

Cystatin C levels in patients with β -thalassemia during deferasirox treatment

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ABSTRACT

Deferasirox (Exjade®) is a once-daily, oral iron chelator approved for the treatment of transfusional iron overload. This study was conducted to analyze changes in cystatin C concentration, an endogenous marker of glomerular filtration rate (GFR), in patients with thalassemia receiving daily deferasirox therapy over a period of at least 9 months. One hundred and fifty β -thalassemia patients were treated with deferasirox at doses of 20–40 mg/kg/day for 9 consecutive months. Cystatin C concentrations were measured at regular intervals and GFR was calculated according to the cystatin C-based prediction equation. Plasma concentrations of NGAL protein and NT-proBNP were also monitored as indicators of renal function and LVEF, respectively. Serum ferritin concentration was also measured to assess iron overload. Throughout the 9 months of deferasirox treatment cystatin C concentration remained stable ($p > 0.850$). The baseline cystatin C mean values were 0.97 ± 0.27 mg/L and reached a maximum of 1.01 ± 0.29 mg/L at 4 months of treatment. No correlation was found between cystatin C and NGAL concentrations ($p > 0.674$). Cystatin C and NT-proBNP concentrations correlated positively with a binomial equation ($p < 0.004$), as also did cystatin C and serum ferritin ($p < 0.001$). These findings suggest that slight changes of cystatin C during deferasirox treatment may not reflect renal injury. However hemodynamic signals such as LVEF alterations and iron mobilization do appear to affect changes in cystatin C concentration.

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Introduction

Cystatin C is a non-glycosylated protein that belongs to the cystatin superfamily of cysteine protease inhibitors. It has been suggested as a sensitive marker of glomerular filtration rate (GFR) providing an early indication of renal impairment, possibly superior to serum creatinine [1].

Deferasirox (Exjade®) is a once-daily, oral iron chelator for the treatment of transfusional iron overload. The efficacy and tolerability of deferasirox have been established in adult and pediatric (≥ 2 years of age) patients with a range of transfusion-dependent anemias, including β -thalassemia [2–5]. Deferasirox is absorbed rapidly, achieving peak plasma concentration within 1–3 h after administration. It has a terminal elimination half-life with repeated doses that

ranges from 8 to 16 h (longer with higher drug doses), which is supportive of a once-daily dosing regimen [6]. Deferasirox is generally well tolerated and has a clinically manageable safety profile across a range of underlying anemias, including β -thalassemia [2,5,7]. Adverse events (AEs) associated with therapy are mostly of mild-to-moderate severity and easily managed even during long periods of treatment [8,9]. During Phase III evaluation of deferasirox, more than one third of patients experienced mild elevations in serum creatinine concentration, but a few patients experienced elevations exceeding the upper limit of normal (ULN; 2–3%) [2]. A cross-study analysis of data from the core registration studies demonstrated that mild, non-progressive and dose-related changes in serum creatinine often resolved spontaneously in the majority of patients (71.3%) or were managed by dose reduction or interruption [9,10]. In the clinical trials, no patients developed acute or chronic renal failure. None of the studies reported renal insufficiency as reflected by decreases in GFR, considered a more accurate and sensitive biomarker where renal disease is suspected.

This study was conducted to observe changes in cystatin C plasma concentration over 9 months in β -thalassemia patients treated with deferasirox. We aimed to determine whether changes in cystatin C concentration were associated with kidney impairment or representative of alternative phenomena.

Abbreviations: GFR, glomerular filtration rate; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal pro-B-type natriuretic peptide; LVEF, left ventricular ejection fraction.

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Patients

This study recruited 150 patients with transfusion-dependent β -thalassemia, who had no prior history of treatment with deferasirox. Patients with either serum creatinine above the ULN, or history of nephrotic syndrome or severe systemic diseases, (such as cardiovascular, renal, and hepatic disease) preventing long-term treatment, or patients who were pregnant, were excluded from the study. Blood from patients was collected before routine transfusion according to the guidelines established by the local Ethics Committee for observational human subject studies. Informed consent was obtained from the patients or from their parents if pediatric patients. Patients were treated with deferasirox at doses of 20–40 mg/kg/day and were followed for 9 consecutive months. Dose of deferasirox was adjusted according to the product prescribing information (<http://www.pharma.us.novartis.com/product/pi/pdf/exjade.pdf>) [11]. Dose reductions were recommended based on continuous assessment of changes in serum creatinine; deferasirox dose was reduced at 5–10 mg/kg/d if serum creatinine concentration was increased >33% above the average of the pretreatment measurements at two consecutive visits, and above the ULN, and it could not be attributed to other causes, and the drug was interrupted following progressive increases in serum creatinine.

Methods

Cystatin C concentration was measured by an immunonephelometric technique using the BN Prospec nephelometer (Dade Behring, Siemens Healthcare Diagnostics, Liederbach, Germany) at regular monthly intervals (5–9 monthly serial measurements per patient). The first measurement was performed before initiation of deferasirox treatment, then subsequently at monthly intervals. With a range of 0.23–7.25 mg/L, this assay is currently the most precise automated assay across the clinical concentration range. The inter-assay coefficient of variation (CV) for the assay was 5.05% and 4.87% at mean concentrations of 0.97 and 1.90 mg/L, respectively.

The GFR was calculated according to the recently proposed cystatin C-based prediction equation using cystatin C concentration in mg/L [12]:

$$\text{GFR} \left[\text{mL} / \text{min} / 1.73 \text{ m}^2 \right] = 76.7 \times \text{Cystatin C}^{-1.19}.$$

This equation was derived when cystatin C concentrations were correlated with GFR values obtained with ^{51}Cr -EDTA clearance.

In a randomly selected group of 15 patients, along with cystatin C concentration measurements, serial measurements (5 measurements per patient) of neutrophil gelatinase-associated lipocalin (NGAL), N-terminal pro-B-type natriuretic peptide (NT-proBNP) and ferritin were also performed. Plasma concentration of NGAL, a protein expressed on tubular cells and increased in response to harmful stimuli such as ischemia or toxicity, was measured as a biomarker of acute kidney injury [13]. NT-proBNP concentration was recorded to correlate cystatin C changes with left ventricular ejection fraction (LVEF). Serum ferritin concentration was monitored as a marker of iron overload/mobilization. Plasma NGAL concentration was determined using a solid phase ELISA technique (R&D Systems). The intra-assay and inter-assay CVs ranged between 3.1% and 4.1% and between 5.6% and 7.9%, respectively, according to the manufacturer. Serum ferritin concentration was measured in duplicate using a two-site chemiluminescence immunoassay (Nichols Institute Diagnostics, CA, USA) and plasma NT-proBNP concentration was quantitatively determined using the Roche Elecsys 2010 immunoassay analyzer (Roche Diagnostics, Mannheim, Germany), using an electrochemiluminescence (ECLIA) technique.

Statistical analysis

Data are presented as mean \pm standard deviation (SD), and the level of statistical significance was set at $p < 0.05$. The ANOVA repeated measures test was used to analyze time-course changes and regression analysis was used to correlate parameters. All statistical procedures were performed using STAT-GRAPHICS PLUS version 5.1 (Graphic Software System).

Results

A total of 150 patients were enrolled in this trial. The mean dose of deferasirox received was 26.2 mg/kg/day (range 20–40 mg/kg/day) and maintained stable through all the study period. The baseline mean concentration of cystatin C was 0.97 ± 0.27 mg/L (Table 1). Cystatin C levels of healthy adults were considered as normal when their levels are less than 0.96 mg/L, according to manufacturer. Considering the cut-off value of 0.96 mg/L, 84/150 (56%) patients had normal renal function, while 66 (44%) patients had mild to moderate decreased glomerular filtration rate based on cystatin C levels. No significant correlations were observed between cystatin C with age, sex, body mass index and/or inflammation expressed by high-sensitivity C-reactive protein ($p > 0.5$). Cystatin C concentration changed during the 9 months of deferasirox therapy (ANOVA repeated measures $p > 0.850$) reaching a maximum of 1.01 ± 0.29 mg/L at 4 months of treatment, without this increase reaching to be significant ($p > 0.08$), (Fig. 1). According to the treatment protocol, the dose of deferasirox was not reduced, as no significant increase in creatinine concentration was observed.

NGAL levels were significantly increased in the thalassemia patients studied compared to normal controls (43.1 ± 25.7 vs. 17.3 ± 3.5 $\mu\text{g/L}$, respectively, $p < 0.00001$). Similarly, NT-proBNP levels were also significantly increased in patients compared to normal controls (145.9 ± 100.2 vs. 40.1 ± 19.7 pg/mL, respectively, $p < 0.0001$). Furthermore, similar profile was observed for ferritin.

Statistical analysis was performed regarding the concentration of cystatin C with respect to concentrations of NGAL, NT-proBNP and serum ferritin (Figs. 2A–C). Analysis of data showed that there was no correlation between all cystatin C changes and NGAL concentrations ($p > 0.674$; Fig. 2A). However, all cystatin C changes and NT-proBNP concentrations correlated positively with a binomial equation ($p < 0.005$; Fig. 2B), as also did all cystatin C changes and serum ferritin concentrations ($p < 0.001$; Fig. 2C).

Discussion

Our results suggest that cystatin C concentration remains stable during deferasirox treatment and that any changes observed do not reflect renal injury but appear to be a consequence of the effects of deferasirox on hemodynamic parameters. In a previous study in randomly selected transfused patients with β -thalassemia treated or not treated with deferasirox, cystatin C levels correlated significantly with NGAL concentrations ($r = 0.740$, $p < 0.0001$) indicating renal insufficiency [14]. However in the present study, the concentration of

Table 1
Demographic and baseline patient characteristics (mean \pm SD, range).

Age (mean, range), years (N = 150)	29.2 (6.4–44.2)
Male/female (N = 150)	67/83
Baseline Cystatin C (mg/L) (N = 150)	0.97 ± 0.27 (0.61–1.46)
GFR (mL/min/1.73 m ²) (N = 150)	78.5 ± 1.8 (127.4–50.7)
Baseline NGAL ($\mu\text{g/L}$) (N = 15)	43.1 ± 25.7 (14.1–102.5)
Baseline NT-proBNP (pg/mL) (N = 15)	145.9 ± 100.2 (29.0–392.0)
Baseline Ferritin (ng/mL) (N = 15)	2657 ± 1784 (509–7873)

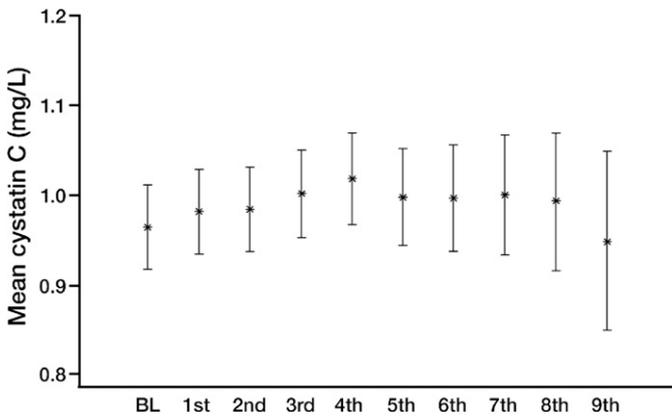


Fig. 1. Follow-up of renal function based on cystatin C concentration. Blood samples were taken at regular monthly intervals to determine the concentration of cystatin C. ANOVA repeated measures, $p > 0.850$; Kruskal–Wallis, $p > 0.146$.

cystatin C did not correlate with NGAL, suggesting that the tubuloglomerular feedback was not activated and that deferasirox treatment had a limited effect on renal function [15]. However, cystatin C concentrations were correlated with other hemodynamic signals such as LVEF, evident by NT-proBNP alterations and iron mobilizations, suggesting that these parameters exert any important role on changes in cystatin C concentrations.

There is no consensus on how renal function should be evaluated [16]. Serum creatinine measurements are a well-established parameter of renal function, being widely available and relatively inexpensive. Nevertheless creatinine measurements can be affected by age, weight, sex and race. Cystatin C has been proposed as a more suitable marker; however, it may still be influenced by age, weight, smoking, sex and C-reactive protein concentration [17,18]. Even so, cystatin C can serve as a useful predictor of adverse outcomes possibly also as a marker of inflammation [19]. In patients with decreasing renal function, assessment of cystatin C has been used in large-scale studies to monitor the risk of death, cardiovascular events, and hospitalization [20]. In a study in 52 thalassemia patients with deferasirox treatment, 40% had cystatin C values greater than the upper limit of normal (ULN, 0.95 mg/L) whereas none of the patients had increased serum creatinine (>1.4 mg/dL) and only 6 had low creatinine clearance (<80 mL/min). Serum concentrations of cystatin C correlated strongly with creatinine ($r = 0.657$, $p < 0.0001$) and creatinine clearance ($r = -0.625$, $p < 0.0001$) [21]. Following 12 months of therapy, levels of serum ferritin, SGOT and SPGT were significantly reduced, and levels of cystatin C and creatinine clearance significantly increased compared with baseline values. At the end of that study 32 patients (61.5%) had increased cystatin C concentration $>ULN$, 10 (19.2%) reduced creatinine clearance and one patient (1.9%) had high levels of serum creatinine [21]. To our knowledge this was the first study to demonstrate that pathologic renal impairment (increased cystatin C) and normal renal function (normal GFR) can occur simultaneously. The results of our study suggest that deferasirox has differing effects on these two markers of renal function supporting the findings from the current study that changes in cystatin C concentration during deferasirox therapy may reflect hemodynamic changes other than renal impairment.

A conclusive theory to explain the effects of deferasirox on renal function is yet to be determined. Our data suggest that, as a consequence of the effects of deferasirox in reducing iron burden, renal hemodynamics are modified as demonstrated by correlations of cystatin C with the hemodynamic parameter NT-proBNP and with serum ferritin. Similar theories exist with regard to alterations in serum creatinine. Data from the deferasirox clinical development program indicated that large increases in serum creatinine corre-

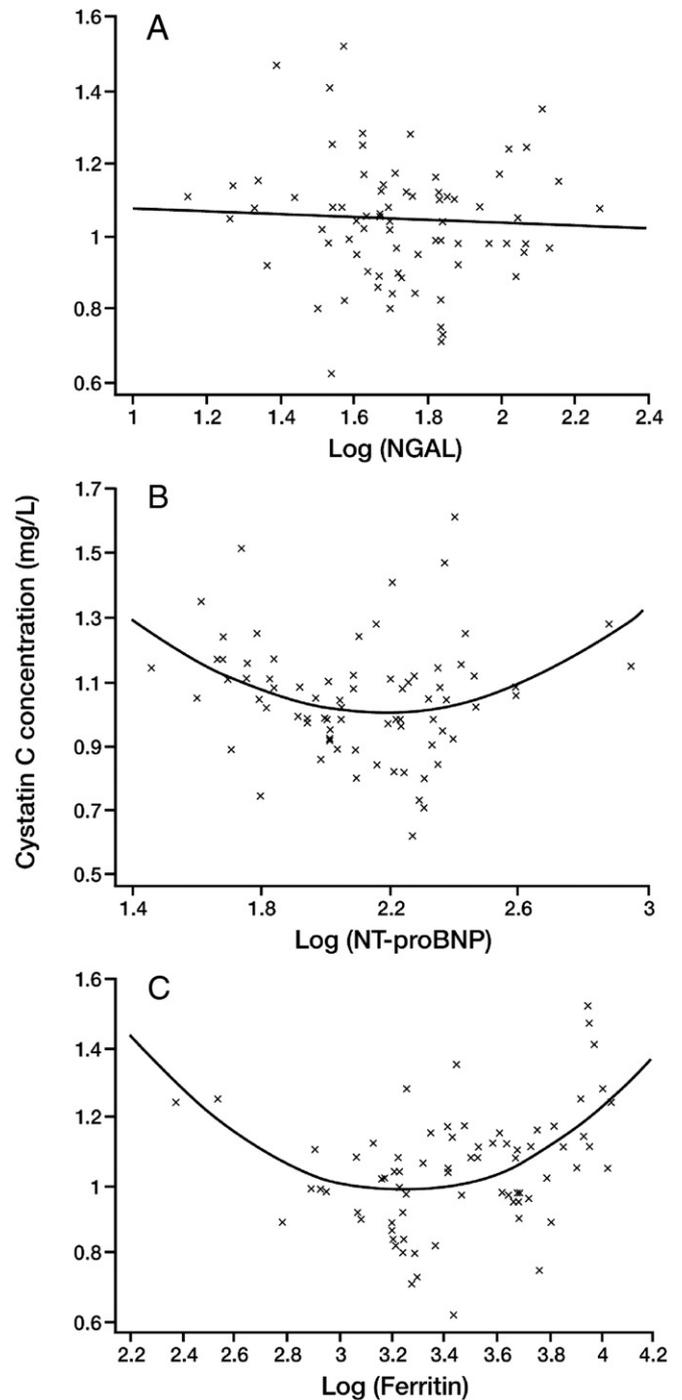


Fig. 2. (A) Correlation of Cystatin C concentration with NGAL values. (B) Correlation of cystatin C concentration with NT-proBNP ($p < 0.005$). (C) Correlation of cystatin C concentration with ferritin ($p < 0.001$). Blood samples were taken at regular intervals and the concentration of cystatin C, NGAL, NT-proBNP and Ferritin determined. Regression analysis was used to correlate parameters.

sponded to patients with the greatest reduction in iron burden [10]. It is possible that removal of iron in some body compartments exceeds that of transfusional iron intake, which could result in exclusion of iron from iron-dependent enzymes and transporters that are associated with kidney function.

In conclusion, cystatin C concentration may be influenced by hemodynamic parameters as a result of therapy with deferasirox. Any changes in cystatin C do not reflect renal impairment and, therefore, measurements of this biomarker should be interpreted

with caution with respect to possible AEs. Further trials evaluating the effects of deferasirox on renal hemodynamics including GFR, plasma flow rate and filtration fraction in patients with β -thalassemia are ongoing.

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