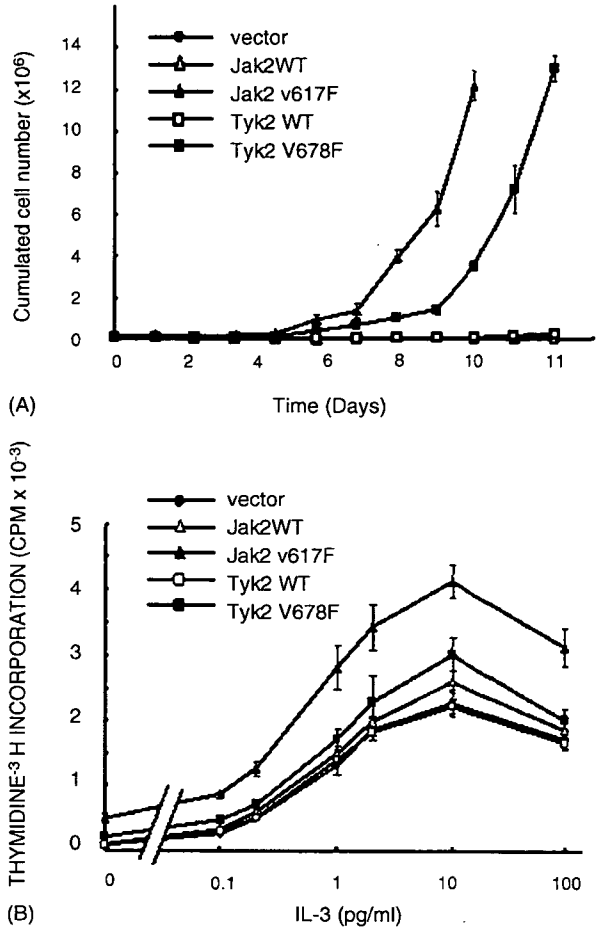

Fig. 3. The effects of V678F Tyk2 on the growth of Ba/F3 cells. (A) Ba/F3 cells expressing vector, WT Jak2, V617F Jak2, WT Tyk2, or V678F Tyk2 were grown in the absence of cytokine. Cell numbers (average of triplicates ± S.D.) were counted on the indicated days. (B) Ba/F3 cells expressing vector, WT Jak2, V617F Jak2, WT Tyk2, or V678F Tyk2 were grown in the absence of cytokine or stimulated with the indicated concentrations of IL-3. IL-3-induced cell proliferation was assessed by the incorporation of [^{3}H] thymidine.

Jak2 is essential for erythropoiesis [18] and somatic mutations of the JH2 region of Jak2; the substitution of valine to phenylalanine at position 617 of Jak2 (V617F Jak2) leads to the constitutive activation of Jak2 [5,6] and the development of myeloproliferative diseases [4–8]. According to five initial reports, the frequency of V617F Jak2 was 77% in PV, 35% in ET, and 43% in primary myelofibrosis. Expression of V617F Jak2 in cytokine-dependent cell lines induced constitutive activation and factor-independent growth (Fig. 2A and previous reports [5,6]). Furthermore, the transplantation of murine bone marrow cells previously transfected with V617F Jak2 induces erythrocytosis in recipient mice [6]. These results clearly indicate that V617F Jak2 causes myeloproliferative diseases.

In contrast to the predominance of the Jak2 mutation in PV patients, only a third of ET and a half of primary myelofibrosis patients harbored the Jak2 mutation. In the patients lacking the Jak2 mutation, signaling molecule besides Jak2 might be involved in the pathogenesis. PV is characterized by an increase in RBC and erythropoiesis is regulated by EPO [6]. ET is characterized by an increase in platelet counts, and TPO is the major cytokine regulating thrombopoiesis [19]. EPO selectively activates Jak2, whereas TPO selectively activates Jak2 and Tyk2 [12,15]. Jak2 valine 617, which was converted to phenylalanine in many patients with myeloproliferative diseases, was conserved in Tyk2 as valine 678. We therefore hypothesized that the Tyk2 mutation homologous to V617F Jak2 might induce the constitutive activation of Tyk2 and induce the cytokine-independent cell growth apparent in ET patients lacking the V617F Jak2 mutation.

To address the question of whether the Tyk2 mutation could be responsible for autonomous cell growth, we first examined the transcriptional activity of V678F Tyk2. As shown in Fig. 1A and B, the transfection of WT Tyk2 into 293T cells activated Stat3 and Stat5, and V678F Tyk2 augmented it about 2-fold greater than did WT Tyk2. But the effect of V678F Tyk2 on Stat3 and Stat5 was much less than that of V617F Jak2, which augmented the transcriptional activity of Stat3 and Stat5 more than 20 times greater than WT Jak2. We next transfected wild-type or VF mutant of Tyk2 into Ba/F3 cells to observe the effect of the V678F Tyk2 mutation in blood cells, and obtained stable cell lines. As shown in Fig. 2, Jak2 is autophosphorylated in Ba/F3 cells harboring V617F Jak2, and Tyk2 is also autophosphorylated in Ba/F3 cells harboring V678F Tyk2 in the absence of IL-3 stimulation. Stat5 is not usually phosphorylated in Ba/F3 cells without cytokine stimulation, and is transiently phosphorylated in response to cytokines such as IL-3. In Ba/F3 cells harboring V617F Jak2 or V678F Tyk2, Stat5 is phosphorylated in the absence of IL-3 stimulation, correlating with the activation of either Jak2 or Tyk2 (Fig. 2). Stat5 plays an essential role in lymphoid or mammary epithelial development and differentiation [28,29]. Furthermore, deletion of Stat5 resulted in perinatal lethality, and Stat5$^{-/-}$ fetuses were anemic [28,30]. The autophosphorylation of Stat5 in Ba/F3 cells harboring Jak2 V617F or Tyk2 V678F might induce the autonomous cell growth in the absence of cytokine stimulation. We next examined cytokine-independent cell growth in Ba/F3 cells harboring V678F Tyk2. Ba/F3 cells transfected with V678F Tyk2 exhibited autonomous cell growth in the absence of IL-3, but the effects of V678F Tyk2 on the proliferation of Ba/F3 cells in the absence of IL-3 was less drastic than that of V617F Jak2 (Fig. 3A). Recently, Staerk et al. also reported that the V678F mutation leads to the constitutive activation of Tyk2, inducing cytokine-independent cell proliferation [31].

One hallmark of myeloproliferative diseases is the hypersensitivity of haematopoietic cells to cytokines. PV bone marrow cells responded to low concentration of EPO, and those from ET responded to low concentrations of TPO, producing higher numbers of colonies in vitro [32,33]. Ba/F3 cells grow in response to IL-3 in a dose-dependent manner and plateau at 10 pg/ml of IL-3. Ba/F3 cells harboring V617F

Jak2 responded more strongly to all concentrations of IL-3 than did those with WT Jak2 (Fig. 3B). Ba/F3 cells harboring V678F Tyk2 also showed greater hypersensitivity to IL-3 than did cells with WT Tyk2, but the degree of hypersensitivity to IL-3 was much less than that of V617F Jak2 (Fig. 3B).

In Ba/F3 cells harboring V678F Tyk2, Tyk2 is constitutively activated even in the absence of cytokine (Fig. 2). Stat5 is also constitutively activated, despite the fact that cytokines that activate Tyk2, such as IFN-γ, IL-12, IL-10, IL-6, and G-CSF do not primarily phosphorylate Stat5 [24]. The phosphorylation of Jak2 was not observed in Ba/F3 cells harboring V678F Tyk2 (Fig. 3), then V678F Tyk2 could directly (not via Jak2 activation) activate Stat5 and other downstream signaling molecules to initiate autonomous cell growth. As the mutation of conserved valine residues corresponding to positions 678 in Tyk2 or 617 in Jak2 causes the constitutive activation of Tyk2 or Jak2 (Fig. 2), respectively, the common function of the JH2 pseudokinase domain, which inhibits the JH1 kinase domain of Jaks, might be present. Accordingly, the deletion of the JH2 region of Jak2 partially activates its kinase activity [34]. On the other hand, the deletion of the pseudokinase domain of Tyk2 inactivates the kinase activity of Tyk2 [35]. Taken together, the correct conformation of the JH2 region of Jak proteins is required for the inhibition of kinase activity of Jaks in the absence of cytokine stimulation, and also the activation of kinase activity in response to cytokine stimulation.

As V678F Tyk2 was constitutively activated in the absence of any cytokine and induced both the phosphorylation of Stat5 and autonomous cell growth, Tyk2 mutation could cause myeloproliferative diseases. We then searched for mutations in Tyk2 in ET patients without the Jak2 mutation. The V617F Jak2 mutation was found in 9 of 15 ET patients. This proportion (60%) was higher than the reported incidence of the Jak2 mutation (35%) in ET patients. But Baxter et al. reported nearly the same positive ratio: 29 of 51 ET patients (57%) had V617F Jak2 [4]. We then examined mutations in Tyk2 in the six remaining ET patients without the Jak2 mutation. Valine 678 of Tyk2, which corresponds to valine 617 of Jak2, was intact, so we extended our search to the full-length coding region of Tyk2. A non-synonymous substitution (1107G > *T*) of Tyk2 was present in 5 of 6 ET patients lacking V617F Jak2. This substitution was also commonly seen in ET patients with the V617F Jak2 mutation (six of nine had this substitution), so we doubted that the substitution was related to the ontogeny of myeloproliferative diseases.

We could not detect somatic mutations of Tyk2 in ET patients without the V617F Jak2 mutation. It requires a 2-bp change (from GTG to TTC) to change valine 678 to phenylalanine in Tyk2, while only a 1-bp change (from GTC to TTC) is required to produce V617F Jak2. The lower occurrence of 2-bp changes might be one reason why the V678F Tyk2 mutation was not found in ET patients. Another reason might be that the effect of V678F Tyk2 on cell proliferation is much less than that of V617F Jak2. The lower effect of V678F Tyk2 on cell proliferation, combined with the lower incidence of the VF mutation in Tyk2, might explain why mutations in the Tyk2 pseudokinase domains are not found in ET patients in this study. When we search for the Tyk2 mutation in MPD patients with mild laboratory findings on a larger scale, there is a possibility that mutations in the Tyk2 pseudokinase domains may be found. Another possibility is that a mutation of signaling molecules downstream of Jaks, such as Stat5, may be a cause of disease in MPD patients lacking the V617F Jak2 mutation.

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Contributions. K. Shide contributed to the concept and design, interpreted and analyzed the data, provided drafting of the article, collected and assembled the data. K. Kamezaki, H. Kakumitsu, T. Kumano, A. Numata, F. Ishikawa, K. Takenaka, K. Yamamoto and T. Matsuda collected clinical samples, interpreted and analyzed the data, and provided critical revisions. K. Shimoda organized this project and gave final approval. M. Harada provided critical revisions and important intellectual content, and obtained a funding source.

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ORIGINAL ARTICLE

Retrospective nationwide survey of Japanese patients with transfusion-dependent MDS and aplastic anemia highlights the negative impact of iron overload on morbidity/mortality

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Abstract

Objective: Myelodysplastic syndromes (MDS) and aplastic anemia (AA) are the most common anemias that require transfusion therapy in Japan. This retrospective survey investigated relationships between iron overload, chelation practices, and morbidity/mortality in patients with these diseases. *Method:* Medical histories of transfusion-dependent patients were assessed at transfusion onset, chelation onset, and study end. *Results:* Data were collected from 292 patients with MDS, AA, pure red cell aplasia, myelofibrosis, and other conditions. Patients received a mean of 61.5 red blood cell units during the previous year. Fewer than half (43%) of patients had previously received deferoxamine (DFO) therapy. Only 8.6% received daily/continuous DFO. In all, 75 deaths were reported, with cardiac and liver failure noted in 24.0 and 6.7% of cases. Of these, 97% had ferritin levels >1000 ng/mL. Abnormal cardiac and liver function was observed in 21.9% (14/64) and 84.6% (11/13) of all patients assessed. Effective chelation with DFO resulted in improved serum ferritin, liver enzymes, and fasting blood sugar. *Conclusions:* Mortality is higher in heavily iron-overloaded patients, with liver and cardiac dysfunction being the primary cause. Daily/continuous chelation therapy was effective at reducing iron burden and improving organ function. Chelation therapy should be initiated once serum ferritin levels exceed 1000 ng/mL.

Key words refractory anemias; myelodysplastic syndromes; aplastic anemia; iron chelation therapy; deferoxamine

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Myelodysplastic syndromes (MDS) and aplastic anemia (AA) are the most common anemias that require transfusion therapy in Japan (1). Because there is no physiological mechanism for iron excretion in humans, as the number of transfused units of blood increases patients rapidly develop iron overload. Excessive accumulation of iron released from aging and damaged erythrocytes by reticuloendothelial macrophages leads to transferrin becoming saturated and the circulation of non-transferrin-bound iron (NTBI) in serum (2). As a result, iron is deposited in the form of ferritin and hemosiderin in the parenchymal cells of the liver, heart, pancreas, brain, and joints (3, 4). Ionic iron-mediated toxicity in these organs such as lysosomal disruption in hepatocytes, collagen formation and fibrogenesis, and lipid peroxidation in heart and spleen cells have been shown to cause various symptoms of congestive heart failure, arrhythmias, cirrhosis, hepatocellular carcinoma, insulin resistance and

diabetes, arthritis, fatigue, and sexual dysfunction (5–8). Iron overload also directly affects the systemic immunity and increases availability of iron to viruses, bacteria, and cancer cells (9, 10).

As phlebotomy is not an option because of their underlying diseases, in anemic patients with iatrogenic iron overload as sequela of repeated transfusions chelation therapy with subcutaneous or intravenous deferoxamine (Desferal®; DFO) has been, to date, the only means of removing the toxic accumulation of NTBI. Daily treatment with DFO has been demonstrated effective; however, in Japan it is common practice to administer DFO fortnightly in a hospital setting because of the risk of infection or bleeding associated with subcutaneous and intramuscular administration. Once-every-2 wk treatment is less than optimal, difficult and inconvenient for patients and physicians, and adversely affects the patient's quality of life (QoL). The goal of this retrospective survey was to investigate the relationships between iron overload, current chelation practices, and morbidity/mortality in Japanese patients with MDS and AA.

Materials and methods

Study design

This retrospective survey investigated the outcomes of iron overload-related morbidity and mortality in Japan from August 2001 to December 2005. A questionnaire was sent to hematology departments in hospitals all over Japan. The medical chart histories of transfusion-dependent (TD) patients were assessed by questionnaire at three time points: (i) transfusion onset, (ii) chelation onset, and (iii) end of study (EOS). TD patients were defined as those receiving >2 packed red blood cell (RBC) units/month for ≥6 months. Data categories on the questionnaire included age, sex, underlying disease, risk of becoming TD, number of RBC units received (in Japan, one RBC unit derives from 200 mL of whole blood), results of laboratory blood tests [serum ferritin, total protein, serum glutamic oxaloacetic transaminase (SGOT; aspartate aminotransferase), serum glutamic pyruvic transaminase (SGPT; alanine aminotransferase), bilirubin, fasting blood sugar (FBS), and HbA_{1c}], cardiac tests (ECHO, ultrasound examination, and electrocardiogram), liver magnetic resonance imaging (MRI), and liver biopsy.

Cardiac function was assessed by each individual patient's physician; in this study there was no preset definition of what constituted cardiac abnormality. Liver MRI assessments were undertaken at radiology departments in each participating hospital. Details of the MRI pulse sequences used and magnetic strengths were not recorded. The prevalence of hepatitis C virus infection in the study population was not recorded.

Statistical analysis

Comparisons between laboratory tests, liver MRI, cardiac examinations, cause of death, patient background, and serum ferritin levels (a measure of iron overload) were performed using Student's *t*-test or the Fisher test. Relationships between EOS ferritin levels and SGOT and SGPT levels were calculated by Cochran–Armitage test. Cutoff values for RBC units were calculated using a logistics model that was evaluated with the Pearson chi-squared test. A *P*-value ≤0.05 was considered significant.

Results

Patients' demography and background characteristics

In response to 173 questionnaires circulated, 43 hospitals replied returning data on 292 patients with a range of underlying conditions. Demographic and background data from these patients are presented in Table 1. Six patients had more than one diagnosis: AA and MDS ($n = 1$); AA and paroxysmal nocturnal hemoglobinuria ($n = 1$); pure red cell aplasia and graft-vs.-host disease ($n = 1$); MDS and myelofibrosis ($n = 2$); and MDS and multiple myeloma ($n = 1$).

Transfusion history

Transfusion history of the patients is summarized in Table 2. Average period of transfusion dependency was

Table 1 Patients' demography and background clinical characteristics

No. of patients	292
Sex *n* (%)	
Male	159 (54.5)
Female	130 (44.5)
Unknown	3 (1.0)
Age in years *n* (%)	
20–29	12 (4.1)
30–39	20 (6.8)
40–49	23 (7.9)
50–59	34 (11.6)
60–69	75 (25.7)
70–79	78 (26.7)
>80	14 (4.8)
Other	2 (0.7)
Unknown	34 (11.6)
Underlying disease[1] *n* (%)	
MDS	152 (52.1)
AA	90 (30.8)
Pure red cell aplasia	15 (5.1)
Myelofibrosis	13 (4.5)
Other	26 (8.9)
Unknown	2 (0.7)

[1] Six patients had more than one disease.
MDS, myelodysplastic syndromes; AA, aplastic anemia.

Table 2 Transfusion history (n = 292)

Parameter	n (%)
Period of transfusion dependency, months	
≤12	87 (29.8)
13–30	106 (36.3)
≥31	92 (31.5)
Unknown	7 (2.4)
Total lifetime no. of RBC[1] units received	
≤40	62 (21.2)
41–160	132 (45.2)
≥161	87 (29.8)
Unknown	11 (3.8)
No. of RBC units[1] in the past year	
≤20	30 (10.3)
21–40	53 (18.2)
41–70	93 (31.8)
≥71	82 (28.1)
Unknown	34 (11.6)

[1] One RBC unit is made from 200 mL of whole blood.

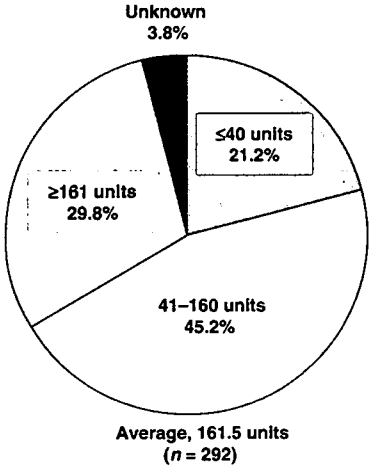

Figure 1 Transfusion history of patients: total lifetime red blood cell units.

32 months. Lifetime transfusions were: ≤40 units in 21.2%; >40–160 units in 45.2%; ≥161 units in 29.8%; and unknown in 3.8% of patients (Fig. 1). On average, patients received a total lifetime transfusions of 161.5 RBC units, and 61.5 RBC units in the year prior to data collection.

DFO chelation therapy

Less than half of the patients (126/292; 43.2%) had received DFO therapy, mostly on an intermittent basis; 164 of 292 patients (56.2%) were chelator-naïve and chelation history was unknown in two patients (0.7%). Among those who received DFO, 11 patients (8.6%) received daily/continuous DFO, with the remainder receiving intermittent DFO (mean; once/1.9 wk) or other regimens

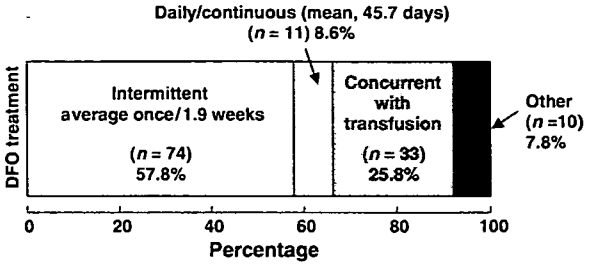

Figure 2 Proportion of patients receiving deferoxamine and regularity of treatment.

Table 3 Number of patients on various deferoxamine 500–1000 mg/d schedules

Route of administration	500 mg/d, n	1000 mg/d, n
Bolus intravenous	7	9
Subcutaneous	9	4
Intravenous (drip)	35	44
Intramuscular	9	0
Total	60	57

(Fig. 2). Intravenous drip infusion was the most common route of DFO administration, as shown in Table 3.

Of the DFO-treated patients, 53.7% had received ≤40 RBC units and 46.3% >40 units at the time of initiating DFO. On average, DFO was initiated in patients after receiving 61.6 RBC units. More than 95% of patients on DFO were monitored by serum ferritin, with 4.1% undergoing liver MRI.

Treatment with DFO was not associated with abnormality of total protein, SGOT, bilirubin, FBS, HbA_{1c}, and liver MRI scans or cause of death but was significantly associated with increased risk of abnormal SGPT (P = 0.0072), serum ferritin ≥1000 ng/mL (P = 0.0385), and cardiac dysfunction (P = 0.0312). Among patients with abnormal SGPT, serum ferritin, and cardiac dysfunction the majority received DFO. On the other hand, in patients receiving daily/continuously administered DFO serum ferritin, SGOT, SGPT, and FBS levels improved during treatment; pair-wise comparison using Wilcoxon two-sample test revealed that the proportion of patients with abnormal parameters in the daily/continuous DFO group was lower that on other DFO regimens (Table 4).

Assessment of serum ferritin

At the time of becoming TD, data on 142 patients revealed that the mean serum ferritin level was 1672.7 ng/mL and rose to 4378.3 ng/mL at EOS (n = 161; Fig. 3).

Serum ferritin levels were increased despite DFO usage. At the time of becoming TD and at EOS mean

Parameter	Intermittent (once/1.9 wk)	Concurrent with transfusion	Daily/continuous
Serum ferritin[1,2] (ng/mL)	+2222.8 (n = 36)	+2204.8 (n = 19)	−1135.2 (n = 9)
SGOT[1,3] (mU/mL)	+28.0 (n = 53)	+40.0 (n = 30)	−9.2 (n = 10)
SGPT (mU/mL)	+28.6 (n = 53)	+10.3 (n = 30)	−28.8 (n = 10)
FBS (mg/dL)	+31.2 (n = 31)	+8.2 (n = 12)	−4.8 (n = 5)

[1] Intermittent vs. continuous, $P < 0.05$.
[2] Continuous vs. concurrent, $P < 0.01$.
[3] Continuous vs. concurrent, $P < 0.05$.

Table 4 Average changes of laboratory values during the period of transfusion dependence in patients receiving deferoxamine treatment regimens

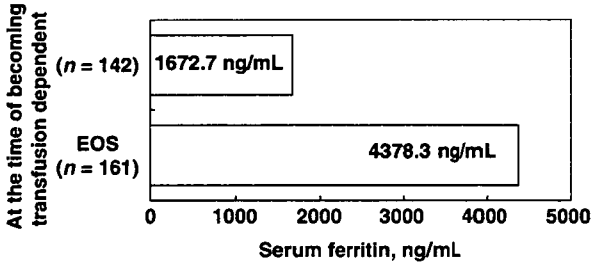

Figure 3 Serum ferritin levels at end of study and at the time of becoming transfusion-dependent.

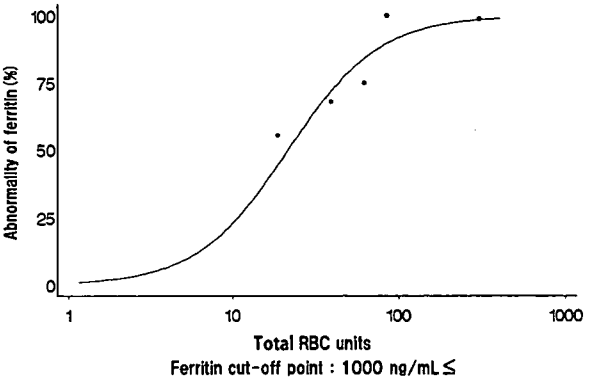

Figure 4 Relationship between end of study serum ferritin and total lifetime number of red blood cell units.

serum ferritin levels were 1590.1 and 4486.7 ng/mL, respectively, in patients who received DFO and 1465.8 and 3303.4 ng/mL, respectively, in chelator-naïve individuals. The proportion of patients with serum ferritin >1000 ng/mL rose from 47.2% at the time of becoming TD to 89.4% at EOS.

Serum ferritin level was significantly correlated with lifetime total number of RBC units received ($P = 0.0072$) and number of RBC units received in the previous year ($P = 0.0004$) but was not correlated with age or underlying diseases. Figure 4 shows the relationship between the number of RBC units received and mean ferritin level, indicating the percentage of patients with an abnormal ferritin level (≥1000 ng/mL) for any total number of RBC units received as analyzed by logistics model. Patients were characterized by the number of RBC units, and the ratio to abnormal ferritin was categorized for each category. The goodness-of-fit of this model between theoretical and actual values was assessed by Pearson chi-squared test. The estimated number of RBC units required to raise ferritin to ≥1000 ng/mL in 50% and 75% of patients was calculated as 21.5 and 43.4 units, respectively.

There was a significant difference between the period of transfusion dependence in patients who received DFO and chelator-naïve patients (37.4 vs. 15.4 months; $P < 0.0001$). When comparing the monthly change of serum ferritin levels during the period of transfusion dependence between these two groups, DFO patients showed a slightly slower increase (77.9 vs. 162.3 ng/mL/month; $P = 0.0248$); this was nonetheless an abnormal increase. Among DFO-treated patients those who received daily/continuous DFO tended to show a slower increase in serum ferritin levels than those who received DFO by other schedules (45.8 vs. 78.5 ng/mL/month; $P = 0.6280$).

Laboratory values

The proportion of patients with abnormal laboratory parameters increased after initiation of transfusions: the proportion of patients in whom SGOT was ≥36 mU/mL [the upper limit of normal (ULN)] increased from 16.8% to 41.8%. Similarly, SGPT was ≥46 mU/mL (ULN) in 16.4% and 36.8% of patients before and after initiation of transfusions, respectively.

SGOT and SGPT abnormalities were significantly correlated with transfusion frequency ($P = 0.0016$ and < 0.0001, respectively), transfusion history ($P = 0.0099$ and 0.0009, respectively) and increased ferritin levels ($P = 0.0003$ and 0.0006, respectively) but not with age or underlying disease. There was a significantly ($P < 0.0001$) higher prevalence of SGOT (Fig. 5A) and SGPT (Fig. 5B) abnormality in patients with high serum ferritin than in those whose serum ferritin was <1000 ng/mL. FBS was ≥121 mg/dL (ULN) in 39.1% and 54.0% of patients before and after initiation of transfusions, respectively. FBS abnormality was correlated with

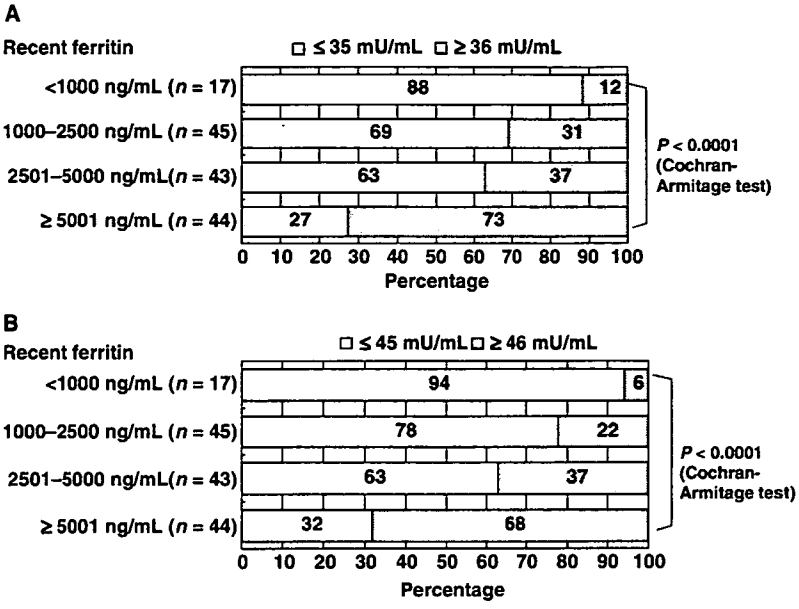

Figure 5 Relationship between serum glutamic oxaloacetic transaminase (A) and serum glutamic pyruvic transaminase (B) abnormality and serum ferritin.

Table 5 Sensitivity and specificity of serum ferritin levels ≥1000 and ≥2500 ng/mL to predict for abnormal SGOT/SGPT and cardiac function

	Serum ferritin (1000 ng/mL)		Serum ferritin (2500 ng/mL)	
Abnormality	Sensitivity	Specificity	Sensitivity	Specificity
SGOT	0.97	0.18	0.75	0.54
SGPT	0.98	0.17	0.55	0.81
Cardiac function	0.92	0.06	0.67	0.36

SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase.

transfusion frequency ($P = 0.0072$) but not with transfusion history, serum ferritin, age, or underlying disease.

Among patients with abnormal laboratory parameters >90% had serum ferritin levels >1000 ng/mL. Serum ferritin was calculated a predictor of abnormality of EOS SGOT, SGPT, and cardiac function with sensitivities and specificities as shown in Table 5.

Assessment of organ function and cause of death

Of patients in whom cardiac function and liver MRI were evaluated, abnormalities were observed in 21.9% (14/64) and 84.6% (11/13), respectively. Among patients with cardiac dysfunction and those who underwent liver MRI, 91.7% (11/12) and 91.9% (10/11), respectively, had serum ferritin levels ≥1000 ng/mL. Cardiac abnormality was weakly correlated with serum ferritin levels. Patients with cardiac abnormality showed greater increases of serum ferritin levels than those who did not (7096.0 vs. 4220.1 ng/mL; $P = 0.0392$). There was no correlation observed between MRI abnormality and patient background or transfusion history.

In total, 75 deaths were reported. Most of the deaths were caused by infection and leukemia; however, cardiac and liver failure was noted in 24.0% and 6.7% of cases, respectively. There was a significant difference between the number of patients who died from leukemia or infection and those whose cause of death was reported as cardiac/liver failure in terms of transfusion history.

There was no significant difference in mean age between patients who died from iron overload (cardiac or liver failure) and those who died from other causes ($P = 0.6767$; Fisher test). Among patients with MDS, 11 died of cardiac or liver failure. Of these, 10 patients (90.9%) were classified as International Prognostic Scoring System low/intermediate-1 risk whereas one patient (9.1%) was intermediate-2/high risk. Sixty-four percent of the patients with MDS who died became TD when aged 50–60 yr, and the mean duration from onset of transfusion dependence to death was 30.5 months.

Patients who died from cardiac or liver failure had more transfusions than those who died from other causes (total RBC units = 289.2 vs. 160.7 units, respectively; $P = 0.0033$). Furthermore, among 38 patients in whom serum ferritin levels were available, almost all ($n = 37$) died with serum ferritin levels ≥1000 ng/mL, whereas only one patient had a serum ferritin level <1000 ng/mL. Twenty four of these patients had serum ferritin levels >5000 ng/mL.

Discussion

This was the first nationwide survey to investigate morbidity/mortality resulting from iron overload in Japanese TD patients with refractory anemias. A high serum

ferritin level was correlated with cardiac and liver dysfunction, and more patients died with serum ferritin levels ≥1000 ng/mL than < 1000 ng/mL. Further observations may clarify the effect of history of transfusions on death. The compelling results of this study suggest that a randomized study comparing effective chelation therapy vs. no treatment would not be ethical. However, a prospective survey of effectively chelated patients, in comparison with the results of this retrospective study, would be of great value to physicians in underlining the devastating impact of iron overload in patients with TD anemias. Overall, the results presented here seem useful for establishing guidelines for the treatment of iron-overloaded patients with MDS and AA in Japan, indicating the need to address iron overload in these patient populations (11–14).

Cause of death was reported as cardiac failure in 24% and liver failure in 6.7% of patients studied. Therefore multiple transfusion therapy for anemias such as MDS and AA was confirmed associated with a high risk for developing fatal comorbidities caused by chronic iron overload. This result is in line with a recent report showing that TD MDS patients exhibited a significantly shorter overall survival than MDS patients who did not require transfusions, and that developing secondary iron overload significantly affected survival (15).

Cardiac examination was conducted in only 64 cases (21.9%); however, among these cases 14 patients (21.9%) showed signs of cardiac abnormality. Cardiac risk was weakly correlated with the presence/absence of DFO therapy, with DFO-treated patients exhibiting a slightly higher risk of cardiac dysfunction. It is not certain as to how far this result reflects the clinical tendency for patients with cardiac dysfunction to be selected as candidates for treatment with DFO. Although DFO is cardioprotective in TD patients (16, 17), the effects of DFO on reversing congestive heart failure could not be assessed in the present series. We recommend that cardiac function be carefully monitored, and the effect of iron overload on the heart examined in detail.

The pathological effects of transfusional iron overload on liver and pancreas are well documented, and major iron deposition in these organs usually precedes that in cardiac myocytes (18). Hemosiderin in pancreatic islet cells has been shown to increase with the number of blood transfusions in iron-overloaded patients with a history of glycosuria and hyperglycemia. Furthermore, in comparison with normal controls, increased glucose intolerance associated with significantly reduced insulin output was observed in non-thalassemic patients with anemias requiring transfusions (18, 19). In the present study of TD patients SGOT, SGPT, and FBS were time-dependently increased from the time of becoming TD. No such effect was observed on total protein, bilirubin, and HbA_{1C}. Abnormality of serum liver enzymes was significantly correlated with history of transfusion and serum ferritin levels but not with age and underlying disease; FBS was also correlated with frequency of transfusion. These observations confirm previous reports that liver and pancreatic dysfunction occur as a result of iron overload from repeated transfusions.

Ferritin tests were conducted in 50–70% of patients. This procedure was very common and seems more practical for monitoring patients than liver MRI and biopsy. Serum ferritin was significantly correlated with frequency and history of transfusion, and appears useful for monitoring iron overload. Ferritin was also well correlated with SGOT/SGPT and cardiac dysfunction. Patients with high serum ferritin levels ≥1000 ng/mL had an increased risk of liver enzyme abnormality as well as increased risk of cardiac dysfunction and death caused by iron overload. Hence serum ferritin is a useful marker to predict clinical comorbidity resulting from iron overload.

The number of RBC units required to raise serum ferritin levels to ≥1000 ng/mL in 50% and 75% of patients was 21.5 and 43.4 units, respectively. Therefore from these data it is reasonable to consider that iron chelation therapy should start when serum ferritin reaches 1000 ng/mL or after transfusion of 20–40 units of total RBC units so as to avoid the risk of end-organ damage caused by toxic free iron. Indeed, it is recommended in various guidelines that serum ferritin levels be maintained < 1000 ng/mL by iron chelation therapy (12, 14, 20, 21).

In the patients assessed in this study the sensitivity and specificity of EOS serum ferritin levels were calculated and correlated with abnormal EOS SGOT, SGPT, and cardiac function. These results show that serum ferritin level is a highly sensitive and useful indicator for monitoring chelation therapy, and strongly suggest that effective chelation therapy with a long period of chelation coverage should be administered to prevent cardiac as well as other organ dysfunction.

It is widely acknowledged that continuous exposure to DFO provides optimal efficacy (14). In the treatment of thalassemia full compliance or increasing the overall period of chelation coverage (> 300 d/yr) is strongly correlated to length of survival (22). However, in our series DFO was mostly given intermittently (once/1.9 wk) or administered concurrently with transfusion. It is difficult to administer DFO daily/continuously especially in MDS and AA patients who may be at risk of infection or bleeding because of peripheral cytopenia. Furthermore, most patients in Japan receive treatment on an outpatient basis, and infusion pumps for DFO therapy are not reimbursed.

DFO-treated patients showed a slightly lower monthly increase in serum ferritin levels during the period of

transfusion dependence than chelator-naïve patients but this was nonetheless abnormally increased. DFO suppressive efficacy was not seen in laboratory values, cardiac/liver function, or death from iron overload. Taken together, these results imply that the current treatment methodology in Japan (often once/2 wk) might not be clinically the most effective. Patients who received daily/continuous (average, 45.7 d) DFO treatment, on the other hand, exhibited decreases in serum ferritin levels; SGOT, SGPT, and FBS and were less likely to display abnormal laboratory values.

Daily DFO treatment (5–7 d/wk) is the reference standard of care for patients with β-thalassemia, and effective DFO therapy has been clearly demonstrated to prolong survival in thalassemic patients as well as those with MDS (23, 24). Furthermore, daily DFO treatment elicits prophylactic effects against cardiac dysfunction and diabetes (25). In a small trial conducted in 11 patients with MDS daily/continuous DFO chelation improved serum ferritin and hemoglobin requirement of the patients (26).

The only other approved chelators are for use as second-line treatment for β-thalassemia. Recently, a novel oral iron chelator, deferasirox, has been approved in >60 countries. Deferasirox is easily absorbed and has a median elimination half-life of 8–16 h, which means that deferasirox is continuously present in the plasma with once-daily dosage (27). In a large phase III trial deferasirox was comparable with DFO at decreasing iron burden in β-thalassemic patients with a liver iron concentration >7 mg Fe/g dry weight (28). Deferasirox also reduced iron burden in patients with various anemias including MDS (29). These findings indicate that the availability of oral iron chelators, especially deferasirox, can improve patients' QoL by ameliorating organ dysfunction and preventing iron damage. This may ultimately prolong survival of patients receiving treatment with an oral iron chelator.

Conclusions

This retrospective analysis of TD patients with anemias such as MDS and AA revealed that the mortality rate is raised in heavily iron-overloaded patients, with liver and cardiac dysfunction being the primary cause of death. Serum ferritin level appears a useful monitor of iron overload. Daily/continuous chelation therapy was effective in reducing iron burden and improving organ function; however, practical implementation of continuous administration is currently difficult. Based on these findings chelation therapy should be initiated at a serum ferritin level of ≥1000 ng/mL, using a regimen that provides the longest possible period of chelation coverage with the least intrusion on patient QoL.

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