Longitudinal changes in liver fibrosis in children with sickle cell disease undergoing chronic transfusion therapy

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Abstract

Background and study aims : **The progression of liver injury from transfusional iron overload in sickle cell disease (SCD) is poorly understood. We sought to identify predictors liver fibrosis development over time.**

Patients and methods : **We performed a retrospective cohort study of chronically transfused SCD patients who had ≥ 2 serial liver biopsies. Core biopsies were scored for fibrosis in a blinded fashion. Primary analyses evaluated longitudinal changes in liver fibrosis and changes in surrogate markers. Secondary analyses determined the relationship between liver iron concentration (LIC) and serum biomarkers.**

Results : **26 people had ≥ 2 serial biopsies for evaluation (n = 70 biopsies total). Fibrosis was Ishak grade 0 or 1 in all biopsies. Evaluation of the first 2 biopsies showed fibrosis regression (n = 6), development (n = 2), persistence (n = 1), and absence (n = 17). There was no consistent association of fibrosis with LIC over time, or between changes in fibrosis status and surrogate markers. For predicting fibrosis on a cross-sectional basis, ALT and ferritin performed moderately (AUCs 0.80 and 0.63, respectively) but LIC performed poorly (AUC 0.30). The highest positive likelihood ratios for fibrosis were for ferritin cutoff of 5000 ng/mL (LR + 5.7) and ALT cutoff of 65 U/L (LR + 5.2).**

Conclusions : **Liver fibrosis progression is minimal in chronically transfused SCD. LIC does not correlate well with fibrosis development. We propose routine liver biopsies are not necessary components in the standard monitoring of chronically transfused SCD patients.** (Acta gastroenterol. belg., **2012**, 75, **419-424**).

Key words : pediatric, liver, iron overload, sickle, fibrosis.

Introduction

Chronic transfusion therapy for patients with sickle cell disease (SCD) is beneficial in selected circumstances such as ischemic cerebrovascular disease (1). Unfortunately, subsequent iron overload in the liver and liver fibrosis are a source of morbidity in patients who receive chronic transfusions, especially for children who may ultimately receive decades of regular transfusion (2). Patients with SCD are already subject to increased iron turnover, absorption and systemic circulation (3). Regular blood transfusions may double this rate of iron accumulation (4).

Serial liver biopsy in the setting of chronic transfusion serves two purposes : evaluating liver injury and quantifying hepatic iron load (5). Liver biopsy has been the gold standard for both purposes. The liver is unique compared to other organs in that the degree of iron content directly correlates with total body iron (6). However, liver biopsy places the patient at risk for complications such as bleeding, perforation of lung, gallbladder, or intestine, and infection. Magnetic resonance imaging (MRI) and superconducting quantum interference device (SQUID) are validated, noninvasive modalities for evaluating liver iron concentration (LIC) (7-9) and they are used as surrogate measures of whole body iron load ; however, these methods do not provide information about hepatic fibrosis. Furthermore, iron-related hepatic fibrosis may be a marker for injury in other organs.

Iron overload leads to increased storage of iron in ferritin and hemosiderin. Subsequently, the predictive value of serum ferritin levels for liver iron overload and fibrosis has been assessed in several studies. However, ferritin does not have sufficient accuracy to be useful diagnostically (10-14). This is in part due to the fact that serum ferritin levels are affected by other factors, including infection, inflammation, liver disease, ascorbate deficiency, or ineffective erythropoiesis (12).

Iron-mediated hepatic injury is a time-dependent process ; to date no study has examined longitudinal changes in quantitative liver iron and fibrosis in patients with SCD receiving chronic transfusions. The primary aim of this study is to evaluate longitudinal changes in liver fibrosis and biomarkers related to fibrosis among chronically transfused people with SCD. Secondary aims are to determine how quantitative liver iron and biomarkers related to liver iron correlate with fibrosis.

Patients and methods

Human subjects

All subjects with sickle cell anemia who were chronically transfused at Johns Hopkins Hospital any point from 1992-2010 and had one or more liver biopsy were identified. These patients were confirmed to be HBV and HCV negative. Archived liver biopsy slides were evaluated in people with two or more core liver biopsies. It

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was general practice for pediatric hematologists to recommend a liver biopsy within 1-2 years of starting chronic transfusion therapy. Subsequent biopsy intervals varied based on multiple factors, including chelation status, ferritin trends, initial biopsy results, and patient acceptance of biopsy procedures. The IRB of Johns Hopkins University School of Medicine approved this study.

Clinical and laboratory data

Biopsy slides were stained with standard Masson's trichrome technique and Prussian blue at the time of clinical acquisition. After collecting all archived samples, scoring was performed by a hepatopathologist (RA) who was blinded to the subject name and could not link serial biopsies to an individual. Scoring for fibrosis used the method of Ishak (15), and histologic iron score (HIS) summarizing the distribution and intensity of iron staining used the method of Deugnier (16).

Laboratory, transfusion, and chelation data were extracted from the electronic medical record, and all data were validated against paper transfusion records. Transfusion was performed using one of three methods : 1) simple transfusion of 10-15 cc/kg, 2) partial exchange transfusion in which approximately 5-7 cc/kg is phlebotomized immediately prior to transfusion of 10-15 cc/kg in order to reduce net iron loading, and 3) erythrocytapheresis, which is an automated euvolemic red cell exchange that typically results in negligible iron loading. Only certain patients with appropriate vascular access are candidates for erythrocytapheresis. Net transfusion volume was calculated as volume transfused minus volume of therapeutic phlebotomy at each transfusion.

Erythrocytapheresis was assumed to have a net transfusion volume of zero. Clinical lab data were recorded in the units measured. In order to reduce variability and the influence of isolated ferritin values, ferritin used in analysis was an average of the three closest values within six weeks before and after the biopsy. Iron concentration of the liver biopsy core was determined by inductively coupled plasma mass spectrometry.

Statistics

The overall analysis plan was to describe the pattern of progression of hepatic fibrosis and to correlate blood and liver biopsy markers with changes in fibrosis. Summary data are presented as mean ± SD or median with interquartile range (IQR) depending on the distribution of data. Paired differences between first and second biopsies were evaluated using the Wilcoxon signed rank test. Tests for differences among any group utilized the Kruskal-Wallis test. Longitudinal changes in biomarkers with LIC were estimated with simple linear regression, and correlations were evaluated by calculating Pearson's r. Diagnostic performance of biomarkers to assess for fibrosis was evaluated with area under receiver operator characteristic curve (AUC) analysis and likelihood ratios for positive tests (LR+). Statistics were calculated using Stata v11.2. (StataCorp, College Station, TX).

Results

Patient and biopsy characteristics

There were 26 subjects who had at least two biopsies available for analysis. A total of 70 biopsies were analyzed ; 52 biopsies (26 pairs comprised of the first and second biopsies) were included in the primary analysis. The clinical and transfusion characteristics are shown in Table 1. The most common indications for chronic transfusion were CNS and pulmonary disease. Chelation therapy was used in 15 of 26 subjects (58%) at the time of first biopsy and 25 of 26 subjects (96%) at the time of second biopsy. Deferoxamine and deferasirox were the two chelation agents used. Initiation of chelation therapy was individualized and depended on ferritin trends and patient and family acceptance of therapy. Among subjects receiving chelation, deferoxamine was used in 14 of 15 subjects (93%) at first biopsy and 14 of 25 subjects (56%) at second biopsy.

Of these 52 biopsies in the primary analysis, trichrome staining for fibrosis was available on all specimens (Table 2). LIC was available on 51 of 52 biopsies. Forty-four had both iron staining for histologic iron

Table 1. — **Patient characteristics of children with SCD and ≥ 2 liver biopsies (n = 26)**

Median age at transfusion start, years (range)	6.7 (2.0-16.3)
Male, n (%)	15 (58)
Median time receiving chronic transfusion, years (range)	8.4 (2.3-24.2)
Indication for chronic transfusion, n	
Stroke	10
Elevated TCD	5
CNS vasculopathy	3
Silent cerebral infarct	2
Pulmonary disease	4
Recurrent Pain	1
Retinal artery infarct	1
Median biopsies per patient, n (range)	2 (2-7)

TCD = transcranial Doppler.

Table 2. — **Histologic and hematologic findings associated with paired, longitudinal liver biopsies**

	Biopsy 1			Biopsy 2			P
	All (n = 26)	No Chelation (n = 11)	Chelation (n = 15)	All (n = 26)	No Chelation (n = 1)	Chelation (n = 25)	
Age at biopsy, years	12.5 (9.2-15.4)	13.3 (8.3-15.4)	12.4 (9.2-13.8)	14.8 (10.4-17.7)	20.8	14.8 (10.4-17.6)	< 0.001
RBC transfusion volume*, cc/kg	373 (321-822)	332 (187-362)	687 (353-1341)	660 (452-1130)	520	693 (452-1130)	< 0.001
LIC, mg/g dry liver wt	14.6 (11.2-16.7)	12.8 (11.0-27.0)	15.0 (12.2-16.3)	11.9 (8.1-28.6)	12.6	11.2 (8.1-28.6)	0.9
Fibrosis, n (%)**	7 (26.9)	2 (18.1)	5 (33.3)	3 (11.5)	0	3 (12.0)	0.16
Histologic Iron Score	39 (33-45)	37 (30-45)	42 (36-44)	36 (30-42)	34	36 (28-43)	0.08
Ferritin***, ng/mL	3289 (2378-3641)	2246 (1711-2652)	3358 (2607-3978)	2694 (1657-3978)	2539	2556 (1657-3978)	0.9
INR	1.1 (1.0-1.2)	1.1 (1.0-1.2)	1.1 (1.0-1.2)	1.1 (1.0-1.1)	1	1.1 (1.0-1.1)	0.13
AST, U/L	45 (36-59)	45 (36-61)	45 (33-58)	38 (29-58)	24	38 (31-58)	0.6
ALT, U/L	22 (17-36)	22 (19-38)	24 (15-47)	21 (18-29)	18	21 (18-29)	0.8

Continuous data are presented as median (interquartile range). *P* value is for the overall comparison of biopsy 1 to biopsy 2 values.
* Cumulative volume is net transfused after subracting phlebotomy volumes, if any.
** All fibrosis was Ishak grade 1.
*** average of 3 ferritin values ± 1 month of biopsy.

score and trichrome staining for fibrosis. Additionally, three or more biopsies were also evaluable in 11 of the 26 patients ; specifically, 4 patients had 4 biopsies, 2 patients had 5 biopsies, and 1 patient had 7 biopsies. No biopsy showed steatosis. Among patients who had started chelation at the time of biopsy 1 (n = 15), the median duration of chelation was 2.3 years (range 0.2-10.8) ; among patients receiving chelation at biopsy 2 (n = 25), the median duration of chelation was 2.9 years (range 0.6-12.3). When feasible, 14 subjects received intermittent erythrocytapheresis procedures as part of their transfusion regimens to reduce iron loading, with a median of 6 procedures (range 1-116).

Longitudinal changes in fibrosis

Analysis of the first two biopsies in these patients showed no fibrosis in 17 patients, fibrosis progression from grade 0 to grade 1 in two patients, stable fibrosis (grade 1) in one patient, and fibrosis regression in six patients. The low prevalence and low grade of fibrosis limited analysis for longitudinal changes. Of note, fibrosis regressed in the one patient who did not receive any chelation or erythrocytapheresis prior to both biopsies. Figure 1 shows serial changes in fibrosis (asterisks) and LIC over time in subjects with ≥ 3 biopsies. In these patients fibrosis appears and disappears without an obvious pattern, except for the suggestion that low grade fibrosis may become more prevalent over time.

Longitudinal changes in biomarkers

Table 2 shows the cross-sectional values of biomarkers used to track liver iron loading and liver injury associated with fibrosis for the first two biopsies. With increasing cumulative transfusion volumes, values of

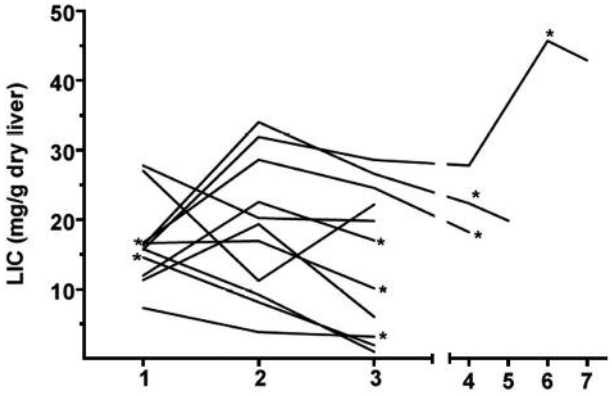

Fig. 1. — Fibrosis does not correlate closely with LIC. The spaghetti plot shows longitudinal changes in LIC among subjects with 3 or more biopsies. * indicates biopsies with fibrosis (all Ishak 1). Remaining biopsies showed no fibrosis.

LIC, HIS, and ferritin decreased as a result of chelation therapy. Biomarkers of liver function (ALT, AST, and INR) remained stable. Furthermore, when stratified by changes in persistence or appearance of fibrosis over the first two biopsies, there were no statistically significant changes in ALT, AST, ferritin, transfusion volume, and histologic iron score, in part due to the low prevalence of fibrosis in this cohort.

The overall trend of changes in LIC as they relate to fibrosis is shown in Figure 1, which includes subjects with ≥ 3 biopsies. Among these subjects with ≥ 3 biopsies, two observations can be made. First, fibrosis can appear and disappear with serial liver biopsies, and second, there is not an obvious association of LIC with fibrosis. At the time of biopsy 3 and subsequent biopsies (Fig. 1), all subjects were being treated with chelation therapy. The sporadic incidence of grade 1 fibrosis

Table 3. — **Association of biomarkers with liver fibrosis (n = 26 biopsy pairs)**

Ferritin Cutoff (ng/mL)*	Proportion ≥ cutoff with fibrosis	%	ALT Cutoff (U/mL)	Proportion ≥ cutoff with fibrosis	%	LIC cutoff (mcg/g)	Proportion ≥ cutoff with fibrosis	%
1000	9/49	18.4	27	7/19	36.8	5	8/49	16.3
2000	8/39	20.5	40	5/12	41.7	10	6/37	16.2
3000	6/20	30	50	4/9	44.4	15	4/23	17.4
4000	4/10	40	60	3/8	37.5	20	2/14	14.3
5000	3/5	60	70	2/2	100	25	2/11	18.2
6000	1/1	100	100	1/1	100	30	2/7	28.6

* average of 3 ferritin values ± 1 month of biopsy.

tended to be observed in later biopsies (4/11 had fibrosis on the last evaluable biopsy), but in the two children with the highest LICs, hepatic fibrosis appeared to regress on the final biopsy.

Cross sectional analysis of biomarkers and fibrosis

Although there were no associations between changes in biomarkers and changes in fibrosis over time, we evaluated cross-sectional associations between biomarkers and fibrosis for the 26 paired biopsies. Ferritin, ALT, and LIC are used to monitor for risk of liver injury during chronic transfusion ; we evaluated the ability of various thresholds of these biomarkers to identify fibrosis (Table 3). AUC analysis was performed to summarize the diagnostic performance of these biomarkers ; as a single marker elevations in ALT were more strongly associated with hepatic fibrosis than ferritin, with ALT having an ROC area of 0.80 (95% CI 0.66-0.94) and ferritin having an ROC area of 0.63 (95% CI 0.38-0.87). LIC performed poorly at discriminating fibrosis from no fibrosis (AUC 0.30 ; 95% CI 0.0-0.82). The optimal cutoffs that maximized the likelihood of fibrosis were a ferritin of 5000 ng/mL (LR+ 5.7) and an ALT of 65 (LR+ 5.2).

Correlation of changes in biomarkers with changes in LIC

Increasing LIC in the setting of chronic transfusion is concerning in part because of the increased risk of liver fibrosis with higher iron burdens. Clinicians track both ferritin and LIC over time to assess for risk of liver fibrosis, but how changes in ferritin over time relate to changes in LIC has not been evaluated quantitatively. Figure 2 shows the relationship between net changes in ferritin and net changes in LIC for all evaluable serial biopsies (n = 38). Net changes in ferritin and LIC were concordant in 18/38 measurements, with an increase in both ferritin and LIC in eight and a decrease in both ferritin and LIC in 10. Of measurements that tracked discordantly, 16 measurements showed ferritin increasing despite a decrease in LIC and four measurements showed an increase in LIC with decreasing ferritin.

Table 4 shows simple linear regression results of how changes between the first and second biopsies in ALT, AST, ferritin, transfusion volume, and histologic iron score change with LIC for the 52 paired biopsies. Ferritin and net volume RBC transfused had a statistically significant positive correlation with net change in LIC.

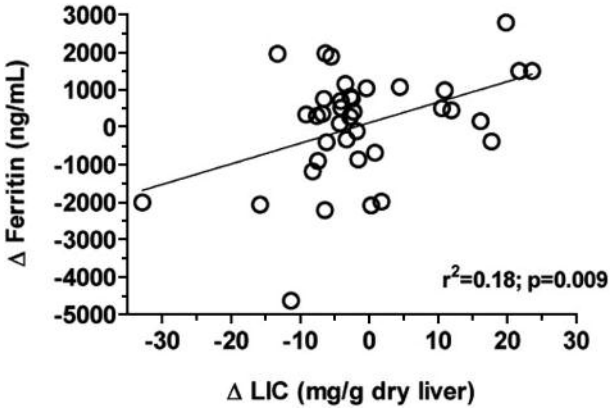

Fig. 2. — Correlation of changes in ferritin with changes in LIC over serial biopsies (n = 38) in patients with ≥ 3 biopsies.

MRI estimates of LIC

Eight patients had Ferriscan liver MRI performed within 3 months of liver biopsy. As with quantitative LIC by liver biopsy, LIC by MRI had poor correlation with fibrosis (data not shown). There was correlation between liver biopsy LIC and Ferriscan MRI (r = 0.75, P = 0.03), but MRI had uniformly higher estimates of LIC (median 31.4 vs 11.7 mcg/g dry liver weight).

Patient Morbidity

In this series we observed two unscheduled hospital admissions for unanticipated morbidity, one for respiratory distress from anesthesia and one for pain. Total charges for outpatient liver biopsies in our institution in 2007 ranged from $2,458-3,919.

Discussion

An important goal in avoiding morbidity in chronically transfused patients is to detect hepatic injury and avoid

Table 4. — **Simple linear regression comparing net parameter changes with net LIC changes in paired biopsies (n = 26)**

	Coefficient*	r^2	*P*
Change in ALT, U/L	0.09	0.02	0.6
Change in AST, U/L	0.09	0.01	0.6
Change in ferritin (per 100 ng/mL)**	0.39	0.23	0.02
Interval increase in RBC tx volume (per 100 cc/kg)	3.7	0.14	0.02
Change in histologic iron score	0.43	0.09	0.2

* per mg/g dry liver.
** average of 3 ferritin values closest to biopsy.
*** Cumulative volume is net transfused after subracting phlebotomy volumes, if any.

progression to cirrhosis. Previous studies evaluated the prevalence of fibrosis among chronically transfused sickle cell patients but not longitudinal changes in fibrosis status or the biomarkers used as surrogates for injury. We find that in the setting of chronic red cell transfusion in SCD for up to 17 years, hepatic fibrosis overall is low grade, does not progress linearly, and does not correlate with LIC well. However, both higher ALT and ferritin were associated with hepatic fibrosis.

We evaluated the change in fibrosis over time. The primary observation was that liver fibrosis appeared to be minimal and does not appear to progress over an average of two years between the first and second biopsies. This lack of progression to fibrosis may be due to the effect of chelation therapy, the patients' relatively young ages, effects of shortterm erythrocytapheresis, which can result in negative iron balance in some people (17), and variation in sampling sites on serial biopsy (18). Prior studies have found no significant fibrosis despite an increased liver iron load (10,13), while others have had a greater number of patients that progressed to fibrosis (11). This discrepancy in progression to fibrosis is certainly multifactorial but cannot be discerned with our study design. In our study, fibrosis was not highly prevalent and was only Ishak 0 or 1.

In contrast to prior studies, we analyzed changes in serum biomarkers over time, and their correlation with changes in LIC, HIS, and fibrosis. There was no statistically significant difference in the change in serum biomarkers and those with no fibrosis, progression or regression of fibrosis, and those with persistence of fibrosis. However, because fibrosis scores were all low (Ishak grade 1), advancement to fibrosis at final biopsy was low (2/26 in the primary analysis), and only 1/26 patients demonstrated persistence of fibrosis, no firm conclusion regarding the change in serum biomarkers and development of fibrosis can be made. Nevertheless, higher ferritin and ALT did have discriminatory ability to predict fibrosis on a cross-sectional basis.

The reduction in LIC, HIS, and ferritin from the first to second biopsy likely reflects the effects of chelation therapy, and reductions in these biomarkers coincides with a reduction in fibrosis prevalence from 7/26 to 3/26 biopsies, suggesting an association between chelation therapy and reduction in fibrosis. Studies in thalassemia show that chelation therapy appears to stabilize or reverse fibrosis independent of LIC (19,20).

Although chelation therapy was recommended to start within 1-2 years of starting chronic transfusion therapy, several factors limit the ability to make inferences about the effect of chelation therapy on fibrosis in this study, including variable adherence to chelation therapy, difficulties in ascertaining type and duration of therapy, the introduction of deferasirox during this study period, the effects of intermittent erythrocytapheresis in a subset of patients, and individual variation in iron loading, as ascertained by initial biopsy LIC, ferritin trends, and extent of prior episodic transfusion history.

MRI has supplanted routine liver biopsy for iron status monitoring in many centers and more recently for assessing hepatic fibrosis as well, but current MRI methods are not sufficiently sensitive for identifying lower grade hepatic fibrosis. In our analysis, there were not sufficient concomitant measurements of liver MRIs with liver biopsies to assess longitudinal changes associated with liver MRI results. The higher LIC estimates based on MRI may be explained by biopsy sampling variability versus MRI techniques that average whole organ content.

There appeared to be a relatively lower hepatic morbidity of chronic red cell transfusion in SCD when compared to disorders characterized by ineffective erythropoiesis. LIC and hepatic fibrosis in the setting of transfusion has been most extensively studied in chronically transfused people with thalassemia, where the association of LIC and liver fibrosis is more direct and of earlier onset compared to liver fibrosis in patients with sickle cell disease and transfusional hepatic iron overload (5). Studies in sickle cell disease have shown variable, but overall lower levels of fibrosis at given LICs. Thus, the utility of LIC in SCD is less clear than in thalassemia, and extrapolation from thalassemia data is imperfect. Others have proposed that differences in iron metabolism due to chronic hemolysis versus ineffective erythropoiesis, in addition to the chronic inflammation of SCD, likely contribute to these differences (21-23).

There are limitations to this study. There was not a standard schedule for biopsy sampling or initiation of

chelation therapy. Patient acceptance of follow up biopsies and chelation therapies also influenced outcome. We did not have sufficient numbers of patients to stratify between deferoxamine and deferasirox in the analysis, some patients crossed over these therapies, and some patients received short periods of erythrocytapheresis. In SCD, iron-mediated liver fibrosis can takes decades to develop, and it is possible that the median of nine years of transfusion observed in this study is insufficient to detect significant trends in liver pathology.

Our data bring into question the utility of routine liver biopsies in this population. Tracking LIC is important for assessing chelation therapy and iron loading, but LIC now can be feasibly determined non-invasively with MRI. We observed that liver fibrosis is minimal and does not progress, and changes in LIC and HIS over time do not correlate with fibrosis. Furthermore, liver biopsy incurs costs in terms of money, risk, and morbidity.

We propose that routine liver biopsies are not necessary components in the standard monitoring of children with SCD undergoing chronic transfusion. Liver biopsy appears most useful in select circumstances where liver MRI is not readily available or when serum biomarkers suggest liver injury associated with fibrosis. Prospective studies and long-term follow up of chronically transfused people with SCD will be useful in further evaluating the utility of liver biopsy in this population.

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