Exploring the Clinical Utility of Renal Safety Biomarkers During Iron Chelation Therapy

M Domenica Cappellini[1]*#, John B Porter[2]#, Erhard Quebe-Fehling[3], Celine Pallaud[3] and Frank Dieterle[3]

[1]University of Milan, Ca Granda Foundation IRCCS, Italy

[2]University College London, UK

[3]Novartis Pharma AG, Switzerland

#These authors contributed equally to this work.

Corresponding author:

M Domenica Cappellini, University of Milan, Ca Granda Foundation IRCCS, Italy.
Email: maria.cappellini@unimi.it

ABSTRACT

Monitoring renal function by assessing serum creatinine is recommended for patients receiving iron chelation therapy with deferasirox. Identifying biomarkers to predict renal function changes and further understanding of the pathophysiology is therefore of potential interest. Exploratory analyses of 19 renal protein biomarkers were conducted using urine samples, at baseline and every 3–6 months during two deferasirox clinical trials (comparative 1-year CICL670A0107 study of β-thalassemia patients randomized to deferasirox or deferoxamine; non-comparative 2-year CICL670A0108 study including β-thalassemia or other anemia [myelodysplastic syndromes, Diamond-Blackfan anemia, rare anemias] patients, excluding sickle cell disease). No progressive changes were identified, including acute renal injury (kidney injury molecule-1, neutrophil gelatinase-associated lipocalin), and inflammatory (clusterin, osteopontin) biomarkers. Overall, biomarker levels at baseline or subsequent changes showed either little or no correlation with changes in serum creatinine. Weak, though consistent correlations were identified between changes in serum creatinine and biomarkers of renal protein reabsorption and glomerular filtration (alpha-1-microglobulin, beta-2-microglobulin, retinol-binding protein, microalbumin, total protein, immunoglobulin G) across patient groups. Better associations were identified in non-β-thalassemic patients. Absence of progressive renal biomarker changes suggests that deferasirox chelation did not lead to acute/chronic renal dysfunction in these patients. The biomarkers did not show sufficiently strong associations with serum creatinine changes to support their clinical utility for monitoring kidney function in routine practice. Sequential measurements of serum creatinine and urine protein/creatinine ratio remain the recommendations for monitoring renal function.

ABBREVIATIONS: alpha1M: alpha-1-microglobulin; AUC: Area Under the Curve; beta2m: Beta-2-Microglobulin; CTGF: Connective Tissue Growth Factor; DBA: Diamond-Blackfan Anemia; DFO: Deferoxamine; GFR: Glomerular Filtration Rate; GSTA: Glutathione S-Transferase Alpha; IgG: Immunoglobulin G; KIM-1: kidney Injury Molecule-1; MDS: Myelodysplastic Syndromes; NAG: N-Acetyl-beta-Glucosaminidase; NGAL: Neutrophil Gelatinase-Associated Lipocalin; RBP: Retinol-Binding Protein; ROC: Receiver Operating Characteristic; SD: Standard

Exploring the Clinical Utility of Renal Safety Biomarkers During Iron Chelation Therapy. Journal of Nephrology & Kidney Diseases. 2018; 1(2):119.

SCIENTIFIC LITERATURE

Deviation; TFF3: Trefoil Factor 3; THP: Tamm–Horsfall Protein; TIMP-1: Tissue Inhibitor of Metalloproteinase-1; ULN: Upper Limit of Normal; VEGF: Vascular Endothelial Growth Factor

INTRODUCTION

The oral iron chelator deferasirox (Exjade®) was first licensed for the treatment of transfusional iron overload in the US in 2005 [1]. During the registration clinical trials, increases in serum creatinine values (defined as >33% above baseline on ≥2 consecutive measurements) were reported in up to 38% of patients [1-4], most frequently at higher doses (30 mg/kg/day). These creatinine increases were non-progressive, within the normal range and did not exceed two times the Upper Limit of Normal (ULN). They predominantly occurred within a few weeks of starting or increasing the dose and were reversible or stabilized with downward dose adjustments. Creatinine increases also tended to be more frequent in patients having the most dramatic reductions in liver iron concentration or serum ferritin [2]. A Phase I, open-label study of renal hemodynamics in patients with β-thalassemia major treated with deferasirox demonstrated a mild and reversible effect of deferasirox on renal function, without worsening over time [5]. Furthermore, a recent analysis of serum creatinine trends in patients enrolled in deferasirox registration studies for up to 13 years, confirmed the non-progressive nature of serum creatinine increases [6]. Development of mild proteinuria during chelation therapy also requires consideration, though interpretation is problematic as one study demonstrated that approximately 1 in 4 thalassemia major patients had average values three times that of healthy controls, irrespective of the underlying chelation modality [7]. Elevation of urine calcium and cystatin C have been reported during chelation with deferasirox, deferiprone and deferoxamine (DFO), with elevation of beta-2-microglobulin (beta2M) seen also in patients receiving deferasirox [7]. Therefore, close monitoring of renal function is recommended by assessment of serum creatinine and estimated creatinine clearance in duplicate before initiating deferasirox therapy, and monthly thereafter. Proteinuria should also be assessed monthly [1]. There have also been occasional case reports of renal tubular acidosis (Fanconi syndrome) with electrolyte imbalance and metabolic acidosis secondary to tubular dysfunction [8-10]. Interpretation of these findings can again be problematic because some patients, especially children, have intercurrent infections associated with Fanconi syndrome. Renal impairment may also develop as part of a generalized delayed hypersensitivity reaction.

More specific screening for renal effects of deferasirox would be of value to distinguish drug-related from other causes of proteinuria or increased serum creatinine and to also identify patients who are at increased risk for acute kidney injury while on therapy. This could prevent unnecessary withholding of chelation treatment and also minimize treatment-related renal toxicity, should it occur. Here we conducted a detailed study of renal biomarkers in patients receiving deferasirox focused on identifying protein biomarkers that might predict changes in renal function before serum creatinine increases, and to better understand the pathophysiology, particularly drug-induced nephrotoxicity [11,12]. In this exploratory analysis of two deferasirox clinical trials over 1 and 2 years (NCT00061750 [Study 107] and NCT00061763 [Study 108], respectively), a panel of 19 renal protein biomarkers were evaluated at baseline and over time. The main objective was to assess their potential as early indicators for an increased risk of acute renal dysfunction in transfusion-dependent patients treated with deferasirox. In particular, the following were analysed; 1) changes in biomarker profiles over time, to investigate progressive changes indicative of potential renal dysfunction; 2) correlations between baseline biomarker levels and changes in serum creatinine during treatment, to assess whether increased biomarker levels before treatment initiation could indicate an increased risk for renal dysfunction during treatment; 3) correlation of changes in biomarker levels with changes in serum creatinine during treatment to explore whether changes in biomarkers can potentially explain changes in renal function; 4) correlation between early biomarker changes during treatment with subsequent changes in serum creatinine, to investigate whether biomarkers could provide an early sign for potential changes in renal function. With weak associations between the renal biomarkers studied and serum creatinine, our findings do not support a role for monitoring additional biomarkers beyond serum creatinine. However, the absence of progressive changes indicate that iron chelation therapy did not lead to acute or chronic renal dysfunction in these patients.

MATERIALS AND METHODS

Study population

Urine samples were collected from male or female patients enrolled in two deferasirox clinical trials (Study 107 and Study 108). Patients were aged ≥2 years, received at least eight blood transfusions per year, and had a liver iron concentration of ≥2 mg Fe/g dry weight. No patient had a baseline serum creatinine above the ULN 1 year prior to enrollment. Other enrollment criteria have been previously described [2,3]. Both trials were conducted in accordance with Good Clinical Practices. Institutional Review Board or Ethics Committee approval was obtained at each participating institution and written informed consent was obtained from all patients or their legal guardians prior to participation in this study.

Additional documentation of informed consent was obtained prior to using biomarker samples.

Study design

A summary of the two deferasirox clinical trials is provided in (Table1). Exploratory analyses were performed on urine samples collected during the 1-year core phase of Study 107 (henceforth referred to as the comparative trial), and during the 1-year core and 1-year extension of Study 108 (henceforth referred to as the non-comparative trial). In this analysis, patients in the comparative trial were grouped according to treatment received (deferasirox versus [DFO]). In the non-comparative trial all patients received deferasirox and were grouped by underlying disease.

Table 1: Summary of the two deferasirox clinical trials.			
Clinical trials.gov Identifier (Novartis study number)	**Study design**	**Patient population**	**Patients exposed to chelation therapy, N Safety set***
NCT00061750 (Study 107) [2, 13]	Phase III trial, open-label, randomized, comparative, deferasirox versus DFO (1 year),[†] followed by deferasirox only (4 years)	Adult and pediatric β-thalassemia patients (≥2 years of age) with transfusional hemosiderosis	586: Deferasirox, n=296 DFO, n=290
NCT00061763 (Study 108)[3]	Phase II, open-label, non-comparative, single-arm trial of deferasirox (1 year) with extension (4 years)[‡]	Adult and pediatric patients (≥2 years of age) with congenital or acquired anemias (other than sickle cell disease) and transfusional hemosiderosis	184: β-thalassemia, n=85 MDS, n=47 DBA, n=30 Other rare anemias,[§] n=22

DBA: Diamond-Blackfan anemia; DFO: Deferoxamine; MDS: Myelodysplastic Syndromes.

*All patients received at least one dose of study treatment.

†Only the 1-year core study was analysed.

‡1-year core and 1-year extension were analysed.

§Other anemias included: aplastic anemia, α-thalassemia, and sideroblastic anemia, myelofibrosis, pure red cell aplasia, pyruvate kinase deficiency, autoimmune hemolytic anemia, Fanconi's anemia, hereditary sideroblastic anemia, erythropenia, and unspecified anemia.

Exploring the Clinical Utility of Renal Safety Biomarkers During Iron Chelation Therapy. Journal of Nephrology & Kidney Diseases. 2018; 1(2):119.

SCIENTIFIC LITERATURE

Assessments

In both studies, serum creatinine was measured at screening, baseline, then monthly at a central laboratory. Urine samples were collected at baseline, then every 3 to 6 months and stored at −80ºC. Four to six years later, the archived urine samples were used to measure a panel of exploratory renal protein biomarkers using a Luminex® platform (Table2). Urine creatinine was used to normalize all biomarkers. Results are reported in normalized units (weight/weight) unless otherwise specified.

Table 2: Urinary renal protein biomarkers.		
	Function	Reference
Progressive biomarkers (inflammatory biomarkers)		
Clusterin	Inflammatory marker; not site-specific	[14]
Connective Tissue Growth Factor (CTGF)	Fibrotic marker of chronic kidney disease	[15]
Osteopontin	Inflammation and macrophage activation	[16]
Tissue Inhibitor of Metalloproteinase-1 (TIMP-1)	Inflammation and fibrotic processes marker	[17]
Non-progressive biomarkers (acute injury biomarkers)		
Alpha-1-Microglobulin (alpha1M)	Proximal tubular functional marker	[14]
Beta-2-Microglobulin (beta2M)	Proximal tubular functional marker	[14]
Cystatin C	Proximal tubular functional marker	[14]
Kidney Injury Molecule-1 (KIM-1)	Proximal tubular injury; acute renal injury	[18,19]
Microalbumin	Proximal tubular function marker; glomerular marker	[20]
Neutrophil Gelatinase-Associated Lipocalin (NGAL)	Proximal tubular injury; acute renal injury	[19,21]
Total protein	Tubular function marker and glomerular marker dependent on size distribution of excreted proteins	[14]
Others		
Calbindin	Distal tubular and vitamin D pathway activation	[22]
Glutathione S-transferase alpha (GSTA)	Proximal tubular injury at brush border	[23]
Immunoglobulin G (IgG)	Glomerular injury	[24]
N-acetyl-beta-glucosaminidase (NAG)	Proximal tubular injury and stress marker	[25]
Retinol-Binding Protein (RBP)	Proximal tubular injury at brush border and stress marker	[26]
Trefoil factor 3 (TFF3)	Increased upon tubular injury; unclear role	[20]
Tamm–Horsfall Protein (THP)	Distal tubular and oxidative stress marker	[27]
Vascular Endothelial Growth Factor (VEGF)	Diabetic nephropathy progression, glomerulosclerosis and interstitial inflammation marker	[28]

Statistical analyses

All analyses were exploratory. The statistical analysis plan was developed before any data were analysed in order to ensure a prospective approach. The full analysis set was comprised of patients who received at least one treatment dose and had at least one baseline and one post-baseline biomarker sample. Results for the two studies are presented separately. Patients at risk of acute renal dysfunction were defined as having serum creatinine increased >33% from baseline on ≥2 consecutive visits, as defined by a renal safety board during the

deferasirox clinical development program and specified within the deferasirox PI as criteria for dose modification for decreases in renal function [1]. Data are summarized descriptively. Scatter plots evaluated correlations between renal biomarkers and serum creatinine, and correlation coefficients (r) were calculated. Statistical analyses were performed using SAS version 9.1.

For each renal biomarker, a receiver operating characteristic (ROC) curve was generated with the biomarkers (at baseline or change from baseline) as continuous classifiers and confirmed increase in serum creatinine as a binary (yes/no) variable to determine predictability for patients at risk of acute renal dysfunction. ROC analyses are routinely used to evaluate the predictive performance of diagnostic tests and biomarkers. The ROC curves show sensitivity versus 1−specificity over a range of cut-off values, so that high sensitivity values (eg, 90% or 0.9), and high specificity (eg, 90% meaning 1−specificity, being 1−0.9 = 0.1) relate to a high area under the ROC curve (AUC). The area under the ROC curve provides a single, numerical representation of the performance of the test; 1.0 represents a perfect test and 0.5 is no better than a random test [29].

RESULTS

Patient characteristics

Of 586 β-thalassemia patients in the comparative trial, 296 were randomized to receive deferasirox and 290 patients received DFO; 48.1% were males and 51.0% were <16 years of age (median 15 years [range 2−49] and 15.5 years [range 2−53] in the deferasirox and DFO arms, respectively). In the non-comparative trial, of 184 patients, 85 had β-thalassemia and 99 had other non-β-thalassemic anemias (MDS, n=47; DBA, n=30; other rare anemias, n=22), excluding sickle cell disease. Overall, 50.5% were male and 19.0% were <16 years of age (median 23 years [range 4−59] and 49 years [range 3−81]), in β-thalassemia and other anemias, respectively.

Changes in serum creatinine

In the comparative trial, 26.3% (n=154/586) of patients experienced at least one confirmed increase in serum creatinine during the 1-year trial; 73.4% (n=113/154) received deferasirox and 26.6% (n=41/154) DFO. Mean ±

standard deviation (SD) serum creatinine increased from 37.6 ± 11.4 μmol/L at baseline to 51.8 ± 16.3 μmol/L in patients receiving deferasirox and from 32.0 ± 11.2 to 41.5 ± 15.7 μmol/L in patients receiving DFO after 1 year. In the non-comparative trial, of 59.2% (n=109/184) who had a confirmed increased serum creatinine over the 2-year trial, 48.6% (n=53/109) had β-thalassemia and 51.4% (n=56/109) had other anemias. Mean ± SD serum creatinine increased from 44.2 ± 12.8 to 59.6 ± 17.3 μmol/L in β-thalassemia patients and from 55.0 ± 21.2 to 69.0 ± 24.3 μmol/L in patients with other anemias.

Renal biomarker trends over time

Over the 1−2 years, available data for the analysis of renal protein biomarkers varied (Tables S1 and S2) because of missed patient visits. In general, all biomarkers showed considerable variability (Tables S1 and S2). No significant progressive changes of the renal biomarkers in the entire treated population were observed. In the comparative trial, in patients with a confirmed increase in serum creatinine, a greater than 2-fold increase in alpha1M, beta2M, KIM-1, and microalbumin were noted in the deferasirox group and an almost 3-fold increase in trefoil factor 3 (TFF3) was noted in the DFO group. In the non-comparative trial, in patients with other anemias who had increased serum creatinine, increases were observed in alpha1M, beta2M, microglobulin, calbindin, clusterin, cystatin C, NGAL, Tamm−Horsfall protein (THP), TFF3, and RBP. Furthermore, beta2M, calbindin, connective tissue growth factor, and vascular endothelial growth factor increased more in patients with β-thalassemia compared to those with other anemias. These effects were not observed in patients without a confirmed increase in serum creatinine. These changes are not considered clinically meaningful when compared to biomarker studies in other disease settings (eg after cardiopulmonary bypass operations or in sepsis patients), where approximately 5- to 100-fold changes in biomarker levels were predictive of renal dysfunction [21, 30-33].

Associations between baseline renal biomarker levels and increased serum creatinine

ROC curves were generated by treatment and by underlying disease to determine whether urinary renal markers at baseline (pre-treatment) could predict an increased risk of acute renal dysfunction, defined as a confirmed increase in serum

creatinine of >33% from baseline (Table S3). The majority of AUC values ranged between 0.45 and 0.60, suggesting that urinary renal biomarkers at baseline (pre-treatment) had limited ability to predict an increased risk of acute renal dysfunction. Renal biomarkers generating higher AUC values were inconsistent between the two studies and underlying disease groups (Figure S1). Even in patients who had a confirmed increase in serum creatinine (data not shown), there was no correlation between baseline renal biomarker levels and relative change in serum creatinine from baseline during chelation therapy. Therefore, in these studies, biomarker values before treatment could not identify patients at greater risk of developing acute renal dysfunction during treatment.

Associations between changes in renal biomarkers and changes in serum creatinine

In the comparative trial, ROC curves identified at least four biomarkers from both the deferasirox and DFO treatment groups with an AUC between 0.6 and 0.7 (Table 3). A confirmed increase in serum creatinine had the most association

with RBP (AUC 0.7021; Figure 1A) and beta2M (AUC 0.6588; Figure 1B) in deferasirox- and DFO-treated patients, respectively. In the non-comparative trial, none of the renal biomarkers showed diagnostic value for confirmed increases in serum creatinine during deferasirox therapy in β-thalassemia patients. The majority of AUC values were between 0.4 and 0.6 (Table 3) and showed the most association with NGAL (AUC 0.6027; Figure 1C). In patients with other anemias, nine renal biomarkers had AUCs >0.6; the strongest association was with alpha1M (AUC 0.7982; Figure 1D) and beta2M (AUC 0.7663). Correlations between logged fold-change from baseline for individual renal biomarkers and relative change in serum creatinine from baseline over time were generally weak in both the comparative and non-comparative studies; r values were between 0.1 and 0.3 (data not shown). In both studies, the majority of renal biomarkers (alpha1M, beta2M, RBP, microalbumin, total protein, IgG) showed a positive correlation with an increased serum creatinine.

Table 3: AUC fold-change from baseline in renal protein biomarkers versus increased serum creatinine.				
	Comparative study		Non-comparative study	
	DFO (n)	Deferasirox (n)	β-thalassemia (n)	Other anemias (n)
Inflammatory biomarkers				
Clusterin	0.5330 (234)	0.4858 (236)	0.4651 (67)	0.5689 (59)
Connective Tissue Growth Factor (CTGF)	0.6442 (234)	0.4729 (236)	0.5343 (29)	0.5647 (22)
Osteopontin	0.5663 (234)	0.5826 (236)	0.3508 (67)	0.5614 (59)
Tissue Inhibitor of Metalloproteinase-1 (TIMP-1)	0.5508 (234)	0.6141 (236)	0.4003 (53)	0.6506 (44)
Acute injury biomarkers				
Alpha-1-Microglobulin (alpha1M)	0.6020 (234)	0.5915 (236)	0.4622 (67)	**0.7982 (59)**
Beta-2-Microglobulin (beta2M)	0.6588 (234)	0.5408 (236)	0.5620 (67)	**0.7663 (55)**
Cystatin C	0.6004 (234)	0.4537 (236)	0.4826 (67)	0.6717 (59)
Kidney Injury Molecule-1 (KIM-1)	0.6248 (234)	0.4065 (236)	0.5426 (67)	0.6704 (59)
Microalbumin	0.5828 (234)	0.5325 (236)	0.5891 (67)	0.5965 (59)
Neutrophil Gelatinase-Associated Lipocalin (NGAL)	0.4885 (234)	0.5246 (236)	0.6027 (67)	0.6402 (57)
Total protein	0.5166 (289)	0.5915 (294)	0.5152 (84)	0.4981 (93)
Others				

	Comparative study		Non-comparative study	
	DFO (n)	Deferasirox (n)	β-thalassemia (n)	Other anemias (n)
Calbindin	0.5878 (234)	0.5913 (236)	0.4254 (67)	0.6190 (59)
Glutathione S-Transferase Alpha (GSTA)	0.4819 (234)	0.5379 (236)	0.5520 (60)	0.6232 (50)
Immunoglobulin G (IgG)	0.5997 (289)	0.6232 (294)	0.5067 (84)	0.4508 (93)
N-Acetyl-Beta-Glucosaminidase (NAG)	0.6089 (289)	0.6541 (294)	0.5727 (84)	0.4677 (93)
Retinol-Binding Protein (RBP)	0.6430 (289)	**0.7021 (294)**	0.4380 (83)	0.5314 (93)
Trefoil Factor 3 (TFF3)	0.5336 (234)	0.5787 (236)	0.4943 (30)	0.5729 (32)
Tamm–Horsfall Protein (THP)	0.5536 (234)	0.5579 (236)	0.4942 (67)	0.5827 (59)
Vascular Endothelial Growth Factor (VEGF)	0.5903 (234)	0.4469 (236)	0.4913 (67)	0.6767 (59)

AUC >0.7 are highlighted.

AUC, area under the plasma concentration–time curve; DFO: Deferoxamine.
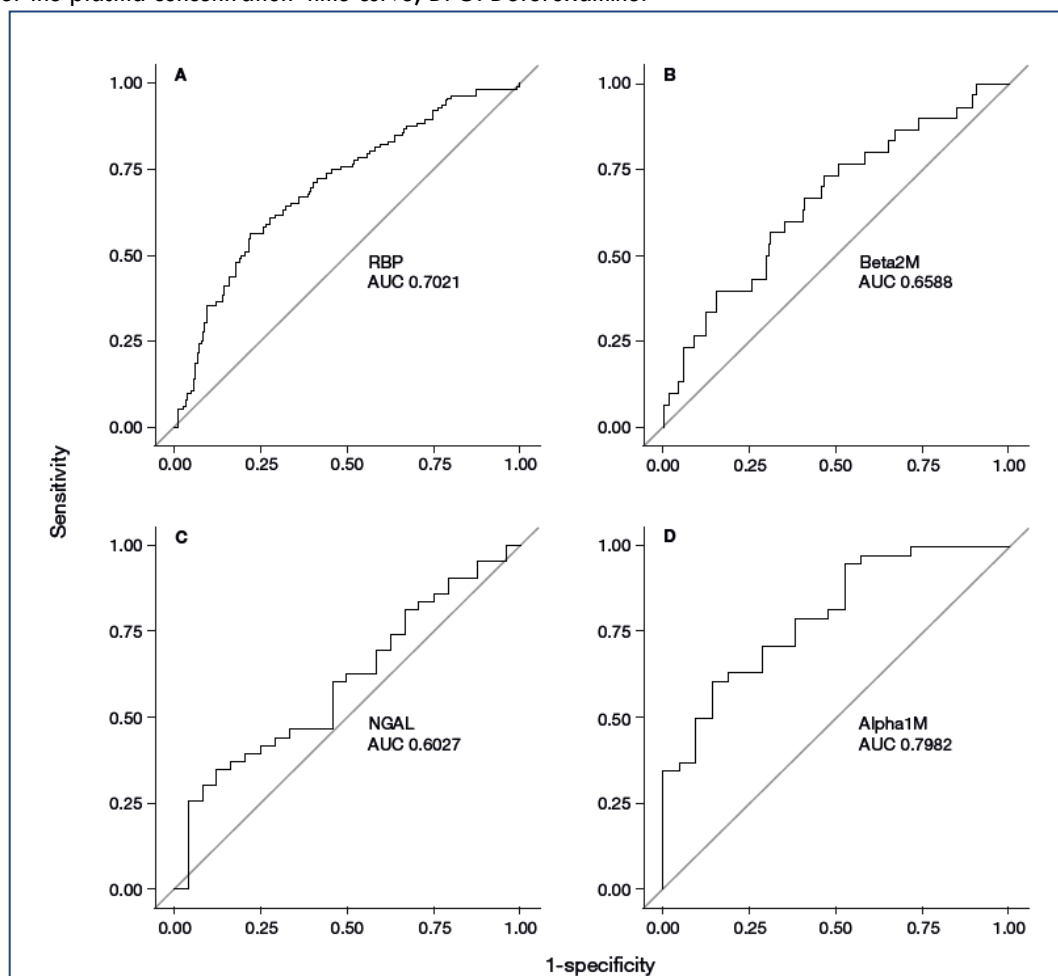

Figure 1: ROC curves for the highest AUC fold-change from baseline in renal protein biomarkers versus increased serum creatinine. Comparative study: A) Retinol-Binding Protein (RBP; deferasirox arm), B)Beta-2-Microglobulin (beta2M; DFO arm). Non-comparative study: C) Neutrophil Gelatinase-Associated Lipocalin (NGAL; β-thalassemia), D) Alpha-1-microglobulin (alpha1M; other anemias)

Note: high sensitivity values (eg, 90% = 0.9) and high specificity (eg, 90% = 1−0.9 = 0.1) relate to a high area under the ROC curve (AUC).

AUC, area under the plasma concentration–time curve; DFO, deferoxamine; ROC, receiver operating characteristic.

ROC curves were also generated for changes in biomarkers 1 month prior to the confirmed increase in serum creatinine. In the comparative study, 10 biomarkers in deferasirox- and seven in DFO-treated patients had an AUC >0.6 (Table 4). The strongest associations were with RBP (AUC 0.6744; Figure 2A) in the deferasirox arm and connective tissue growth factor (AUC 0.7031; Figure 2B) in the DFO arm. In the non-comparative study, patients with β-thalassemia had four biomarkers with AUC >0.6; the strongest association was with TFF3 (AUC 0.7619; Figure 2C). In patients with other anemias, 17 renal biomarkers had an AUC >0.6;alpha1M (AUC 0.9000; Figure 2D) and beta2M (AUC 0.8594) were statistically significant.

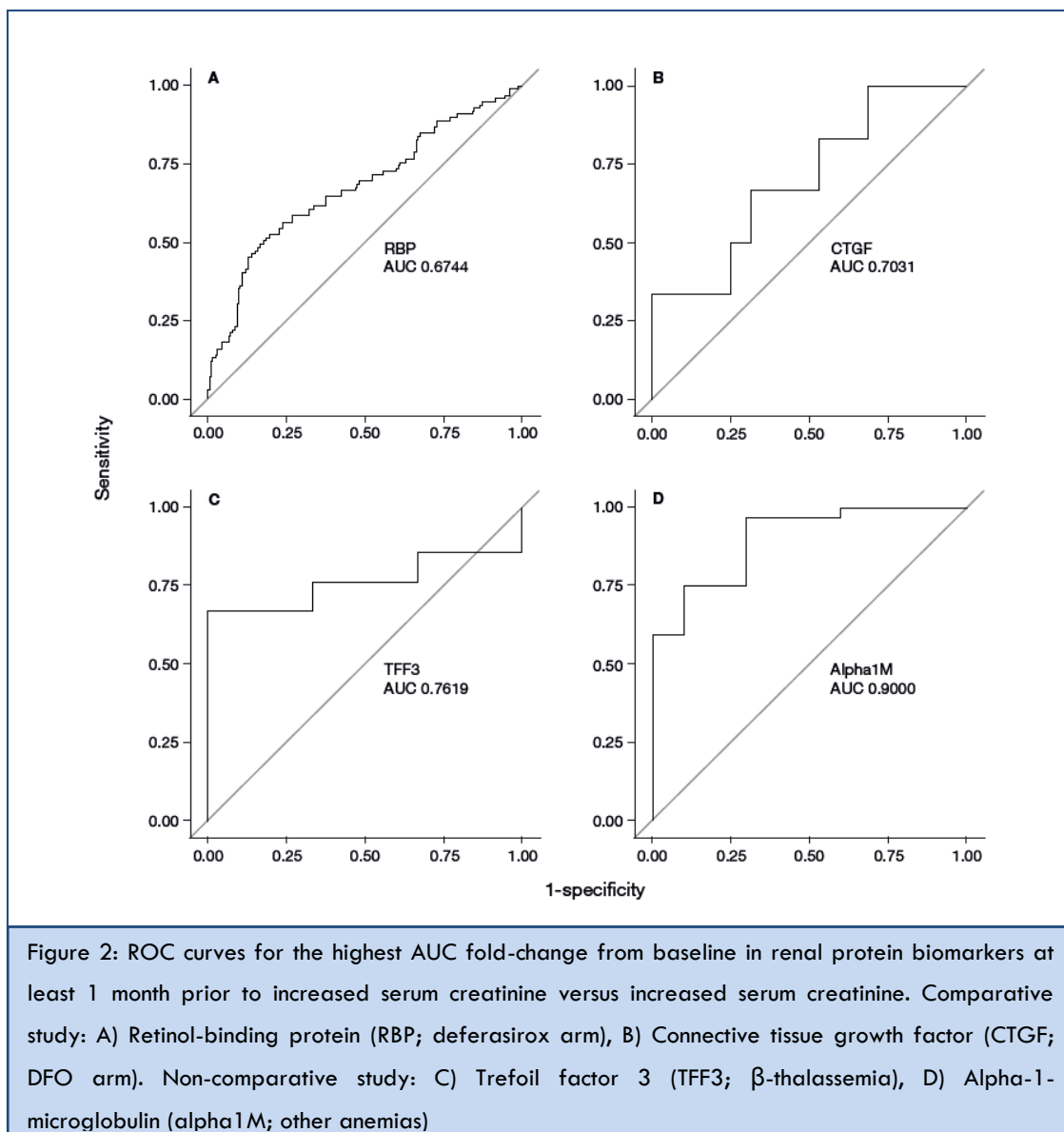| Table 4: AUC fold-change from baseline in renal protein biomarkers at least 1 month prior to increased serum creatinine versus increased serum creatinine.				
	Comparative study		Non-comparative study	
	DFO (n)	Deferasirox (n)	β-thalassemia (n)	Other anemias (n)
Inflammatory biomarkers				
Clusterin	0.5430 (155)	0.4155 (146)	0.5198 (60)	0.6719 (42)
Connective tissue growth factor (CTGF)	**0.7031 (38)**	0.5413 (44)	0.4833 (23)	**0.8571 (15)**
Osteopontin	0.6026 (157)	0.5860 (146)	0.4299 (60)	0.6781 (42)
Tissue inhibitor of metalloproteinase-1 (TIMP-1)	0.5087 (79)	0.6479 (79)	0.4083 (46)	**0.8087 (28)**
Acute injury biomarkers				
Alpha-1-microglobulin (alpha1M)	0.5752 (157)	0.6678 (146)	0.5278 (60)	**0.9000 (42)**
Beta-2-microglobulin (beta2M)	0.6054 (135)	0.6094 (132)	0.6190 (60)	**0.8594 (40)**
Cystatin C	0.6128 (149)	0.5938 (143)	0.5344 (60)	**0.7656 (42)**
Kidney injury molecule-1 (KIM-1)	0.5792 (157)	0.6211 (146)	0.6058 (60)	**0.7406 (42)**
Microalbumin	0.6434 (156)	0.4062 (146)	0.5899 (60)	**0.7594 (42)**
Neutrophil gelatinase-associated lipocalin (NGAL)	0.5631 (142)	0.5675 (140)	0.6944 (60)	**0.7867 (40)**
Total protein	0.5240 (255)	0.5412 (261)	0.5757 (80)	0.5805 (79)
Others				
Calbindin	0.5960 (157)	0.6232 (146)	0.4921 (60)	**0.7406 (42)**
Glutathione S-transferase alpha (GSTA)	0.4437 (122)	0.4385 (118)	0.5414 (53)	0.6158 (36)
Immunoglobulin G (IgG)	0.5941 (237)	0.6269 (238)	0.5277 (80)	0.6873 (79)
N-acetyl-beta-glucosaminidase (NAG)	0.5476 (256)	0.6391 (261)	0.5720 (80)	0.5962 (79)
Retinol-binding protein (RBP)	0.6251 (256)	0.6744 (260)	0.3897 (79)	0.6460 (79)

	Comparative study		Non-comparative study	
	DFO (n)	Deferasirox (n)	β-thalassemia (n)	Other anemias (n)
Trefoil factor 3 (TFF3)	0.5113 (49)	0.5453 (47)	**0.7619 (24)**	0.6481 (21)
Tamm–Horsfall protein (THP)	0.6179 (157)	0.6267 (146)	0.5701 (60)	0.6531 (42)
Vascular endothelial growth factor (VEGF)	0.5274 (157)	0.6006 (146)	0.5503 (60)	**0.8156 (42)**

AUC >0.7 are highlighted.

AUC, area under the plasma concentration–time curve; DFO: Deferoxamine.

Figure 2: ROC curves for the highest AUC fold-change from baseline in renal protein biomarkers at least 1 month prior to increased serum creatinine versus increased serum creatinine. Comparative study: A) Retinol-binding protein (RBP; deferasirox arm), B) Connective tissue growth factor (CTGF; DFO arm). Non-comparative study: C) Trefoil factor 3 (TFF3; β-thalassemia), D) Alpha-1-microglobulin (alpha1M; other anemias)

Note: high sensitivity values (eg, 90% = 0.9) and high specificity (eg, 90% = 1–0.9 = 0.1) relate to a high area under the ROC curve (AUC).

AUC: Area Under the Plasma Concentration–Time Curve; DFO: Deferoxamine; ROC: Receiver Operating Characteristic.

DISCUSSION

In patients with transfusion-dependent anemias, a range of renal protein biomarkers indicative of pathological processes in different regions of the nephron were evaluated as predictors for patients at risk of acute renal dysfunction before and during iron chelation therapy. There were no progressive or late increases in any renal biomarker examined. Alpha1M, beta2M, RBP, microalbumin, total protein, and IgG, all showed weak, though consistent, positive correlations with increased serum creatinine, which may indicate impaired protein reabsorption in the proximal tubule in some patients within 3 to 6 months of initiating chelation therapy. The acute kidney injury biomarkers KIM-1 and NGAL did not change, suggesting that deferasirox does not cause severe tubular cell injury [12]. No change in inflammatory markers clusterin and osteopontin suggests that deferasirox did not cause chronic kidney injury within the 2-year follow-up of the study. Changes in alpha1M, beta2M, RBP, and microalbumin observed in this study might link to a reversible partial blockage of the tubular reabsorption complex, such as that observed in Fanconi syndrome. Rare reports of acquired Fanconi syndrome, a functional tubular cell abnormality, have been published in patients treated with deferasirox [34,35], and is generally reversible with treatment interruption or discontinuation.

In routine clinical practice, kidney function is commonly evaluated using serum creatinine as an estimation of glomerular filtration rate (GFR) [36] as direct measurements of GFR are not widely available and burdensome; however, over- and under-estimation of GFR, inter-personal variability due to differences in muscle mass, and inability to identify the site of impaired kidney function have led to the evaluation of alternative markers (see Table 2), which may also provide information on whether renal dysfunction is progressive or non-progressive, acute or chronic, or is a functional impairment. In this exploratory analysis, renal biomarkers at baseline were unable to predict increases in serum creatinine during treatment. Renal biomarkers, even with the highest AUC values, were weak and inconsistent between trials and patient groups in predicting increases in serum creatinine. Since no correlation was observed in β-thalassemia patients, there appears to be limited clinical utility for the renal biomarkers investigated to identify patients at risk of developing renal dysfunction in this population. Interestingly, patients with other anemias from the non-comparative study had higher AUCs across all biomarkers compared with β-thalassemia patients in both the comparative and the non-comparative trials, suggesting better predictability for patients with MDS, DBA, and aplastic anemia. In both trials, renal biomarker levels obtained 1 month prior to a confirmed increase in serum creatinine were not predictive of changes in serum creatinine in β-thalassemia patients. In the non-comparative study, better predictability was observed for alpha1M and beta2M in patients with other anemias. However, this was a smaller, more diverse patient population. In addition, as there was no adjustment for multiple testing, findings could have been spurious. While for most kidney biomarkers very limited degradation has been reported when stored at −80°C during multiple years [37-43], variation during sample collection and before freezing cannot be excluded and might contribute to the overall variability of the biomarker data. Together with the irregular collection of urine samples because of missed clinic visits, robust conclusions on the clinical utility of alpha1M and beta2M as more sensitive biomarkers compared with serum creatinine cannot be drawn from these data. Nevertheless, further examination of their clinical utility should be considered alongside other, novel biomarkers [44].

CONCLUSIONS

The absence of progressive changes in these renal biomarkers, investigated for their predictability for changes in serum creatinine, at the population level did not show a clear association between iron chelation therapy and acute or chronic renal dysfunction in these studies. Independently of the underlying disease, the weak association between renal biomarkers and serum creatinine was insufficiently strong to support the clinical utility of routinely monitoring these renal biomarkers in patients receiving deferasirox. In patients with other anemias (myelodysplastic syndromes, Diamond-Blackfan anemia, rare anemias), there was a stronger association shown by a concomitant increase in serum creatinine and renal biomarkers both simultaneously and 1 month preceding a change in serum creatinine; however, this needs to be interpreted with caution because of the small, heterogeneous patient population. Additional, longitudinal investigation of the potential role of renal biomarkers, particularly alpha1M and beta2M, in predicting patients at risk of renal dysfunction

SCIENTIFIC LITERATURE

during deferasirox treatment diagnosed by changes of measured GFR instead of changes of serum creatinine is still needed. Therefore, measurement of serum creatinine and sequential monitoring of urine protein/creatinine ratio remain the current recommendations for the monitoring of kidney function in patients receiving deferasirox.

DATA SHARING

Novartis is committed to sharing with qualified external researchers access to patient-level data and supporting clinical documents from eligible studies. These requests are reviewed and approved by an independent review panel on the basis of scientific merit. All data provided are anonymised to respect the privacy of patients who have participated in the trial, in line with applicable laws and regulations. This trial data availability is in accordance with the criteria and process described on www.clinicalstudydatarequest.com.

AVAILABILITY OF DATA AND MATERIALS

The datasets supporting the conclusions of this article are available on clinicaltrials.gov (https://clinicaltrials.gov/ct2/show/NCT00061750 and https://clinicaltrials.gov/ct2/show/NCT00061763) and/or are included within the article (and supplementary information).

REFERENCES

1. Novartis Pharmaceuticals. (2018). EXJADE® (deferasirox) US Prescribing Information.

2. Cappellini MD, Cohen A, Piga A, Bejaoui M, Perrotta S, et al. (2006). A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with beta-thalassemia. Blood. 107: 3455-3462.

3. Porter J, Galanello R, Saglio G, Neufeld EJ, Vichinsky E, et al. (2008). Relative response of patients with myelodysplastic syndromes and other transfusion-dependent anaemias to deferasirox (ICL670): a 1-yr prospective study. Eur J Haematol. 80: 168-176.

4. Vichinsky E, Onyekwere O, Porter J, Swerdlow P, Eckman J, et al. (2007). A randomized comparison of deferasirox versus deferoxamine for the treatment of transfusional iron overload in sickle cell disease. Br J Haematol. 136: 501-508.

5. Piga A, Fracchia S, Lai ME, Cappellini MD, Hirschberg R, et al. (2015). Deferasirox effect on renal haemodynamic parameters in patients with transfusion-dependent beta thalassaemia. Br J Haematol. 168: 882-890.

6. Origa R, Piga A, Tartaglione I, Della CG, Forni GL, et al. (2018). Renal safety under long-course deferasirox therapy in iron overloaded transfusion-dependent beta-thalassemia and other anemias. Am J Hematol. 93: E172-E175.

7. Economou M, Printza N, Teli A, Tzimouli V, Tsatra I, et al. (2010). Renal dysfunction in patients with beta-thalassemia major receiving iron chelation therapy either with deferoxamine and deferiprone or with deferasirox. ActaHaematol. 123: 148-152.

8. Grange S, Bertrand DM, Guerrot D, Eas F, Godin M. (2010). Acute renal failure and Fanconi syndrome due to deferasirox. Nephrol Dial Transplant. 25: 2376-2378.

9. Rheault MN, Bechtel H, Neglia JP, Kashtan CE. (2011). Reversible Fanconi syndrome in a pediatric patient on deferasirox. Pediatr Blood Cancer. 56: 674-676.

10. Wei HY, Yang CP, Cheng CH, Lo FS. (2011). Fanconi syndrome in a patient with beta-thalassemia major after using deferasirox for 27 months. Transfusion. 51: 949-954.

011

Exploring the Clinical Utility of Renal Safety Biomarkers During Iron Chelation Therapy. Journal of Nephrology & Kidney Diseases. 2018; 1(2):119.

SCIENTIFIC LITERATURE

11. Bonventre JV, Vaidya VS, Schmouder R, Feig P, Dieterle F. (2010). Next-generation biomarkers for detecting kidney toxicity. Nat Biotechnol. 28: 436-440.

12. Ferguson MA, Vaidya VS, Bonventre JV. (2008). Biomarkers of nephrotoxic acute kidney injury. Toxicology. 245: 182-193.

13. Cappellini MD, Bejaoui M, Agaoglu L, Canatan D, Capra M, et al. (2011). Iron chelation with deferasirox in adult and pediatric patients with thalassemia major: efficacy and safety during 5 years' follow-up. Blood. 118: 884-893.

14. Dieterle F, Perentes E, Cordier A, Roth DR, Verdes P, et al. (2010). Urinary clusterin, cystatin C, b2-microglobulin and total protein as markers to detect drug-induced kidney injury. Nat Biotechnol. 28: 463-469.

15. O'Seaghdha CM, Hwang SJ, Larson MG, Meigs JB, Vasan RS, et al. (2013). Analysis of a urinary biomarker panel for incident kidney disease and clinical outcomes. J Am SocNephrol. 24: 1880-1888.

16. Xie Y, Sakatsume M, Nishi S, Narita I, Arakawa M, et al. (2001). Expression, roles, receptors, and regulation of osteopontin in the kidney. Kidney Int. 60: 1645-1657.

17. Eddy A. (2001). Role of cellular infiltrates in response to proteinuria. Am J Kidney Dis. 37: S25-S29.

18. Vaidya VS, Ozer JS, Dieterle F, Collings FB, Ramirez V, et al. (2010). Kidney injury molecule-1 outperforms traditional biomarkers of kidney injury in preclinical biomarker qualification studies. Nat Biotechnol. 28: 478-485.

19. Siew ED, Ware LB, Ikizler TA. (2011). Biological markers of acute kidney injury. J Am SocNephrol. 22: 810-820.

20. Yu Y, Jin H, Holder D, Ozer JS, Villarreal S, et al. (2010). Urinary biomarkers trefoil factor 3 and albumin enable early detection of kidney tubular injury. Nat Biotechnol. 28: 470-477.

21. Mishra J, Dent C, Tarabishi R, Mitsnefes MM, Ma Q, et al. (2005). Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet. 365: 1231-1238.

22. Sourial S, Marcusson-Stahl M, Cederbrant K. (2009). Meso scale discovery and luminex comparative analysis of calbindin D28K. J Biomed Biotechnol. 2009: 187426.

23. Branten AJ, Mulder TP, Peters WH, Assmann KJ, Wetzels JF. (2000). Urinary excretion of glutathione S transferases alpha and pi in patients with proteinuria: reflection of the site of tubular injury. Nephron. 85: 120-126.

24. Nauta FL, Boertien WE, Bakker SJ, van GH, van OW, et al. (2011). Glomerular and tubular damage markers are elevated in patients with diabetes. Diabetes Care. 34: 975-981.

25. Skalova S. (2005). The diagnostic role of urinary N-acetyl-beta-D-glucosaminidase (NAG) activity in the detection of renal tubular impairment. ActaMedica (Hradec Kralove). 48: 75-80.

26. Adiyanti SS, Loho T. (2012). Acute Kidney Injury (AKI) biomarker. Acta Med Indones. 44: 246-255.

27. Chakraborty J, Below AA, Solaiman D. (2004). Tamm-Horsfall protein in patients with kidney damage and diabetes. Urol Res. 32: 79-83.

28. Schrijvers BF, Flyvbjerg A, De Vriese AS. (2004). The role of vascular endothelial growth factor (VEGF) in renal pathophysiology. Kidney Int. 65: 2003-2017.

29. Carter JV, Pan J, Rai SN, Galandiuk S. (2016). ROC-ing along: Evaluation and interpretation of receiver operating characteristic curves. Surgery. 159: 1638-1645.

30. Koyner JL, Vaidya VS, Bennett MR, Ma Q, Worcester E, et al. (2010). Urinary biomarkers in the clinical prognosis and early detection of acute kidney injury. Clin J Am SocNephrol. 5: 2154-2165.

31. Nickolas TL, O'Rourke MJ, Yang J, Sise ME, Canetta PA, et al. (2008). Sensitivity and specificity of a single emergency department measurement of urinary neutrophil gelatinase-associated lipocalin for diagnosing acute kidney injury. Ann Intern Med. 148: 810-819.

32. Nickolas TL, Schmidt-Ott KM, Canetta P, Forster C, Singer E, et al. (2012). Diagnostic and prognostic stratification in the emergency department using urinary biomarkers of nephron damage: a multicenter prospective cohort study. J Am CollCardiol. 59: 246-255.

33. Parikh CR, Devarajan P, Zappitelli M, Sint K, Thiessen-Philbrook H, et al. (2011). Postoperative biomarkers predict acute kidney injury and poor outcomes after pediatric cardiac surgery. J Am SocNephrol. 22: 1737-1747.

012

Exploring the Clinical Utility of Renal Safety Biomarkers During Iron Chelation Therapy. Journal of Nephrology & Kidney Diseases. 2018; 1(2):119.

SCIENTIFIC LITERATURE

34. Chuang GT, Tsai IJ, Tsau YK, Lu MY. (2015). Transfusion-dependent thalassemic patients with renal Fanconi syndrome due to deferasirox use. Nephrology (Carlton). 20: 931-935.

35. Dee CM, Cheuk DK, Ha SY, Chiang AK, Chan GC. (2014). Incidence of deferasirox-associated renal tubular dysfunction in children and young adults with beta-thalassaemia. Br J Haematol. 167: 434-436.

36. Ferguson MA, Waikar SS. (2012). Established and emerging markers of kidney function. ClinChem. 58: 680-689.

37. Giesen C, Lieske JC. (2016). The Influence of Processing and Storage Conditions on Renal Protein Biomarkers. Clin J Am SocNephrol. 11: 1726-1728.

38. Grenier FC, Ali S, Syed H, Workman R, Martens F, et al. (2010). Evaluation of the ARCHITECT urine NGAL assay: assay performance, specimen handling requirements and biological variability. ClinBiochem. 43: 615-620.

39. Han WK, Wagener G, Zhu Y, Wang S, Lee HT. (2009). Urinary biomarkers in the early detection of acute kidney injury after cardiac surgery. Clin J Am SocNephrol. 4: 873-882.

40. Juraschek SP, Coresh J, Inker LA, Rynders GP, Eckfeldt JH, et al. (2012). The effects of freeze-thaw on beta-trace protein and beta2-microglobulin assays after long-term sample storage. ClinBiochem 45: 694-696.

41. Nauta FL, Bakker SJ, Lambers HH, de ZD, van OW, et al. (2012). Effect of frozen storage on urinary concentration of kidney damage markers. Am J Kidney Dis. 59: 586-589.

42. Schuh MP, Nehus E, Ma Q, Haffner C, Bennett M, et al. (2016). Long-term Stability of Urinary Biomarkers of Acute Kidney Injury in Children. Am J Kidney Dis. 67: 56-61.

43. Van D, V, Deegens JK, van d, V, Hilbrands LB. (2014). Effect of long-term storage of urine samples on measurement of kidney injury molecule 1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL). Am J Kidney Dis. 63: 573-576.

44. Cheon JH, Kim SY, Son JY, Kang YR, An JH, et al. (2016). Pyruvate Kinase M2: A Novel Biomarker for the Early Detection of Acute Kidney Injury. Toxicol Res. 32: 47-56.

Supporting information

S1 Table: Renal protein biomarker levels (normalized) at baseline, 6 months, and 9 months in the comparative study.						
Renal biomarkers	**Deferasirox** normalized by urinary creatinine, median (range), weight/weight			**DFO** normalized by urinary creatinine, median (range), weight/weight		
	Baseline	**6 months**	**9 months**	**Baseline**	**6 months**	**9 months**
Clusterin $\times 10^{-6}$	40.0 (0, 650.0) (n=226)	30.0 (0, 1220.0) (n=126)	20.0 (0, 4130.0) (n=149)	30.0 (0, 750.0) (n=232)	20.0 (0, 220.0) (n=115)	10.0 (0, 2050.0) (n=151)
Connective tissue growth factor (CTGF) $\times 10^{-8}$	111.0 (24.0, 739.0) (n=226)	118.0 (26.0, 1130.0) (n=126)	90.0 (29.0, 1071.0) (n=149)	115.0 (29.0, 1186.0) (n=232)	90.0 (26.0, 864.0) (n=115)	103.0 (23.0, 2763.0) (n=151)
Osteopontin $\times 10^{-4}$	13.4 (0.1, 68.1) (n=226)	12.3 (0.1, 58.0) (n=126)	10.1 (0.1, 51.9) (n=149)	11.8 (0.2, 68.8) (n=232)	12.2 (0.5, 44.8) (n=115)	12.4 (0.2, 60.0) (n=151)
Tissue inhibitor of metalloproteinase-1 (TIMP-1) $\times 10^{-8}$	93.0 (27.0, 5200.0) (n=226)	94.0 (17.0, 3667.0) (n=126)	85.0 (21.0, 1500.0) (n=149)	97.0 (21.0, 3559.0) (n=232)	83.0 (27.0, 1875.0) (n=115)	80.0 (15.0, 8438.0) (n=151)
Alpha-1-microglobulin (alpha1M) $\times 10^{-3}$	10.8 (0.1, 104.2) (n=226)	12.3 (0.1, 154.8) (n=126)	8.3 (0.1, 102.6) (n=149)	10.2 (0.0, 104.9) (n=232)	10.7 (0.1, 97.0) (n=115)	8.5 (0.0, 104.6) (n=151)
Beta-2-microglobulin (beta2M) $\times 10^{-6}$	60.0 (0, 1420.0) (n=226)	60.0 (0, 26000.0) (n=126)	60.0 (0, 30770.0) (n=149)	70.0 (0, 7550.0) (n=232)	50.0 (0, 20340.0) (n=115)	40.0 (0, 21280.0) (n=151)
Cystatin C $\times 10^{-6}$	49.7 (1.2, 1268.7) (n=226)	35.4 (0.8, 2850.0) (n=126)	30.6 (1.1, 3149.3) (n=149)	46.3 (1.0, 189.8) (n=232)	39.2 (1.1, 2534.4) (n=115)	25.0 (0.7, 1800.0) (n=151)
Kidney injury molecule-1 (KIM-1) $\times 10^{-8}$	78.0 (0, 419.0) (n=226)	93.0 (0, 800.0) (n=126)	75.0 (0, 644.0) (n=149)	67.0 (0, 583.0) (n=232)	71.0 (3.0, 518.0) (n=115)	70.0 (0, 441.0) (n=151)
Microalbumin $\times 10^{-4}$	93.2 (1.4, 4216.2) (n=226)	68.0 (1.8, 3666.7) (n=126)	70.5 (1.8, 3250.0) (n=149)	83.1 (1.5, 6500.0) (n=232)	77.2 (1.3, 1733.3) (n=115)	70.3 (1.1, 8571.4) (n=151)
Neutrophil gelatinase-associated lipocalin (NGAL) $\times 10^{-6}$	20.2 (1.8, 4028.6) (n=226)	20.8 (2.1, 824.8) (n=126)	18.4 (1.7, 600.0) (n=149)	20.0 (1.8, 932.7) (n=232)	19.4 (1.7, 435.9) (n=115)	16.5 (1.3, 494.9) (n=151)
Total protein $\times 10^{-2}$	16.0 (0.5, 56.0) (n=607)	18.0 (2.0, 118.0) (n=852)	18.0 (1.5, 146.0) (n=874)	18.0 (1.5, 94.0) (n=608)	18.0 (1.0, 272.0) (n=834)	18.0 (1.5, 217.0) (n=836)
N-acetyl-beta-glucosaminidase (NAG) $\times 10^{-2}$	96.8 (3.8, 771.0) (n=606)	136.8 (20.3, 1133.8) (n=852)	130.0 (10.6, 1112.8) (n=874)	98.9 (7.0, 866.7) (n=608)	106.9 (4.6, 1357.5) (n=834)	96.4 (19.3, 1400.3) (n=836)
Retinol-binding protein (RBP) $\times 10^{-1}$	151.0 (6.0, 1511.0) (n=606)	151.0 (2.0, 3339150.0) (n=836)	141.0 (5.5, 5154040.0) (n=862)	158.0 (3.5, 18847.0) (n=613)	158.0 (3.0, 125160.0) (n=822)	147.0 (2.0, 30838.0) (n=824)
Calbindin $\times 10^{-5}$	15.9 (0.1, 156.0) (n=226)	12.1 (0.1, 164.8) (n=126)	11.6 (0.1, 292.5) (n=149)	14.1 (0.1, 149.0) (n=232)	15.8 (0.2, 289.9) (n=115)	10.2 (0.1, 73.6) (n=151)
Tamm–Horsfall protein (THP) $\times 10^{-3}$	43.0 (0.2, 307.7) (n=226)	29.6 (0.1, 200.0) (n=126)	29.3 (0.3, 128.1) (n=149)	36.4 (0.1, 310.0) (n=232)	34.4 (0.2, 167.7) (n=115)	29.4 (0.2, 144.7) (n=151)
Trefoil factor 3 (TFF3) $\times 10^{-4}$	16.4 (4.1, 587.0) (n=226)	21.4 (4.4, 797.3) (n=126)	17.5 (4.9, 1761.2) (n=149)	18.6 (4.8, 912.5) (n=232)	20.6 (5.8, 806.5) (n=115)	16.9 (3.7, 388.1) (n=151)

Renal biomarkers	Deferasirox normalized by urinary creatinine, median (range), weight/weight			DFO normalized by urinary creatinine, median (range), weight/weight		
	Baseline	6 months	9 months	Baseline	6 months	9 months
Vascular endothelial growth factor (VEGF) $\times 10^{-8}$	29.3 (0.4, 642.5) (n=226)	25.5 (0.3, 330.3) (n=126)	23.7 (0.3, 315.9) (n=149)	32.6 (0.2, 282.0) (n=232)	26.7 (0.3, 263.3) (n=115)	23.5 (0.2, 372.3) (n=151)
Immunoglobulin G (IgG) $\times 10^{-2}$	79.0 (11.5, 859.0) (n=607)	84.5 (11.5, 1458.0) (n=852)	90.0 (11.5, 174570.0) (n=874)	79.0 (11.5, 757.0) (n=608)	90.0 (11.5, 3672.0) (n=834)	90.0 (11.5, 4328.0) (n=835)
Glutathione S-transferase alpha (GSTA) $\times 10^{-7}$	44.8 (2.8, 9523.8) (n=226)	28.3 (2.4, 16132.0) (n=126)	24.0 (2.7, 28,128.0) (n=149)	69.2 (3.0, 26,756.0) (n=232)	15.0 (3.5, 2983.1) (n=115)	18.2 (2.1, 6190.5) (n=151)

Abbreviation: DFO: Deferoxamine

Renal biomarkers	β-thalassemia normalized (×10^{-3}) by urinary creatinine median (range), weight/weight			Other anemias normalized (×10^{-3}) by urinary creatinine median (range), weight/weight		
	Baseline	6 months	24 months	Baseline	6 months	24 months
Clusterin ×10^{-6}	47.0 (0.2, 664.0) (n=75)	38.5 (4.0, 221.0) (n=27)	53.4 (0.8, 263.0) (n=13)	28.0 (0.3, 424.0) (n=70)	60.0 (0.6, 1570.0) (n=29)	19.4 (15.3, 137.0) (n=3)
Connective tissue growth factor (CTGF) ×10^{-8}	85.5 (24.5, 1710.0) (n=75)	100.0 (37.8, 500.0) (n=27)	127.0 (33.7, 657.0) (n=13)	119.0 (25.7, 3070.0) (n=70)	123.0 (40.1, 1180.0) (n=29)	144.0 (46.8, 342.0) (n=3)
Osteopontin ×10^{-4}	12.3 (0.1, 80.4) (n=75)	9.9 (0.8, 34.2) (n=27)	12.9 (0.2, 49.6) (n=13)	12.9 (1.1, 58.1) (n=70)	14.9 (1.5, 50.7) (n=29)	16.8 (13.2, 30.4) (n=3)
Tissue inhibitor of metalloproteinase-1 (TIMP-1) ×10^{-8}	104.0 (16.0, 730.0) (n=75)	95.2 (32.2, 393.0) (n=27)	205.0 (40.5, 1140.0) (n=13)	111.0 (24.6, 11,500.0) (n=70)	210.0 (34.8, 5280.0) (n=29)	331.0 (74.6, 632.0) (n=3)
Alpha-1-microglobulin (alpha1M) ×10^{-3}	10.0 (0.2, 68.4) (n=75)	11.7 (2.18, 100.0) (n=27)	23.3 (2.76, 53.7) (n=13)	3.4 (0.1, 32.2) (n=70)	13.2 (0.0, 50.0) (n=29)	6.8 (1.1, 14.4) (n=3)
Beta-2-microglobulin (beta2M) ×10^{-6}	80.6 (2.6, 522.0) (n=75)	149.0 (6.9, 33,300.0) (n=27)	138.0 (6.7, 5190.0) (n=13)	75.0 (4.1, 1130.0) (n=70)	113.0 (5.2, 9170.0) (n=29)	144.0 (21.6, 895.0) (n=3)
Cystatin C ×10^{-6}	54.4 (0.7, 209.0) (n=75)	61.3 (2.2, 396.0) (n=27)	63.0 (3.7, 321.0) (n=13)	50.0 (1.1, 149.0) (n=70)	76.9 (1.6, 1500) (n=29)	89.5 (33.1, 91.2) (n=3)
Kidney injury molecule-1 (KIM-1) ×10^{-8}	87.9 (0.4, 543.0) (n=75)	105.0 (5.1, 481.0) (n=27)	105.0 (8.8, 426.0) (n=13)	69.3 (0.7, 410.0) (n=70)	129.0 (0.5, 548.0) (n=29)	71.9 (35.1, 511.0) (n=3)
Microalbumin ×10^{-4}	89.4 (2.3, 946.0) (n=75)	128.0 (17.0, 778.0) (n=27)	109.0 (5.0, 4000.0) (n=13)	90.2 (3.0, 1160.0) (n=70)	133 (3.9, 1030.0) (n=29)	105.0 (75.4, 403.0) (n=3)
Neutrophil gelatinase-associated lipocalin (NGAL) ×10^{-6}	26.5 (1.8, 588.0) (n=75)	25.7 (4.6, 1110.0) (n=27)	31.5 (4.6, 207.0) (n=13)	33.3 (2.3, 480.0) (n=70)	47.6 (2.1, 237.0) (n=29)	44.7 (21.1, 878.0) (n=3)
Total protein ×10^{-2}	14.0 (3.0, 37.0) (n=178)	16.0 (2.0, 146.0) (n=244)	16.0 (3.0, 103.0) (n=232)	11.0 (1.0, 110.0) (n=210)	16.0 (1.0, 244.0) (n=268)	13.0 (1.0, 91.0) (n=249)
N-acetyl-beta-glucosaminidase (NAG) ×10^{-2}	101.0 (22.9, 548.0) (n=178)	118.0 (24.3, 881.0) (n=244)	120.0 (15.6, 741.0) (n=232)	64.2 (18.9, 433.0) (n=210)	97.5 (12.3, 1020.0) (n=268)	79.4 (9.4, 576.0) (n=249)
Retinol-binding protein (RBP) ×10^{-1}	137.0 (12.0, 1250.0) (n=174)	137.0 (6.0, 97,500.0) (n=241)	112.0 (5.0, 108,000.0) (n=230)	119.0 (8.0, 14,300.0) (n=207)	240.0 (8.0, 53,400.0) (n=259)	163.0 (7.0, 122,000.0) (n=244)
Calbindin ×10^{-5}	12.3 (0.2, 133.0) (n=75)	16.7 (0.3, 138.0) (n=27)	14.5 (1.4, 104.0) (n=13)	13.3 (0.0, 106.0) (n=70)	15.0 (0.2, 112.0) (n=29)	21.6 (20.9, 61.1) (n=3)
Tamm–Horsfall protein (THP) ×10^{-3}	34.9 (0.3, 165.0) (n=75)	34.8 (3.64, 144.0) (n=27)	46.5 (21.9, 130.0) (n=13)	24.2 (0.7, 144.0) (n=70)	30.8 (0.7, 85.0) (n=29)	44.2 (36.0, 64.9) (n=3)
Trefoil factor 3 (TFF3) ×10^{-4}	15.7 (3.9, 595.0) (n=75)	16.2 (6.1, 139.0) (n=27)	20.6 (5.4, 106.0) (n=13)	19.8 (3.9, 228.0) (n=70)	38.8 (6.5, 184.0) (n=29)	18.4 (7.6, 55.3) (n=3)
Vascular endothelial growth factor (VEGF) ×10^{-8}	34.2 (0.3, 180.0) (n=75)	25.5 (0.6, 117.0) (n=27)	49.5 (4.5, 99.8) (n=13)	26.5 (1.3, 1490.0) (n=70)	57.5 (1.4, 231.0) (n=29)	44.6 (25.9, 104.0) (n=3)

S2 Table: Renal protein biomarker levels (normalized) at baseline, 6 months, and 24 months in the non-comparative study.

| Renal biomarkers	β-thalassemia normalized ($\times 10^{-3}$) by urinary creatinine median (range), weight/weight			Other anemias normalized ($\times 10^{-3}$) by urinary creatinine median (range), weight/weight		
	Baseline	6 months	24 months	Baseline	6 months	24 months
Immunoglobulin G (IgG) $\times 10^{-2}$	90.0 (23.0, 6130.0) (n=178)	90.0 (23.0, 1160.0) (n=244)	102 .0 (23.0, 768.0) (n=232)	79.0 (11.0, 2260) (n=210)	90.0 (23.0, 2580.0) (n=268)	90.0 (23.0, 418.0) (n=248)
Glutathione S-transferase alpha (GSTA) $\times 10^{-7}$	44.4 (2.3, 9710.0) (n=75)	35.0 (5.9, 7900.0) (n=27)	183.0 (5.7, 3500.0) (n=13)	41.2 (3.5, 3150.0) (n=70)	44.2 (4.5, 7950.0) (n=29)	10.5 (4.3, 474.0) (n=3)

Abbreviation: DFO: Deferoxamine

S3 Table: AUC at baseline for renal protein biomarkers versus increased serum creatinine.				
	Comparative study		Non-comparative study	
	DFO (n)	Deferasirox (n)	β-thalassemia (n)	Other anemias (n)
Inflammatory biomarkers				
Clusterin	0.4990 (231)	0.5745 (225)	0.4685 (74)	0.5356 (67)
Connective tissue growth factor (CTGF)	0.4910 (231)	0.5621 (225)	0.5292 (74)	0.5216 (67)
Osteopontin	0.4783 (231)	0.5156 (225)	0.6032 (74)	0.4681 (67)
Tissue inhibitor of metalloproteinase-1 (TIMP-1)	0.4591 (231)	0.4621 (225)	0.6032 (74)	0.4226 (67)
Acute injury biomarkers				
Alpha-1-microglobulin (alpha1M)	0.5354 (231)	0.5180 (225)	0.4791 (74)	0.5994 (67)
Beta-2-microglobulin (beta2M)	0.5111 (231)	0.5701 (225)	0.4708 (74)	0.4719 (67)
Cystatin C	0.5661 (231)	0.4537 (225)	0.4870 (74)	0.4906 (67)
Kidney injury molecule-1 (KIM-1)	0.5394 (231)	0.5380 (225)	0.5954 (74)	0.5235 (67)
Microalbumin	0.4723 (231)	0.5504 (225)	0.4764 (74)	0.5023 (67)
Neutrophil gelatinase-associated lipocalin (NGAL)	0.5526 (231)	0.5366 (225)	0.5177 (74)	0.6229 (67)
Total protein	0.6330 (289)	0.5642 (294)	0.5761 (84)	0.4288 (93)
Others				
Calbindin	0.4810 (231)	0.5273 (225)	0.5910 (74)	0.5394 (67)
Glutathione S-transferase alpha (GSTA)	0.5228 (231)	0.5481 (225)	0.4232 (74)	0.6004 (67)
Immunoglobulin G (IgG)	0.5189 (289)	0.5575 (294)	0.4848 (84)	0.5232 (93)
N-acetyl-beta-glucosaminidase (NAG)	0.4743 (289)	0.4868 (294)	0.4659 (84)	0.4879 (93)

	Comparative study		Non-comparative study	
	DFO (n)	Deferasirox (n)	β-thalassemia (n)	Other anemias (n)
Retinol-binding protein (RBP)	0.4981 (289)	0.5130 (293)	0.6824 (83)	0.3974 (93)
Trefoil factor 3 (TFF3)	0.4149 (231)	0.5474 (225)	0.4137 (74)	0.4676 (67)
Tamm–Horsfall protein (THP)	0.5544 (231)	0.5057 (225)	0.5493 (74)	0.5568 (67)
Vascular endothelial growth factor (VEGF)	0.5336 (231)	0.5617 (225)	0.5091 (74)	0.5553 (67)

AUC: Area Under the Plasma Concentration–Time Curve; DFO: Deferoxamine

Figure S1: ROC curves for highest AUC baseline renal biomarkers versus increased serum creatinine. Comparative study: A) Clusterin (deferasirox arm), B) Total protein (DFO arm). Non-comparative study: C) Retinol-binding protein (RBP; β-thalassemia), D) Neutrophil gelatinase-associated lipocalin (NGAL; other anemias).
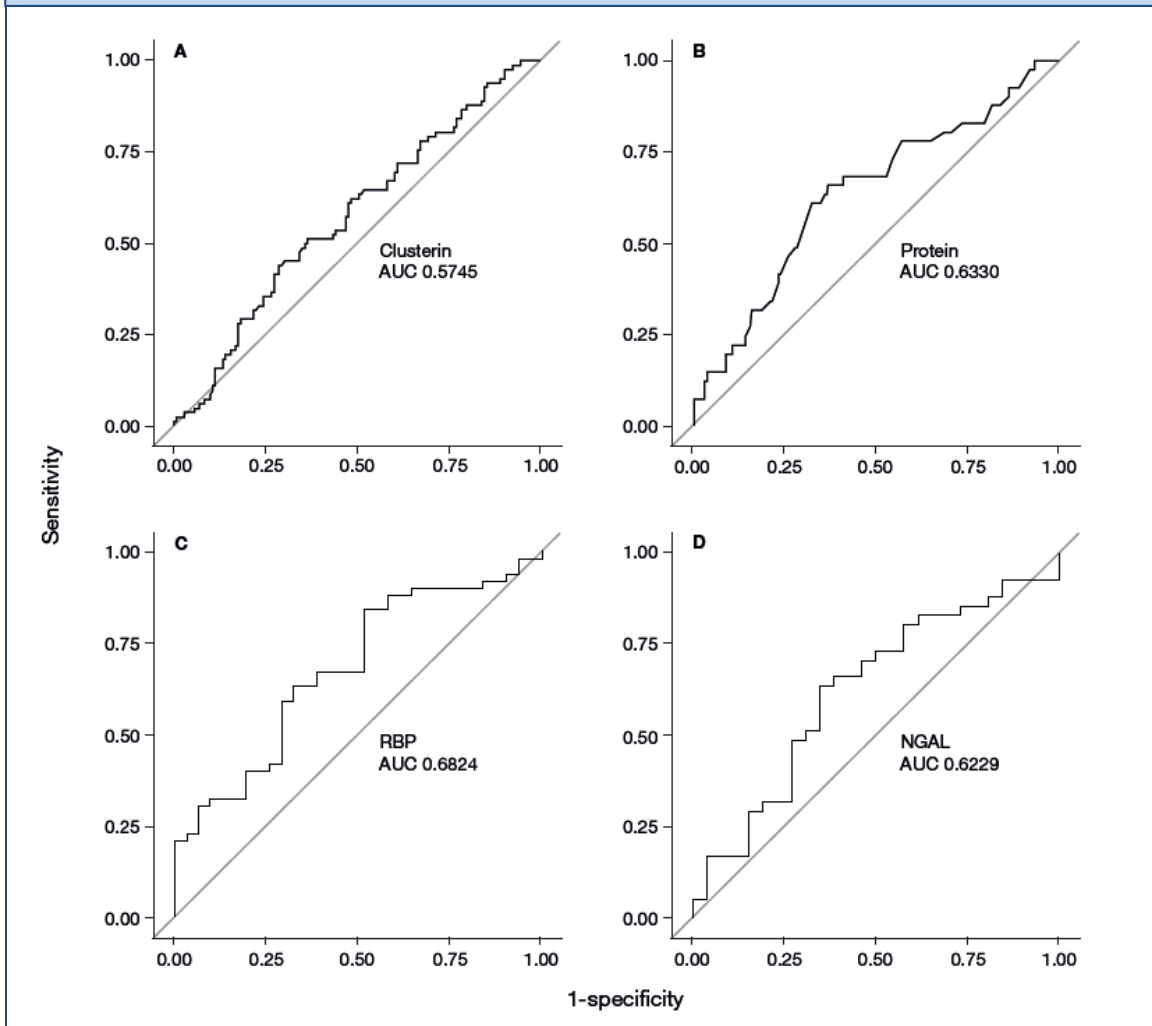
Note: high sensitivity values (eg, 90% = 0.9) and high specificity (eg, 90% = 1−0.9 = 0.1) relate to a high area under the ROC curve (AUC).

AUC: Area Under the Plasma Concentration–Time Curve; DFO: Deferoxamine; ROC: Receiver Operator Characteristic