatin C · Glomerular function · Idiopathic thrombocytopenic purpura · Leukemic cell burden

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Introduction

Determination of serum cystatin C concentration (cysC) provides a rapid and more accurate assessment of glomerular function than measurement of serum creatinine concentration and creatinine clearance in children with cancer. Stable production rate except for thyroid malfunction and corticosteroid medication and free filtration by the glomeruli due to the low molecular weight and positive charge represent the advantages of cysC as a serum marker of glomerular function. Creatinine-based equations to estimate the glomerular filtration rate (GFR) are sensitive to some nonrenal factors such as age, sex, race and lean muscle mass. Calculation of endogenous creatinine clearance (C_{Cr}) requires a precise 24 h urine sample collection, a difficult task before toilet-trained age. Diarrhea and non compliance may adversely affect urine collection even in older children. Other established methods of assessing GFR are based on the application of exogenous substances, some of them radioactive ones [1]. The concentration of serum cysC in healthy individuals ranges around 0.8–1.2 mg/L depending on analytical methods between age 1–50 [2]. In

our previous study we have also examined cystatin C values in children with cancer, in age and sex matched healthy children (negative controlls) and in patients with end stage renal failure with different origin under dialysis therapy (positive controlls). CysC of patients, negative and positive controls were 1.13±0.54 mg/L, 0.95±0.19 mg/L and 4.69±2.19 mg/L, respectively. CysC of positive controls was significantly higher than cysC of either patients ($p<0.001$) or negative controls ($p<0.001$) [1]. However, corticosteroids, frequently applied in many forms of childhood cancer, have been suggested to influence cysC independently of glomerular function [3–6]. To test this hypothesis, we investigated changes of cysC in two different groups of pediatric patients receiving corticosteroid monotherapy.

Materials and Methods

Patients and Treatment

Between September 1, 2004 and January 31, 2008, 17 patients with acute idiopathic thrombocytopenic purpura (ITP; girls: boys=5:12, mean age: 7.6 yrs, range between 1 to 17 years) and 18 patients with de novo acute lymphoblastic leukemia (ALL; girls: boys=6:12, mean age: 6.3 yrs, range between 2 to 17 years) were investigated in the Department of Pediatric Hematology-Oncology at the Institute of Pediatrics of the Medical and Health Science Center of the University of Debrecen (MHSC UD). Patients with severe ITP (platelet count ≤ 20 G/L and spontaneous bleeding) received 100 mg/m^2 6-methylprednisolone (6-MP) daily in infusion for 3 days (cumulative dose of 300 mg/m^2) followed by a gradually decreasing dose of the drug for at least 4 weeks. Patients with ALL were treated according to the BFM ALL-IC 2002 protocol. The induction phase of this treatment is similar to previous BFM protocols starting with gradually increasing doses of oral prednisolone (PRED) for 7 days (30 mg/m^2 on day 1 to be escalated to 60 mg/m^2 by day 5; cumulative dose of 344 mg/m^2) followed by a four drug combination of prednisolone, vincristine, daunorubicine and L-asparaginase until day 33 [7].

Laboratory Evaluation

Blood samples were drawn before any treatment and on day 4 and day 8 in patients with ITP and ALL, respectively. Serum and urinary creatinine concentrations were determined by the kinetic Jaffe method [8]. Creatinine clearance based on the Counahan formula ($C_{Counahan}$) was calculated from S_{cr} and body length (L, [cm]), as: 38×L (cm)/S_{Cr} (μmol/l) and gives an estimate in ml/min/1.73 m^2 [9]. CysC was determined by immunnephelometric assay of serum samples with N Latex cystatin C reagent (Dade Behring, Deerfiels, IL) using a BN ProSpec apparatus (Dade Behring). White blood cell count (WBC) was determined form EDTA-anticoagulated peripheral blood samples of patients using Sysmex KX-21 apparatus (Sysmex, Kobe, Japan).

Written informed consent was obtained from parents or responsible caretakers. The study was accepted by the Ethical Committee of MHSC UD.

Statistical Analysis

Data were analyzed with SAS 8.2 statistical software package. Differences between groups were evaluated by one-way ANOVA with Duncan post-hoc test, after logarithmic transformation. The relationship between cysC and WBC was examined by Spearman correlation. The p<=0.05 probability level was accepted as significant.

Results

Monotherapy with a cumulative dose of 300 mg/m^2 6-MP increased significantly cysC in patients with ITP from 0.96±0.27 mg/L to 1.16±0.30 mg/L ($p=0.02$). In contrast, the effect of a cumulative dose of 344 mg/m^2 PRED monotherapy did not result in a significant difference in the day 1 vs. day 8 cysC values (1.23±1.12 mg/L vs.1.24± 0.49 mg/L; $p=0.99$) of patients with ALL. Pretreatment cysC was within the reference range in patients with ITP and it was significantly lower ($p=0.02$) than that of patients with ALL, exceeding the reference range. Similarly, pretreatment WBC of patients with ITP was within the reference range and it was significantly lower ($p=0.03$) than initial WBC of patients with ALL (7.46±1.23 G/L vs. 22.58±17.30 G/L, respectively). There was a significant correlation ($r^2=0.06$, $p=0.04$) between pretreatment cysC and WBC values in patients with ALL (Fig. 1.). As expected serum creatinine levels and $C_{Counahan}$ in both groups of patients were within the normal range, but not significantly higher in ALL patients, and did not changed significantly during the treatment (Table 1).

Discussion

CysC proved an excellent marker of glomelural function of children with cancer in our hands and by other investigators [1, 10]. However, corticosteroids, frequently applied in this patient population may adversely affect the reliability of the cysC assay as an estimate of glomerular function [4]. In vivo corticosteroid treatment was shown to be associated

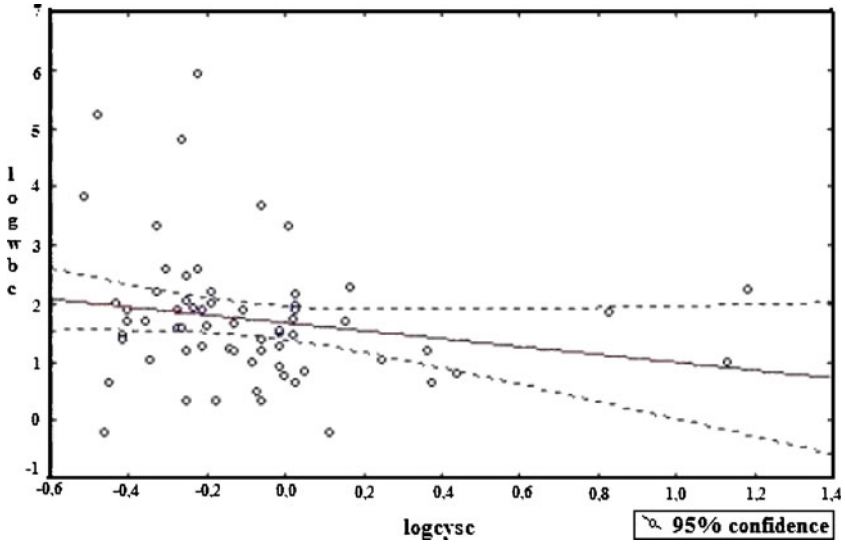

Fig. 1 Correlation of cystatin C concentration and initial white blood cell count in children with ALL. Scatter plot analysis shows significant correlation between log values of white blood cell count (logwbc, vertical axis) and log concentration of serum cystatin C (logcysc, horizontal axis); (p=0.04) in children with ALL: log wbc= 1.6688-0.6751*logcysc. Correlation: r=-0.1952. Continuous line shows mean values, dotted lines show 95% confidence intervals

with an elevation in cysC and this increase seemed to be unrelated to or inappropriately high to a parallel deterioration of the renal function in a variety of pediatric patients, such as children with asthma, glomerulonephritis and with renal transplants [3, 5, 6]. In vitro investigations suggested a promoter mediated increase in dexamethasone-induced cysC production in HeLa cells [11].

In this study, we investigated, for the first time, changes in cysC in two different pediatric populations receiving corticosteroid monotherapy in similar cumulative doses. The first population of children suffered from ITP, representing an immune platelet destruction without any known adverse effect on kidney function. Similar to the cited studies, in this patient population initial cysC levels were within the normal range and elevated significantly after 3 days of corticosteroid treatment.

The second patient population was represented by children with ALL. In this clonal hematopoietic malignancy renal function of patients is frequently impaired because of an increased cell turn-over, resulting in tumor lysis syndrome in the most severe cases and because of the infiltration of the kidneys by the leukemic blast cells. Both conditions are proportional to WBC. CysC was, indeed, elevated in this group of patients when compared both to the reference range of cysC and to the cysC levels of patients with ITP. Moreover, pretreatment cysC in children with ALL correlated significantly with the initial WBC. In this group of patient, no significant change in cysC was observed after 7 days of corticosteroid monotherapy, although both the cumulative dose and the activity (344 mg/m^2 PRED) was similar to the treatment intensity of the patients with ITP (300 mg/m^2 6-MP). Here we propose a dual effect of corticosteroid therapy. Lack of elevation of cysC was in patients with ALL upon PRED treatment may be due to decrease of the leukemic burden and the infiltration of the kidneys, resulting in an improving glomerular function associated with an expected decrease in cysC. The unchanged cysC in children with ALL might have been the net result of these two opposite biological effects.

Serum concentration of cys C, a low molecular weight protein (13.3 kDa) correlates better with GFR than creatinine-based methods, because its production rate is stable except for thyroid malfunction and corticosteroid medication [3, 12]. Our study suggested that in patients with corticosteroid medication cys C is more sensitive method than serum creatinin or creatinine-based methods.

Table 1 Pre- and postreatment values of cystatin C, serum creatinine, creatinine clearence and white blood cell count in ITP and ALL patients

	ITP	ALL
Pretreatment cystatin C values	0.96±0.27 mg/L	1.23±1.12 mg/L
Posttreatment cystatin C values	1.16±0.30 mg/L	1.24±0.49 mg/L
Pretreatment serum creatinine values	64±21 umol/L	76±17 umol/L
Posttreatment serum creatinine values	62±25 umol/L	66±20 umol/L
Pretreatment creatinin clearence values	103±31 ml/perc/1.73 m^2	80±35 ml/perc/1.73 m^2
Posttreatment creatinin clearence values	100±29 ml/perc/1.73 m^2	89±40 ml/perc/1.73 m^2
Pretreatment WBC values	7.46±1.23 G/L	22.58±17.30 G/L

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