Annals of Hematology (2022) 101:1941–1950
https://doi.org/10.1007/s00277-022-04915-7

ORIGINAL ARTICLE

# Frequency, pattern, and associations of renal iron accumulation in sickle/β-thalassemia patients

**Antonella Meloni[1,2] · Luigi Barbuto[3] · Laura Pistoia[1] · Vincenzo Positano[1,2] · Stefania Renne[4] · Giuseppe Peritore[5] · Priscilla Fina[6] · Anna Spasiano[7] · Massimo Allò[8] · Giuseppe Messina[9] · Tommaso Casini[10] · Antonella Massa[11] · Luigia Romano[3] · Alessia Pepe[12] · Filippo Cademartiri[1]**

Received: 1 April 2022 / Accepted: 4 July 2022 / Published online: 11 July 2022


## Abstract

We evaluated frequency, pattern, and associations of renal iron accumulation in sickle/β-thalassemia. Thirty-three sickle/β-thalassemia patients (36.5 ± 14.7 years; 13 females), 14 homozygous sickle cell disease (SCD) patients, and 71 thalassemia major (TM) patients, enrolled in the E-MIOT Network, underwent magnetic resonance imaging. Iron overload (IO) was quantified by the T2* technique. Sickle/β-thalassemia patients had a significantly lower frequency of renal IO (T2* < 31 ms) than homozygous SCD patients (9.1% vs. 57.1%; $P = 0.001$), besides having similar hepatic, cardiac and pancreatic IO. Kidney T2* values were comparable between regularly transfused sickle/β-thalassemia and TM patients but were significantly lower in regularly transfused homozygous SCD patients than in the other two groups. In sickle/β-thalassemia patients, global renal T2* values were not associated with age, gender, splenectomy, and presence of regular transfusions or chelation. No correlation was detected between renal T2* values and serum ferritin levels or iron load in the other organs. Global renal T2* values were not associated with serum creatinine levels but showed a significant inverse correlation with serum lactate dehydrogenase ($R = -0.709$; $P < 0.0001$) and indirect bilirubin ($R = -0.462$; $P = 0.012$). Renal IO is not common in sickle/β-thalassemia patients, with a prevalence significantly lower compared to that of homozygous SCD patients, but with a similar underlying mechanism due to the chronic hemolysis.

**Keywords** Sickle/β-thalassemia · Magnetic resonance imaging · Iron overload · Kidneys

✉ Filippo Cademartiri
fcademartiri@ftgm.it

[1] Department of Radiology, Fondazione G. Monasterio CNR-Regione Toscana, Via Moruzzi, 1 – 56124 Pisa, Italy

[2] U.O.C. Bioingegneria, Fondazione G. Monasterio CNR-Regione Toscana, Pisa, Italy

[3] U.O.C. Radiologia Generale E Di Pronto Soccorso, Azienda Ospedaliera Di Rilievo Nazionale "A. Cardarelli", Naples, Italy

[4] Struttura Complessa Di Cardioradiologia-UTIC, Presidio Ospedaliero "Giovanni Paolo II", Lamezia Terme, Italy

[5] Unità Operativa Complessa Di Radiologia, "ARNAS" Civico, Di Cristina Benfratelli, Palermo, Italy

[6] Unità Operativa Complessa Diagnostica Per Immagini, Ospedale "Sandro Pertini", Rome, Italy

[7] U.O.S.D. Malattie Rare del Globulo Rosso, Azienda Ospedaliera Di Rilievo Nazionale "A. Cardarelli", Naples, Italy

[8] Ematologia Microcitemia, Ospedale San Giovanni di Dio – ASP Crotone, Crotone, Italy

[9] Centro Microcitemie, Grande Ospedale Metropolitano "Bian chi-Melacrino-Morelli", Reggio Calabria, Italy

[10] Centro Talassemie Ed Emoglobinopatie, Ospedale "Meyer", Florence, Italy

[11] Servizio Trasfusionale, Ospedale "Giovanni Paolo II", Olbia, Italy

[12] Institute of Radiology, Department of Medicine, University of Padua, Padua, Italy

## Introduction

Sickle cell disease (SCD) and thalassemia syndromes represent the most common inherited disorders of hemoglobin. While SCD is a structural disorder characterized by the production of abnormal hemoglobin called hemoglobin S (HbS) [1], thalassemias are caused by a quantitative deficiency of structurally normal globin chains [2]. The prototypical, most common, and most severe form of SCD is the homozygous state for the HbS mutation, called sickle cell anemia (SCA), or HbSS [3]. However, SCD can occur also due to compound heterozygosity with other hemoglobin mutations. In sickle/β-thalassemia, the HbS is accompanied by either reduced (β +) or absent (β0) production of normal adult hemoglobin. It represents the most prevalent form of sickling syndromes among people of Mediterranean descent, due to the high frequency of the β-thalassemia trait [4]. The clinical phenotype of sickle/β-thalassemia is chronic hemolytic anemia that resembles mainly that of homozygous SCD, especially when the interacting β-thalassemia allele is β0 [5]. HbS/β + thalassemia is clinically heterogeneous, and it is usually associated with a milder clinical course [6].

In the current context of limited therapeutic strategies acting on the complex pathophysiology of the disease, transfusions can play a vital role in the treatment and prevention of both the acute and chronic complications of SCD [7]. Blood transfusions are not without immunologic and nonimmunologic complications, with iron overload representing a major drawback [8]. Besides transfusions, also the increased iron absorption due to ineffective erythropoiesis, a clinical feature of SCD patients [9], can disrupt the iron metabolism and cause excessive tissue iron accumulation. Since iron overload strongly contributes to increasing the morbidity and mortality of patients with sickle/β-thalassemia, its quantification is of utmost importance for a better patient management.

Magnetic resonance Imaging (MRI) using T2* technique allows the non-invasive, reproducible, and accurate iron content assessment in multiple organs [10–13]. While hepatic and cardiac T2* measurements are widely applied in the clinical routine of SCD and thalassemia patients [14, 15], the measurement of renal T2* is not standard or routinely available, although the kidneys constitute another site of iron accumulation. Hence, very limited information on renal T2* is available in the literature. Kidney iron deposition was found to be common in chronically transfused homozygous SCD patients but not in thalassemia major (TM) patients, and in both diseases, a significant association between renal T2* and hemolysis was detected [16, 17]. Moreover, it has been suggested that kidney iron deposition impairs renal glomerular and tubular functions in pediatric and adult patients with β-TM [18, 19]. Progressive renal failure is one of the main complications in sickle/β-thalassemia [20], making the identification of early markers of renal dysfunction and the understanding of the significance of iron overload in its development of great importance. However, no data are available about renal iron deposition in patients with sickle/β-thalassemia.

The aim of this multicenter study was to evaluate frequency, pattern, and associations of renal iron accumulation in sickle/β-thalassemia.

## Methods

### Study population

We retrospectively studied 33 sickle/β-thalassemia patients (13 females, 36.49 ± 14.72 years, age range: 9–61 years, 87.9% adults). Moreover, 14 homozygous SCD patients and 73 β-TM patients were included as comparison groups.

All patients were consecutively enrolled in the Extension-Myocardial iron Overload in Thalassemia (E-MIOT) network, constituted by 66 hematological or pediatric centers and 11 MRI sites where T2* MRI exams were performed using homogeneous, standardized, and validated procedures [21–23]. All centers were linked by a shared database, collecting all clinical, laboratory, and instrumental data.

The study complied with the Declaration of Helsinki and was approved by the ethical committees of all the MRI sites involved in the study. All patients gave written informed consent.

### Biochemical analysis

All laboratory investigations were performed as standard of care at the thalassemia centers wherein the patients were treated.

The average value of hemoglobin and ferritin levels over the last year prior to the MRI was considered, while the other laboratory parameters were measured once within 3 months from MRI.

Serum creatinine and uric acid were used as markers of renal function. Moreover, the estimated glomerular filtration rate (eGFR) was calculated by means of the Modification of Diet in Renal Disease (MDRD) formula [24]. Lactate dehydrogenase (LDH) served as a clinical marker for intravascular hemolysis and normal range was 120–250 U/l. Other hemolytic markers were increased reticulocytes, an indicator of marrow compensatory response, and increased unconjugated hyperbilirubinemia.

### MRI protocol

MRI was performed using conventional clinical 1.5 T scanners of three main vendors (GE Healthcare, Philips Healthcare, Siemens Healthineers) equipped with phased-array receiver surface coils.

Five or more axial slices covering the whole abdomen and including the kidneys and the pancreas [25, 26], a mid-transverse hepatic slice [27], and three parallel short-axis views (basal, medium, and apical) of the left ventricle (LV) [11, 21] were obtained by a T2* gradient–echo multiecho sequence.

T2* image analysis was performed using custom-written, previously validated software (HIPPO MIOT®) [28].

For each kidney, the slice in which the renal parenchyma was better represented was considered. A region of interest (ROI) was manually placed over the renal parenchyma, taking care to avoid areas affected by susceptibility artifacts from colonic intraluminal gas identified on images at later echo times. The barycenter of the ROI was computed, and the ROI was automatically divided in three regions: anterior, postero-lateral, and postero-medial. T2* values were calculated in the three ROIs and values were averaged to obtain a representative value for the kidney. The global kidney T2* value was calculated as average of T2* values in the two kidneys. The lower limit of normal for the global kidney T2* value was previously demonstrated to be 31 ms [25].

Hepatic T2* values were calculated in a circular region of interest [29] and were converted into liver iron concentration (LIC) using the Wood's calibration curve [30, 31]. A LIC higher than 3 mg/g dry weight indicated hepatic iron overload [32].

Three small regions of interest (ROIs) were manually drawn over the pancreatic head, body, and tail, encompassing parenchymal tissue and taking care to avoid large blood vessels or ducts and areas involved in susceptibility artefacts from gastric or colonic intraluminal gas [33]. Global pancreatic T2* value was calculated as the mean of T2* values from the three regions. Twenty-six ms was previously demonstrated to be the lowest threshold of normal T2* pancreatic value [26].

The myocardial T2* distribution was mapped into a 16-segment LV model, according to the AHA/ACC model [34]. The global heart T2* value was obtained by averaging all segmental T2* values. A T2* measurement > 20 ms was taken as "conservative" normal value [28, 35].

### Statistical analysis

All data were analyzed using SPSS version 27.0 statistical package.

Continuous variables were described as mean ± standard deviation and categorical variables were expressed as frequencies and percentages.

The normality of the distribution of the parameters was assessed by using the Kolmogorov–Smirnov test or the Shapiro–Wilk test for a sample size ≤ 50.

For continuous values with normal distribution, comparisons between groups were made by independent-samples *t*-test (for 2 groups) or one-way ANOVA (for more than 2 groups). Wilcoxon's signed rank test or Kruskal–Wallis test was applied for continuous values with non-normal distribution. Discrete variables were compared using the chi-square test or Fisher's exact probability test as appropriate. Bonferroni post hoc test was used for multiple comparisons between pairs of groups.

Correlation analysis was performed using Pearson's test or Spearman's test where appropriate.

In all tests, a 2-tailed probability value of 0.05 was considered statistically significant.

## Results

### Comparison between sickle/β-thalassemia and homozygous SCD patients

Table 1 shows the comparison between sickle/β-thalassemia and homozygous SCD patients. The two groups were homogeneous for age, sex, frequency of regular transfusion (> 4 transfusions per year), and chelation therapy but sickle/β-thalassemia patients were more frequently splenectomized. No difference was detected in terms of hepatic, pancreatic, and myocardial iron overload. Sickle/β-thalassemia patients showed significantly higher global kidney T2* values than homozygous SCD patients (Fig. 1A), with a frequency of a pathological global kidney T2* value about six times lower (9.1% vs 57.1%; $P = 0.001$).

### Comparison between regularly transfused sickle/β-thalassemia, homozygous SCD, and TM patients

Among the SCD patients, only those regularly transfused (23 sickle/β-thalassemia and 11 homozygous SCD) were selected and compared with transfusion-dependent TM patients. Key differences among the three patient populations are as follows (Table 2). Compared to TM patients, both sickle/β-thalassemia and homozygous SCD patients were started on regular transfusion therapy significantly later ($P < 0.0001$ and $P = 0.006$, respectively) and were less frequently chelated ($P = 0.009$ and $P = 0.048$, respectively). Splenectomy was significantly more frequent in sickle/β-thalassemia patients than in homozygous SCD patients

**Table 1** Demographic, clinical, and MRI data of SCD patients

| | Sickle/β-thalassemia ($N=33$) | Homozygous SCD ($N=14$) | $P$ |
|---|---|---|---|
| *Age (yrs)* | 36.49 ± 14.72 | 36.35 ± 14.70 | 0.975 |
| *Females, N (%)* | 13 (39.4) | 8 (57.1) | 0.263 |
| *Splenectomized, N (%)* | 20 (60.6) | 3 (21.4) | 0.024 |
| *Regularly transfused, N (%)* | 23 (69.7) | 11 (78.6) | 0.726 |
| *Chelated, N (%)* | 23 (69.7) | 10 (71.4) | 0.906 |
| *Mean serum hemoglobin (g/dl)* | 9.53 ± 0.90 | 9.72 ± 0.90 | 0.526 |
| *Mean serum ferritin (ng/ml)* | 771.75 ± 808.18 | 930.54 ± 766.30 | 0.591 |
| *Serum creatinine (mg/dl)* | 0.82 ± 1.12 | 0.64 ± 0.15 | 0.811 |
| *eGFR (ml/min/1.73 $m^2$)* | 140.24 ± 56.67 | 141.19 ± 38.34 | 0.874 |
| *Serum uric acid (mg/dl)* | 4.38 ± 1.47 | 4.77 ± 1.13 | 0.564 |
| *Serum lactate dehydrogenase (U/l)* | 603.93 ± 291.01 | 710.57 ± 395.73 | 0.674 |
| *MRI LIC values (mg/g dw)* | 5.13 ± 5.98 | 2.67 ± 2.17 | 0.340 |
| *Hepatic IO, N (%)* | 13 (39.4) | 3 (21.4) | 0.321 |
| *Global pancreas T2* values (ms)* | 30.06 ± 10.29 | 34.77 ± 8.36 | 0.138 |
| *Pancreatic IO, N (%)* | 11 (33.3) | 3 (21.4) | 0.503 |
| *Global heart T2* values (ms)* | 40.02 ± 4.69 | 42.05 ± 3.69 | 0.157 |
| *Myocardial IO, N (%)* | 0 (0.0) | 0 (0.0) | - |
| *Global kidney T2* values (ms)* | 43.19 ± 8.07 | 26.21 ± 17.07 | 0.002 |
| *Renal IO, N (%)* | 3 (9.1) | 8 (57.1) | 0.001 |

*SCD*, sickle cell disease; *N*, number; *eGFR*, estimated glomerular filtration rate; *MRI*, magnetic resonance imaging; *LIC*, liver iron concentration; *IO*, iron overload

($P=0.030$). SCD patients had significantly higher LDH values than TM patients ($P=0.036$).

Besides having comparable hepatic iron, sickle/β-thalassemia and homozygous SCD patients had significantly higher global pancreas T2* values than TM patients ($P<0.0001$ for both comparisons). Global kidney T2* values were significantly lower in homozygous SCD patients than in sickle/β-thalassemia patients ($P=0.009$) and TM patients ($P=0.006$) but were comparable between sickle/β-thalassemia patients and TM patients (Fig. 1B).

## Demographic and clinical correlates of kidney T2* in sickle/β-thalassemia

Our analysis was focused on sickle/β-thalassemia patients whose characteristics are summarized in Table 1. There was no significant difference between left and right kidney T2* values (43.28 ± 10.05 ms vs. 43.11 ± 9.09 ms; $P=0.492$).

Global kidney T2* values were not significantly different between males and females (42.85 ± 8.19 ms vs 43.73 ± 8 0.17 ms; $P=0.941$) and were not correlated with age ($R=-0.163$; $P=0.365$). Patients with and without splenectomy showed comparable global kidney T2* values (44.67 ± 6.37 ms vs. 40.93 ± 10.01 ms; $P=0.247$).

Out of the 23 regularly transfused patients, 15 received simple transfusions and 8 exchange transfusions. When compared to never or sporadically transfused patients, no significant difference was detected in terms of age (36.82 ± 15.85 years vs 35.73 ± 12.49 years; $P=0.784$), sex (47.8% vs 20.0%; $P=0.245$), and laboratory parameters. Regularly transfused patients were more frequently chelated (82.6% vs. 40.0%; $P=0.035$). Regularly transfused and non-transfused patients showed comparable MRI LIC values (5.48 ± 6.61 mg/g dw vs. 4.35 ± 4.44 mg/g dw; $P=0.638$) and global kidneys T2* values (44.44 ± 6.52 ms vs. 40.34 ± 10.71 ms; $P=0.368$). All the three patients with renal iron overload were not transfused and two of them were not chelated.

Out of the 23 chelated patients, 8 were treated with desferrioxamine (DFO), one with deferiprone (DFP), two with combined DFO + DFP, and 8 with deferasirox (DFX). No significant difference between non chelated and chelated patients was detected in MRI LIC values (3.83 ± 4.29 mg/g dw vs 5.70 ± 6.59 mg/g dw; $P=0.481$), global pancreas T2* values (31.78 ± 6.85 ms vs 29.32 ± 11.53 ms; $P=0.597$), global heart T2* values (38.48 ± 4.70 ms vs 40.68 ± 4.64 ms; $P=0.308$), and global kidneys T2* values (45.16 ± 9.60 ms vs 42.34 ± 7.38 ms; $P=0.273$).

Table 3 shows the correlation of global kidney T2* values with laboratory and MRI data. Global kidney T2*values did not correlate with serum creatinine or eGFR, while the correlation with uric acid was at the limits of the statistical significance. A significant inverse correlation was detected between global kidneys T2* values and LDH ($R=-0.709$;

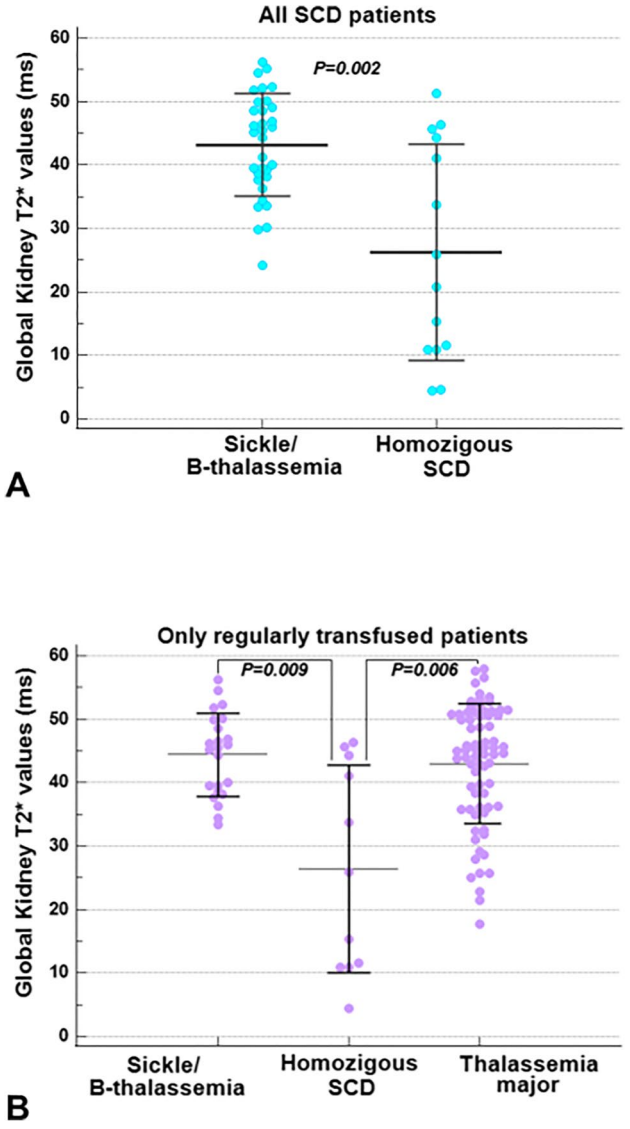


**Fig. 1** **A** Global kidney T2* values in sickle/β-thalassemia and homozygous SCD patients. **B** Global kidney T2* values in the subgroups of regularly transfused sickle/β-thalassemia, homozygous SCD, and thalassemia major patients

$P < 0.0001$) (Fig. 2A). Of note, all of the three patients with detectable renal iron overload had an elevated LDH and in two of them a LDH > 1000 U/l was detected. A normal LDH showed a negative predictive value of 100% for renal iron. Global kidney T2* values were not associated with reticulocyte count but showed a significant inverse correlation with indirect bilirubin (Fig. 2B).

No significant association was detected between serum ferritin levels and global kidney T2* values.

Global kidney T2* values were not correlated with iron deposition in the liver, the pancreas, and the heart. Out of the three patients with renal iron overload, only one also had hepatic iron overload.

## Discussion

We evaluated for the first time renal T2* values in sickle/β-thalassemia, demonstrating that cortical kidney iron deposition can occur in this population, but it is not so frequent, with a prevalence < 10%.

When we compared sickle/β-thalassemia patients with homozygous SCD patients homogeneous for age and sex, we found out that although the two groups were rather similar in respect to hepatic, pancreatic, and cardiac iron deposition, sickle/β-thalassemia patients had significantly less renal iron overload. Afterward, we selected only regularly transfused SCD patients, and the comparison was extended to thalassemia major patients. As expected, both multitransfused sickle/β-thalassemia and homozygous SCD patients begun transfusion therapy at a later age. However, they were also less frequently chelated since not only the use of transfusions but also the practice of chelation in SCD is variable [15, 36]. In the present study, we confirmed the disparity in terms of pancreatic iron overload between homozygous SCD and TM patients previously shown by Noetzli et al. and attributed to the different transfusional burden (entity and duration) and to the innate differences in iron handling and elimination [37]. In addition, we showed that also multitransfused sickle/β-thalassemia patients have less pancreatic iron overload than TM patients. However, in respect of renal iron deposition, our multitransfused sickle/β-thalassemia patients resulted rather similar to patients with TM, with both groups having significantly higher global kidney T2* values than homozygous SCD patients. Unlike iron deposition within the liver, spleen, and pancreas, renal iron loading is caused almost entirely by elevated circulating cell-free hemoglobin and heme species. Circulating free hemoglobin is generally maintained at low levels through scavenging by circulating haptoglobin. The stable and irreversible haptoglobin-hemoglobin complexes circulate in the plasma and are cleared by hepatic and reticuloendothelial macrophages [38]. This buffering system becomes defective in presence of chronic or massive hemolysis (as in SCD). Free hemoglobin binds to megalin and cubulin receptors and is taken up by endocytosis into the kidney where heme iron is extracted and deposited in proximal and distal convoluted tubules [39, 40]. This phenomenon is the basis of the typical cortical loading pattern detected by MRI [16]. The thalassemia acts on sickled red blood cells reducing the hemolysis, thus explaining our data. Indeed, both sickle/β-thalassemia and homozygous SCD patients had higher LDH values than TM patients (percentage difference 33% and 81%, respectively), although the statistical significance was reached only for the comparison versus homozygous SCD.

**Table 2** Comparison of demographic, clinical, and MRI findings among regularly transfused homozygous SCD, sickle/β-thalassemia, and thalassemia major patients

| | Regularly transfused sickle/β-thalassemia ($N=23$) | Regularly transfused homozygous SCD ($N=11$) | Thalassemia major ($N=73$) | $P$ |
|---|---|---|---|---|
| *Age (yrs)* | 36.82 ± 15.85 | 36.98 ± 15.87 | 35.65 ± 10.18 | 0.739 |
| *Females, N (%)* | 11 (47.8) | 7 (63.6) | 37 (50.7) | 0.673 |
| *Age at start of regular transfusions (months)* | 95.20 ± 152.52 | 148.80 ± 138.54 | 23.53 ± 56.67 | 0.001 |
| *Splenectomized, N (%)* | 15 (65.2) | 2 (18.2) | 36 (49.3) | 0.037 |
| *Chelated, N (%)* | 19 (82.6) | 9 (81.8) | 73 (100.0) | 0.001 |
| *Mean serum hemoglobin (g/dl)* | 9.57 ± 0.84 | 9.61 ± 0.80 | 9.74 ± 0.58 | 0.355 |
| *Mean serum ferritin (ng/ml)* | 859.56 ± 884.57 | 1125.10 ± 762.66 | 1549.61 ± 2340.14 | 0.101 |
| *Serum creatinine (mg/dl)* | 0.95 ± 1.34 | 0.65 ± 0.17 | 0.85 ± 0.85 | 0.839 |
| *eGFR (ml/min/1.73 m$^2$)* | 125.43 ± 55.89 | 137.03 ± 39.40 | 123.92 ± 53.91 | 0.599 |
| *Serum uric acid (mg/dl)* | 4.09 ± 1.06 | 4.87 ± 1.24 | 4.25 ± 1.23 | 0.328 |
| *Serum lactate dehydrogenase (U/l)* | 526.94 ± 231.53 | 718.00 ± 432.96 | 396.73 ± 191.92 | 0.011 |
| *MRI LIC values (mg/g dw)* | 5.48 ± 6.60 | 2.99 ± 2.35 | 8.01 ± 10.18 | 0.059 |
| *Hepatic IO, N (%)* | 9 (39.1) | 3 (27.3) | 41 (56.2) | 0.108 |
| *Global pancreas T2* values (ms)* | 28.43 ± 11.39 | 35.13 ± 8.15 | 9.05 ± 7.07 | < 0.0001 |
| *Pancreatic IO, N (%)* | 10 (43.5) | 2 (18.2) | 69 (94.5) | < 0.0001 |
| *Global heart T2* values (ms)* | 39.59 ± 5.05 | 41.39 ± 3.42 | 35.59 ± 10.61 | 0.106 |
| *Myocardial IO, N (%)* | 0 (0.0) | 0 (0.0) | 10 (13.7) | 0.077 |
| *Global kidney T2* values (ms)* | 44.44 ± 6.52 | 26.38 ± 16.32 | 43.01 ± 9.48 | 0.004 |
| *Renal IO, N (%)* | 0 (0.0) | 6 (54.5) | 9 (12.3) | < 0.0001 |

*SCD*, sickle cell disease; *N*, number; *eGFR*, estimated glomerular filtration rate; *MRI*, magnetic resonance imaging; *LIC*, liver iron concentration; *IO*, iron overload

**Table 3** Correlation of global kidney T2* values with laboratory and MRI parameters in sickle/β-thalassemia

| Variable | All patients ($N=33$) | | Only adult patients ($N=29$) | |
|---|---|---|---|---|
| | Correlation coefficient | $P$ | Correlation coefficient | $P$ |
| *Mean serum hemoglobin* | 0.312 | 0.077 | 0.277 | 0.145 |
| *Mean serum ferritin* | 0.162 | 0.367 | 0.222 | 0.247 |
| *Serum creatinine* | 0.052 | 0.778 | 0.072 | 0.717 |
| *eGFR* | −0.016 | 0.930 | −0.023 | 0.908 |
| *Serum uric acid* | −0.357 | 0.058 | −0.342 | 0.088 |
| *Serum lactate dehydrogenase* | −0.709 | < 0.0001 | −0.706 | < 0.0001 |
| *Reticulocyte count* | 0.320 | 0.118 | 0.342 | 0.119 |
| *Indirect bilirubin* | −0.462 | 0.012 | −0.417 | 0.034 |
| *MRI LIC values* | 0.094 | 0.603 | 0.101 | 0.602 |
| *Global pancreas T2* values* | 0.010 | 0.954 | −0.099 | 0.609 |
| *Global heart T2* values* | −0.095 | 0.600 | −0.025 | 0.897 |

*eGFR*, estimated glomerular filtration rate; *MRI*, magnetic resonance imaging; *LIC*, liver iron concentration

We evaluated the correlates of kidney T2* values among sickle/β-thalassemia patients.

As for healthy subjects [25], homozygous SCD [16, 41], and TM patients [18, 42], no correlation was found between renal T2* and age or gender. The finding of comparable renal T2* values between regularly transfused and never or sporadically transfused patients further confirms that in SCD transfusional iron is not the dominant causative agent for kidney iron deposition. Of note, no difference between the two subgroups was detected in MRI LIC values, likely because transfused patients were also more frequently chelated.

No correlation was found between global kidney T2* values and serum creatinine or eGFR. Although universally

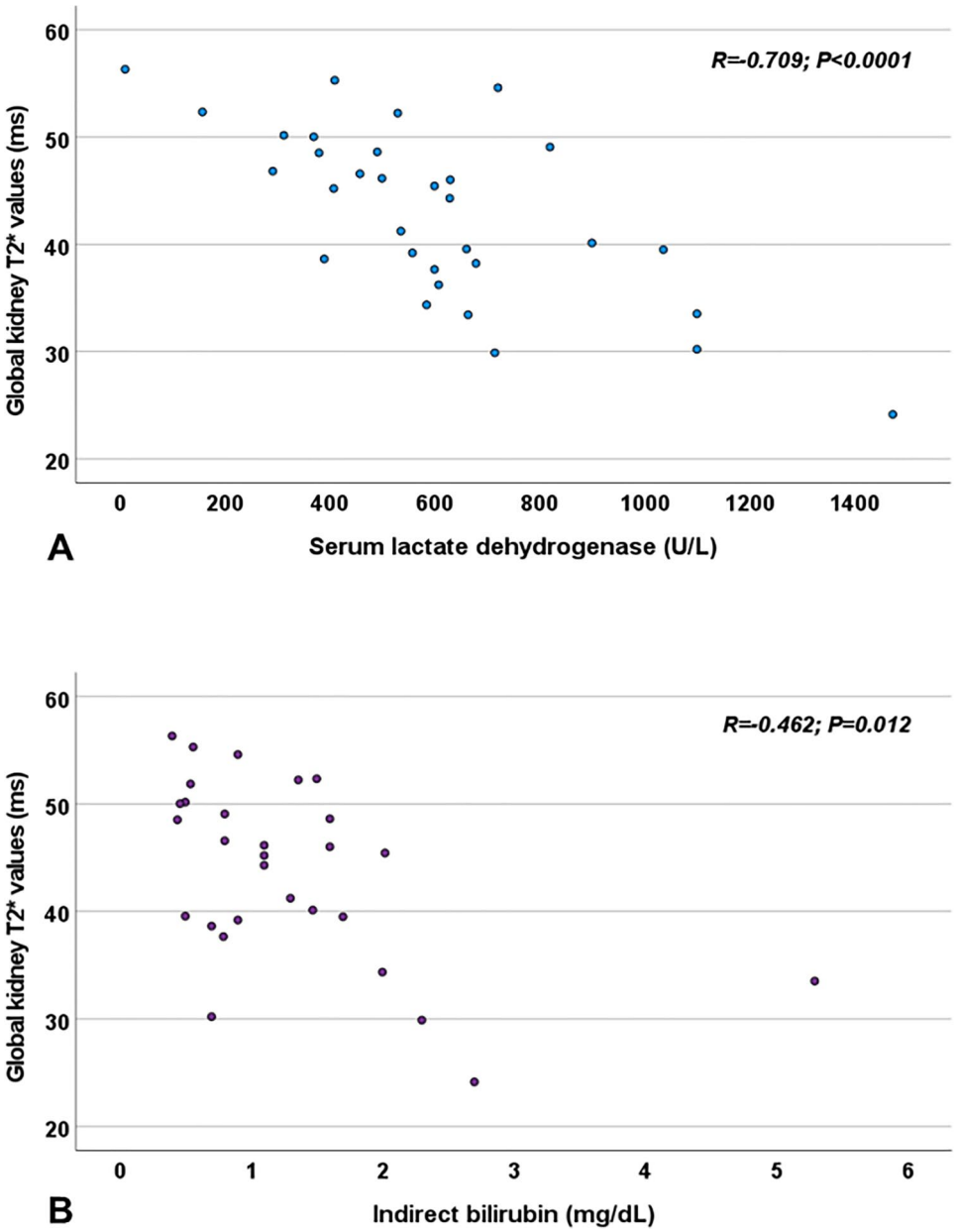


**Fig. 2** Global Kidney T2* as a function of LDH (**A**) and indirect bilirubin (**B**) in sickle/β-thalassemia patients

used, serum creatinine is an imperfect GFR biomarker, not very sensitive [43]. Moreover, its concentration can be influenced by several extra-renal factors and by different treatments. Unfortunately, we did not measure in our population cystatin C and β2-microglobulin, demonstrated to be more robust and sensitive early biomarkers of glomerular and tubular dysfunction than creatinine [44]. In TM patients, a significant negative correlation between serum cystatin C and renal T2* was detected [42]. So, also taking into account the limited number of patients with detectable iron overload, our study cannot rule out a direct, toxic role for kidney iron.

The inverse correlation between kidney T2* and LDH as well as indirect bilirubin values and the negative predictive value of 100% of a normal LDH for renal iron detected in our study further strengthens the role of hemolysis on renal iron overload in sickle/β-thalassemia. The absence of an association between kidney T2* values and reticulocyte count may be explained by the fact that, although increased reticulocyte count is a criterion for hemolysis, it is not specific for hemolysis [45].

In line with previous studies on homozygous SCD [16] and TM patients [18], we found out that in sickle/β-thalassemia renal iron overload cannot be estimated by serum ferritin levels. We failed to detect an association between renal T2* values and MRI LIC values, pancreatic or cardiac T2* values, mainly due to the different mechanisms and kinetics of iron uptake, storage, and clearance among the organs [17, 46]. Moreover, as in thalassemia,

renal iron toxicity might occur at concentrations below MRI detection limits. Anyway, our results suggest that in sickle/β-thalassemia the most reliable approach for evaluating renal iron overload is to image directly the kidney, without relying on the total body iron load or iron levels in other organs.

## Limitations

This study has some limitations.

The main limitation is the relatively small sample size.

Our T2* sequence is designed to accurately quantify high levels of tissue iron but it is less sensitive in detecting changes associated with mild or early iron overload.

We did not strictly divide regions of interest in the kidney into cortex and medulla because partial volume effects made this distinction subjective.

More sophisticated acquisition techniques such as three dimensional or Dixon acquisitions may be necessary to help correct for susceptibility artifacts.

TM patients begun transfusion therapy at an earlier age and received higher transfusion volumes than SCD patients. So, we could not "match" the SCD and TM populations for the cumulative transfusional iron to maintain a comparable age.

We did not quantify serum cystatin C and β2-microglobulin or urinary albumin/creatinine ratio as sensitive markers of renal function. Future studies including these parameters may help to better clarify the clinical significance of renal iron deposition.

## Conclusions

Renal iron deposition is not common in sickle/β-thalassemia patients, with a prevalence significantly lower compared to that of homozygous SCD patients. Kidney iron results from chronic hemolysis and is uncorrelated to iron levels in the liver, pancreas, and heart, suggesting that performing MRI T2* in other organs is not a reliable approach to predict the exact renal iron state. Prospective studies are needed to determine if kidney iron deposition impairs renal function.

**Acknowledgements** We would like to thank all the colleagues involved in the E-MIOT project (https://emiot.ftgm.it/). We thank Silvia Miconi for her skillful secretarial work and all patients for their cooperation.

**Author contribution** A. M. designed the study, analyzed the data, and drafted the initial manuscript. L. B. analyzed the images. L. P. was responsible for data collection. V. P. developed the software for image analysis and assisted with the methods. S. R., G. P., P. F., A. S., M. A., G. M., T. C., A. M., and L. R. collected the data. A. P. contributed to the design and supervision of the study. F. C. is the guarantor of this work. All authors assisted with interpretation, commented on drafts of the manuscript, and approved the final version.

**Funding** The E-MIOT project receives "no-profit support" from industrial sponsorships (Chiesi Farmaceutici S.p.A. and Bayer). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## Declarations

**Ethical approval** The study complied with the Declaration of Helsinki. The institutional review board (Azienda Ospedaliero-Universitaria Pisana) approved this study (protocol number 56664; date of approval: October 8, 2015).

**Informed consent** All patients gave written informed consent to the protocol.

**Conflicts of interest** The authors declare no competing interests..

## References

1. Bunn HF (1997) Pathogenesis and treatment of sickle cell disease. N Engl J Med 337(11):762–769
2. Weatherall DJ (1997) The thalassaemias Bmj 314(7095):1675–1678
3. da Guarda CC, Yahouedehou S, Santiago RP, Neres J, Fernandes CFL, Aleluia MM, Figueiredo CVB, Fiuza LM, Carvalho SP, Oliveira RM, Fonseca CA, Ndidi US, Nascimento VML, Rocha LC, Goncalves MS (2020) Sickle cell disease: a distinction of two most frequent genotypes (HbSS and HbSC). PLoS One 15(1):e0228399. https://doi.org/10.1371/journal.pone.0228399
4. Adekile AD, Akbulut N, Azab AF, Al-Sharida S, Thomas D (2017) The Sickle beta-thalassemia phenotype. J Pediatr Hematol Oncol 39(5):327–331. https://doi.org/10.1097/MPH.0000000000000747
5. Weatherall DJ (2007) Current trends in the diagnosis and management of haemoglobinopathies. Scand J Clin Lab Invest 67(1):1–2. https://doi.org/10.1080/00365510601051532
6. Benites BD, Bastos SO, Baldanzi G, Dos Santos AO, Ramos CD, Costa FF, Gilli SC, Saad ST (2016) Sickle cell/beta-thalassemia: comparison of Sbeta(0) and Sbeta(+) Brazilian patients followed at a single institution. Hematology 21(10):623–629. https://doi.org/10.1080/10245332.2016.1187843
7. Salinas Cisneros G, Thein SL (2020) Recent advances in the treatment of sickle cell disease. Front Physiol 11:435. https://doi.org/10.3389/fphys.2020.00435
8. Wanko SO, Telen MJ (2005) Transfusion management in sickle cell disease. Hematol Oncol Clin North Am 19(5):803–826. https://doi.org/10.1016/j.hoc.2005.07.002
9. El Hoss S, Cochet S, Godard A, Yan H, Dussiot M, Frati G, Boutonnat-Faucher B, Laurance S, Renaud O, Joseph L, Miccio A, Brousse V, Mohandas N, El Nemer W (2020) Fetal hemoglobin rescues ineffective erythropoiesis in sickle cell disease. Haematologica. https://doi.org/10.3324/haematol.2020.265462
10. Anderson LJ, Holden S, Davis B, Prescott E, Charrier CC, Bunce NH, Firmin DN, Wonke B, Porter J, Walker JM, Pennell DJ (2001) Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. Eur Heart J 22(23):2171–2179
11. Meloni A, Positano V, Pepe A, Rossi G, Dell'Amico M, Salvatori C, Keilberg P, Filosa A, Sallustio G, Midiri M, D'Ascola D, Santarelli MF, Lombardi M (2010) Preferential patterns of myocardial

iron overload by multislice multiecho T*2 CMR in thalassemia major patients. Magn Reson Med 64(1):211–219
12. Wood JC (2007) Magnetic resonance imaging measurement of iron overload. Curr Opin Hematol 14(3):183–190
13. Pepe A, Pistoia L, Gamberini MR, Cuccia L, Peluso A, Messina G, Spasiano A, Allo M, Bisconte MG, Putti MC, Casini T, Dello Iacono N, Celli M, Vitucci A, Giuliano P, Peritore G, Renne S, Righi R, Positano V, De Sanctis V, Meloni A (2020) The close link of pancreatic iron with glucose metabolism and with cardiac complications in thalassemia major: a large, multicenter observational study. Diabetes Care 43(11):2830–2839
14. Pennell DJ, Udelson JE, Arai AE, Bozkurt B, Cohen AR, Galanello R, Hoffman TM, Kiernan MS, Lerakis S, Piga A, Porter JB, Walker JM, Wood J (2013) Cardiovascular function and treatment in beta-thalassemia major: a consensus statement from the American Heart Association. Circulation 128(3):281–308
15. Coates TD, Wood JC (2017) How we manage iron overload in sickle cell patients. Br J Haematol 177(5):703–716. https://doi.org/10.1111/bjh.14575
16. Schein A, Enriquez C, Coates TD, Wood JC (2008) Magnetic resonance detection of kidney iron deposition in sickle cell disease: a marker of chronic hemolysis. J Magn Reson Imaging 28(3):698–704
17. Denton CC, Detterich JA, Coates TD, Wood JC (2021) Kidney iron deposition by R2* is associated with haemolysis and urinary iron. Br J Haematol 193(3):633–636
18. ElAlfy MS, Khalil Elsherif NH, Ebeid FSE, Ismail EAR, Ahmed KA, Darwish YW, Ibrahim AS, Elghamry IRF, Shokrey NA, Alajeil DN (2018) Renal iron deposition by magnetic resonance imaging in pediatric beta-thalassemia major patients: relation to renal biomarkers, total body iron and chelation therapy. Eur J Radiol 103:65–70. https://doi.org/10.1016/j.ejrad.2018.04.007
19. Koliakos G, Papachristou F, Koussi A, Perifanis V, Tsatra I, Souliou E, Athanasiou M (2003) Urine biochemical markers of early renal dysfunction are associated with iron overload in beta-thalassaemia. Clin Lab Haematol 25(2):105–109
20. Voskaridou E, Terpos E, Michail S, Hantzi E, Anagnostopoulos A, Margeli A, Simirloglou D, Loukopoulos D, Papassotiriou I (2006) Early markers of renal dysfunction in patients with sickle cell/beta-thalassemia. Kidney Int 69(11):2037–2042. https://doi.org/10.1038/sj.ki.5000248
21. Pepe A, Positano V, Santarelli F, Sorrentino F, Cracolici E, De Marchi D, Maggio A, Midiri M, Landini L, Lombardi M (2006) Multislice multiecho T2* cardiovascular magnetic resonance for detection of the heterogeneous distribution of myocardial iron overload. J Magn Reson Imaging 23(5):662–668
22. Ramazzotti A, Pepe A, Positano V, Rossi G, De Marchi D, Brizi MG, Luciani A, Midiri M, Sallustio G, Valeri G, Caruso V, Centra M, Cianciulli P, De Sanctis V, Maggio A, Lombardi M (2009) Multicenter validation of the magnetic resonance t2* technique for segmental and global quantification of myocardial iron. J Magn Reson Imaging 30(1):62–68
23. Meloni A, De Marchi D, Pistoia L, Grassedonio E, Peritore G, Preziosi P, Restaino G, Righi R, Riva A, Renne S, Schicchi N, Vallone A, Peluso A, Gerardi C, Positano V, Pepe A (2019) Multicenter validation of the magnetic resonance T2* technique for quantification of pancreatic iron. Eur Radiol 29(5):2246–2252
24. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D (1999) A more accurate method to estimate glomerular filtration rate from serum creatinine a new prediction equation Modification of Diet in Renal Disease Study Group. Ann Intern Med 130(6):461–470
25. Grassedonio E, Meloni A, Positano V, De Marchi D, Toia P, Midiri M, Pepe A (2015) Quantitative T2* magnetic resonance imaging for renal iron overload assessment: normal values by age and sex. Abdom Imaging 40:1700–1704
26. Restaino G, Meloni A, Positano V, Missere M, Rossi G, Calandriello L, Keilberg P, Mattioni O, Maggio A, Lombardi M, Sallustio G, Pepe A (2011) Regional and global pancreatic T*(2) MRI for iron overload assessment in a large cohort of healthy subjects: Normal values and correlation with age and gender. Magn Reson Med 65(3):764–769
27. Positano V, Salani B, Pepe A, Santarelli MF, De Marchi D, Ramazzotti A, Favilli B, Cracolici E, Midiri M, Cianciulli P, Lombardi M, Landini L (2009) Improved T2* assessment in liver iron overload by magnetic resonance imaging. Magn Reson Imaging 27(2):188–197
28. Positano V, Pepe A, Santarelli MF, Scattini B, De Marchi D, Ramazzotti A, Forni G, Borgna-Pignatti C, Lai ME, Midiri M, Maggio A, Lombardi M, Landini L (2007) Standardized T2* map of normal human heart in vivo to correct T2* segmental artefacts. NMR Biomed 20(6):578–590
29. Meloni A, Luciani A, Positano V, De Marchi D, Valeri G, Restaino G, Cracolici E, Caruso V, Dell'amico MC, Favilli B, Lombardi M, Pepe A (2011) Single region of interest versus multislice T2* MRI approach for the quantification of hepatic iron overload. J Magn Reson Imaging 33(2):348–355
30. Wood JC, Enriquez C, Ghugre N, Tyzka JM, Carson S, Nelson MD, Coates TD (2005) MRI R2 and R2* mapping accurately estimates hepatic iron concentration in transfusion-dependent thalassemia and sickle cell disease patients. Blood 106(4):1460–1465
31. Meloni A, Rienhoff HY Jr, Jones A, Pepe A, Lombardi M, Wood JC (2013) The use of appropriate calibration curves corrects for systematic differences in liver R2* values measured using different software packages. Br J Haematol 161(6):888–891
32. Angelucci E, Brittenham GM, McLaren CE, Ripalti M, Baronciani D, Giardini C, Galimberti M, Polchi P, Lucarelli G (2000) Hepatic iron concentration and total body iron stores in thalassemia major. N Engl J Med 343(5):327–331
33. Meloni A, De Marchi D, Positano V, Neri MG, Mangione M, Keilberg P, Lendini M, Cirotto C, Pepe A (2015) Accurate estimate of pancreatic T2* values: how to deal with fat infiltration. Abdom Imaging 40(8):3129–3136
34. Cerqueira MD, Weissman NJ, Dilsizian V, Jacobs AK, Kaul S, Laskey WK, Pennell DJ, Rumberger JA, Ryan T, Verani MS (2002) Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart: a statement for healthcare professionals from the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association. Circulation 105(4):539–542
35. Meloni A, Maggio A, Positano V, Leto F, Angelini A, Putti MC, Maresi E, Pucci A, Basso C, Marra MP, Pistoia L, de Marchi D, Pepe A (2020) CMR for myocardial iron overload quantification: calibration curve from the MIOT network. Eur Radiol 29(5):2246–2252
36. Vichinsky EP, Ohene-Frempong K (2011) Approaches to transfusion therapy and iron overload in patients with sickle cell disease: results of an international survey. Pediatr Hematol Oncol 28(1):37–42
37. Noetzli LJ, Coates TD, Wood JC (2011) Pancreatic iron loading in chronically transfused sickle cell disease is lower than in thalassaemia major. Br J Haematol 152(2):229–233
38. Kristiansen M, Graversen JH, Jacobsen C, Sonne O, Hoffman HJ, Law SK, Moestrup SK (2001) Identification of the haemoglobin scavenger receptor. Nature 409(6817):198–201. https://doi.org/10.1038/35051594
39. Gburek J, Verroust PJ, Willnow TE, Fyfe JC, Nowacki W, Jacobsen C, Moestrup SK, Christensen EI (2002) Megalin and cubilin are endocytic receptors involved in renal clearance of hemoglobin. J Am Soc Nephrol 13(2):423–430. https://doi.org/10.1681/ASN.V132423

40. Kovtunovych G, Eckhaus MA, Ghosh MC, Ollivierre-Wilson H, Rouault TA (2010) Dysfunction of the heme recycling system in heme oxygenase 1-deficient mice: effects on macrophage viability and tissue iron distribution. Blood 116(26):6054–6062. https://doi.org/10.1182/blood-2010-03-272138
41. Wood JC, Cohen AR, Pressel SL, Aygun B, Imran H, Luchtman-Jones L, Thompson AA, Fuh B, Schultz WH, Davis BR, Ware RE, Investigators TW (2016) Organ iron accumulation in chronically transfused children with sickle cell anaemia: baseline results from the TWiTCH trial. Br J Haematol 172(1):122–130. https://doi.org/10.1111/bjh.13791
42. Hashemieh M, Azarkeivan A, Akhlaghpoor S, Shirkavand A, Sheibani K (2012) T2-star (T2*) magnetic resonance imaging for assessment of kidney iron overload in thalassemic patients. Arch Iran Med 15 (2):91-94. doi:012152/AIM.009
43. Waikar SS, Betensky RA, Emerson SC, Bonventre JV (2012) Imperfect gold standards for kidney injury biomarker evaluation. J Am Soc Nephrol 23(1):13–21. https://doi.org/10.1681/ASN.2010111124
44. Behairy OG, AbdAlmonaem ER, Abed NT, Abdel Haiea OM, Zakaria RM, AbdEllaty RI, Asr EH, Mansour AI, Abdelrahman AM, Elhady HA (2017) Role of serum cystatin-C and beta-2 microglobulin as early markers of renal dysfunction in children with beta thalassemia major. Int J Nephrol Renovasc Dis 10:261–268. https://doi.org/10.2147/IJNRD.S142824
45. Barcellini W, Fattizzo B (2015) Clinical applications of hemolytic markers in the differential diagnosis and management of hemolytic anemia. Dis Markers 2015:635670. https://doi.org/10.1155/2015/635670
46. Noetzli LJ, Carson SM, Nord AS, Coates TD, Wood JC (2008) Longitudinal analysis of heart and liver iron in thalassemia major. Blood 112(7):2973–2978