University, Hiroshima University, Hokkaido University, Hyogo Medical University, Iwate Medical School, Jichi Medical School, Jikei University, Juntendo University, Kagawa Children's Hospital, Kagawa University, Kagoshima University, Kanazawa University, Kanazawa Medical School, Kansai Medical University, Kawasaki Medical School, Keio University, Kinki University, Kitazato University, Kobe University, Kochi University, Kumamoto University, Kurume University, Kyoto Prefectural University, Kyoto University, Kumamoto Medical Center, Kyushu University, Mie University, Nagasaki University, Nagoya City University, Nagoya Medical Center, Nagoya University, Nara Medical University, National Cancer Center, National Institute of Infectious Diseases, Niigata University, Nishi Sapporo National Hospital, Nippon Medical School, Nippon University, NTT Kanto Medical Center, Oita University, Okayama Medical Center, Okayama University, Osaka City University, Osaka Medical School, Osaka National Hospital, Osaka University, Ryukyu University, Saga University, Saitama Medical School, Sapporo Medical School, Sendai Medical Center, Shimane University, Shinsyu University, Showa University, St. Marianna University, Teikyo University, Toho University, Tohoku University, Tokai University, Tokushima University, Tottori University, Tokyo Medical Center, Tokyo Medical School, Tokyo Medical and Dental University, Tokyo University, Tokyo Women's Medical School, Tsukuba University, University of Occupational and Environmental Health, Wakayama Medical University, Waseda University, Yamagata University, Yamaguchi University, Yamanashi University, Yokohama City University.

Authorship and Disclosures

NF: designed the research, analyzed data and wrote the paper; KS and MH: designed the research, analyzed data and contributed to writing the paper; KO, KS, AM, MT, MK, AA, YY, SN, AU, MO, and KO: designed the research and helped organize this collaborative study.

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Japanese epidemiological survey with consensus statement on Japanese guidelines for treatment of iron overload in bone marrow failure syndromes

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Abstract Many patients with bone marrow failure syndromes need frequent transfusions of red blood cells, and most of them eventually suffer from organ dysfunction induced by excessively accumulated iron. The only way to treat transfusion-induced iron overload is iron chelating therapy. However, most patients have not been treated effectively because daily/continuous administration of deferoxamine is difficult for outpatients. Recently, a novel oral iron chelator, deferasirox, has been developed, and introduction of the drug may help many patients benefit from iron chelation therapy. In this review, we will discuss the current status of iron overload in transfusion-dependent patients, and the development of Japanese guidelines for the treatment of iron overload in Japan, which were established by the National Research Group on Idiopathic Bone Marrow Failure Syndromes in Japan.

Keywords Bone marrow failure syndrome · Iron overload · Iron chelation · Guidelines

1 Introduction

Many patients with aplastic anemia (AA) or myelodysplastic syndromes (MDS) need frequent transfusions of red blood cells (RBCs). One unit (derived from 200 mL of whole blood) of RBC transfusion in Japan contains about 100 mg of iron. Because there is no physiological mechanism for iron excretion in humans, and daily iron excretion is no more than 1 mg in a healthy man, repeated RBC transfusions will soon result in iron overload. Excess iron is mainly deposited in the liver, heart and pancreas, and causes organ dysfunction [1, 2].

As phlebotomy is not an option because of the underlying bone marrow failure, the only way to treat iron overload is by iron chelation therapy. However, difficulty in optimal administration of deferoxamine (DFO, Desferal®) in Japan has hampered effective chelation, and currently most patients are not treated effectively [3].

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Recently, a novel oral iron chelator, deferasirox (Exjade®), has been introduced in more than 60 countries, including Japan. The introduction of deferasirox may improve compliance with iron chelation therapy [4]. Under these circumstances, the National Research Group on Idiopathic Bone Marrow Failure Syndromes in Japan drew up Japanese guidelines for the treatment of transfusion-induced iron overload. Herein, we describe the current status of iron overload in transfusion-dependent patients in Japan, and development of the proposed guidelines for the treatment of transfusion-induced iron overload.

2 Current status of transfusion-induced iron overload in Japan

In 2005, the first nationwide survey on iron overload in transfusion-dependent patients in Japan was carried out [3]. This retrospective survey investigated the outcomes of iron overload-related morbidity and mortality from August 2001 to December 2005. A questionnaire was sent to hematology departments in hospitals all over Japan, and 43 hospitals responded by returning data on 292 patients.

Demographic data showed that MDS and AA accounted for about 80% of the underlying diseases: MDS, 52.1%; AA, 30.8%; pure red cell aplasia (PRCA), 5.1%; and myelofibrosis (MF), 4.5%. Serum ferritin levels were significantly correlated with the lifetime total number of RBC transfusion units received. Figure 1 shows the relationship between the number of RBC units and mean ferritin level, indicating the percentage of patients with an abnormal ferritin level (≥1,000 ng/mL) for any total number of RBC units received as analyzed by a logistics model. The goodness-of-fit of this model between theoretical and actual values was assessed by Pearson chi-squared test, and the estimated number of RBC units required to raise ferritin to ≥1,000 ng/mL in 50 and 75% of patients was calculated as 21.5 and 43.4 units, respectively.

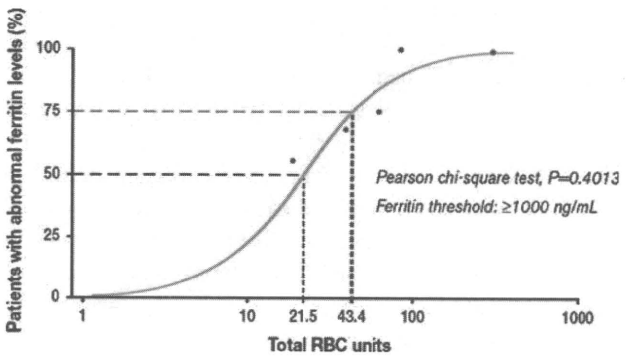

Fig. 1 Relationship between serum ferritin and total number of red blood cell units. [3] Modified with permission from Takatoku et al. *Eur J Haematol.* 2007;78:487–494. ©2007 Blackwell Publishing

Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) abnormalities were significantly correlated with transfusion frequency and increased ferritin levels; there was a significantly ($P < 0.0001$) higher prevalence of SGOT and SGPT abnormality in patients with high serum ferritin than in those whose serum ferritin was <1,000 ng/mL (Fig. 2). Moreover, among patients in whom cardiac function was evaluated, abnormalities were found in 21.9%, and cardiac abnormality was weakly correlated with serum ferritin levels. These data indicate that ferritin levels can be a useful predictor of hepatic and cardiac dysfunction. Fasting blood sugar (FBS) abnormality was also correlated with transfusion frequency.

In the survey, 75 deaths were reported, most of which were caused by infection and leukemia. However, cardiac and hepatic failure was noted in 24% and 6.7% of cases, respectively. Patients who died from cardiac or hepatic failure had received more transfusions than those who died from other causes, and among 38 patients in whom serum ferritin levels were available, 37 patients died with serum ferritin levels ≥1,000 ng/mL; the majority of patients (24 patients) had serum ferritin levels >5,000 ng/mL. These data indicate that multiple transfusion therapy is associated with a high risk of fatal complications caused by iron overload. Recently, similar analyses have been reported describing that transfusion-dependent MDS patients show significantly shorter survival than those who do not require transfusions and that transfusion-induced iron overload significantly affects survival [5].

3 Iron chelation therapy

As phlebotomy is not an option because of underlying bone marrow failure, the only way to treat iron overload is with iron chelation therapy. Until recently, the only available iron chelating agent in Japan was DFO. Because of the limited absorption from the gastrointestinal tract and short biological half-life of the agent, the drug must be administered by parenteral injections at least 5–7 times a week, or continuously for optimal effectiveness [6]. In the survey, 43.2% of patients received DFO, but only 8.6% received DFO daily or continuously; most of the patients were administered the drug intermittently (average once per 1.9 weeks) or concurrently with transfusion [3]. While improvements in serum ferritin, SGOT, SGPT and FBS were noted in the patients who received DFO daily or continuously, these data did not improve, and rather worsened, in those without optimal administration (Table 1). This indicates that appropriate administration of the chelating agent is needed for sufficient therapeutic results.

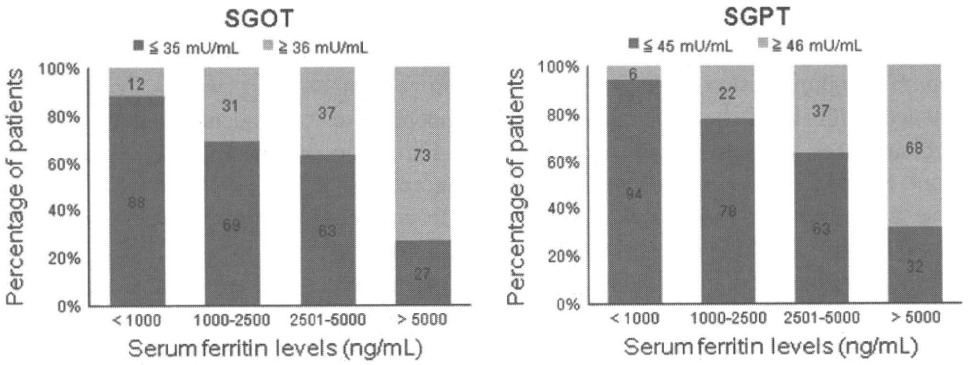

Fig. 2 Relationship between serum transaminase abnormality and serum ferritin levels. [3] Modified with permission from Takatoku et al. *Eur J Haematol.* 2007;78:487–494. ©2007 Blackwell Publishing

Table 1 Average changes in laboratory values during the period of transfusion dependence in patients receiving deferoxamine treatment

Parameter	Intermittent (once/1.9 week)	Concurrent with transfusion	Daily/continuous
Serum ferritin[a,b] (ng/mL)	+2222.8 ($n = 36$)	+2204.8 ($n = 19$)	−1135.2 ($n = 9$)
SGOT[a,c] (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 ($n = 5$)

[3] Modified with permission from Takatoku et al. *Eur J Haematol.* 2007;78:487–494. ©2007 Blackwell Publishing

[a] Intermittent versus continuous, $P < 0.05$

[b] Continuous versus concurrent, $P < 0.01$

[c] Continuous versus concurrent, $P < 0.05$

Moreover, it has also been reported that iron chelation not only reduced iron burden and improved organ dysfunction, but also ameliorated the hemoglobin levels of iron-overloaded patients [7, 8]. Although the biological mechanism of the hematopoietic recovery remains to be elucidated, this fact indicates that iron itself negatively impacts on hematopoiesis, and in some conditions removal of iron burden from the hematopoietic environment can restore normal hematopoiesis.

Deferasirox is easily absorbed in the gastrointestinal tract and has an elimination half-life of 8–16 h, which means that deferasirox is continuously present in the plasma with once-daily dosing [9]. In a large Phase III trial, deferasirox was comparable with DFO at decreasing iron burden in β-thalassemic patients [10]. Deferasirox also reduced iron burden in patients with various anemias including MDS [11]. These findings indicate that oral iron chelators can improve patients' quality of life by ameliorating organ dysfunction and preventing iron damage, even improving hematopoiesis itself. Oral iron chelators are expected to prolong survival of transfusion-dependent patients.

4 Japanese guidelines for the treatment of iron overload in transfusion-dependent patients

The clinical significance of iron chelation is undeniable and requires attention. With the availability of deferasirox in Japan, the frequency of continuous treatment may be strengthened and many more patients can benefit from chelation therapy. To help optimal iron chelation therapy, the National Research Group on Idiopathic Bone Marrow Failure Syndromes drew up the Japanese guidelines for the treatment of transfusion-induced iron overload. To date, guidelines for iron overload have been developed in several countries [6, 12–14], and the Japanese guidelines were designed to align with the international guidelines (see the paper by Dr. Gattermann in this issue). The essential features of the Japanese guidelines are depicted in Fig. 3 and Table 2.

The contents of the guidelines are as follows:

Patients who may benefit from chelation therapy: The guidelines are applicable to transfusion-dependent patients with primary (MDS, AA, PRCA, MF, etc.) and secondary (chemotherapy-induced, etc.) bone marrow failure. Transfusion-dependent patients are defined as those receiving >2 RBC units/month for ≥6 months. Because organ dysfunction becomes symptomatic after a certain period of time, it is suggested that iron chelation therapy is offered to patients with an expected survival of more than 1 year. The international guidelines for MDS patients also recommend that they should have a life expectancy of ≥1 year.

Diagnosis of iron overload: After patients become transfusion dependent, regular examination of serum ferritin is required to monitor iron burden at least once every 3 months. For early diagnosis of organ dysfunction,

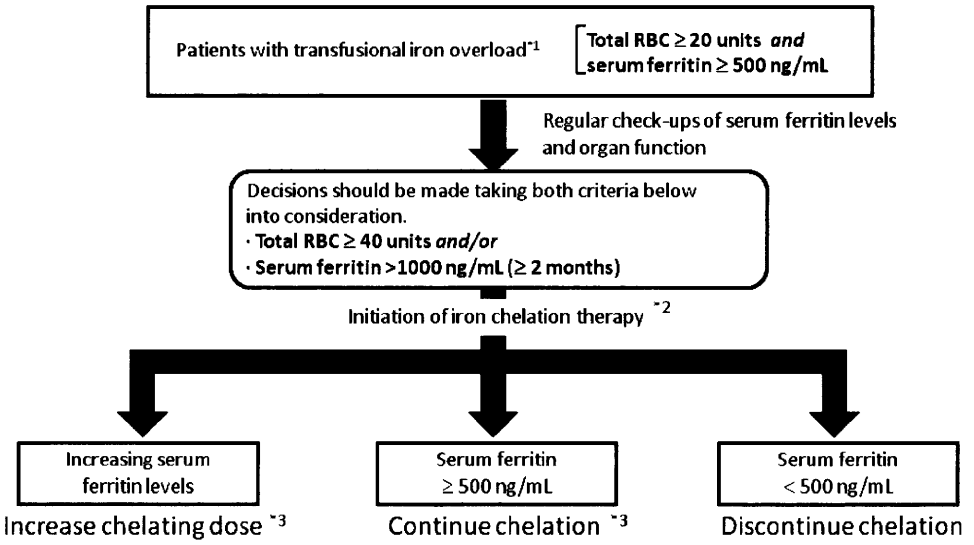

Fig. 3 A flow chart for the treatment of transfusion-dependent iron overload

*1 Patients who are transfusion dependent (≥ 2 RBC units/month for ≥ 6 months) and are expected to survive for >1 year.
*2 Monitoring serum ferritin levels at least once in 3 months is required.
*3 Regular check-ups of renal and hepatic function, and annual eye and hearing tests are necessary.

periodic check-ups of cardiac, hepatic and pancreatic endocrine functions are recommended.

Patients can be said to be iron overloaded when their serum ferritin levels reach >500 ng/mL and when they have received >20 Japanese RBC units (in pediatric patients, >50 mL/kg body weight). Severity of iron overload is determined by serum ferritin levels and organ dysfunction (Table 2, lower part).

Initiating iron chelation therapy: Administration of an iron chelator is the only recommended treatment for iron overload in patients with bone marrow failure. To initiate iron chelation therapy, confirmation of serum ferritin levels >1,000 ng/mL for more than 2 months, at least in two successive examinations, is recommended. The nationwide survey reported that more than 90% of patients who suffered from organ dysfunction had serum ferritin levels >1,000 ng/mL, and prevalence of hepatic dysfunction increases in parallel with ferritin levels [3] (Fig. 2). Therefore, a serum ferritin level >1,000 ng/mL is considered the appropriate point to initiate iron chelation. However, serum ferritin levels are not reliable in patients with inflammatory conditions such as Still's disease and hemophagocytic syndrome, or in those with malignancies. In these cases, transfusion history should be taken into account. Therefore, receiving a total of more than 40 Japanese RBC transfusion units (in pediatric patients, >100 mL/kg body weight) was included as another recommended criterion. As mentioned previously, about 75% of patients who received >40 RBC units have serum ferritin levels >1,000 ng/mL, indicating that 40 units of RBC transfusion can be a good indicator of transfusion-induced hyperferritinemia. However, transfusion history alone is also not reliable, because serum ferritin levels may not increase in patients with chronic bleeding and hemolysis. Furthermore, patients who have already discontinued transfusion therapy with successful treatment may not require iron chelation therapy. If neither of these two criteria is applicable, chelation therapy should not be started.

Target ferritin maintenance levels and adverse effects of iron chelators: During chelation therapy, monitoring of iron burden and organ functions should be continued. After initiating chelation therapy, serum ferritin levels should decrease, but if they continue to increase, even 3–6 months after starting treatment, an increase in dose is necessary. When patients are minimally transfusion dependent (<2 RBC units/month) or already free of transfusions, dose adjustment must be determined carefully.

It is recommended that serum ferritin levels are maintained at 500–1,000 ng/mL, and when ferritin levels are below 500 ng/mL at two successive examinations, chelators should be discontinued. As an excessive reduction in iron burden is harmful, the guidelines have determined this target value (500–1,000 ng/mL) with a safety margin.

As iron chelating agents can induce adverse effects on the kidney, liver and sensory organs [10], regular examination of renal and hepatic functions, and periodical (prior to treatment and annually after initiation) ophthalmologic examinations and hearing tests, are recommended. If an abnormal increase in serum creatinine level is noticed, the drug should be decreased or discontinued. In patients with a high risk of renal dysfunction, weekly monitoring of creatinine level is recommended, at least during the first

Table 2 Japanese guidelines for transfusional iron overload (main points)

Patients	Transfusion-dependent patients with bone marrow failure syndromes who are likely to survive for >1 year
Diagnosis of iron overload	1. Total RBC >20 units[a] (in pediatric patients, RBCs >50 mL/kg body weight) *and* 2. Serum ferritin >500 ng/mL
Criteria for initiating chelation therapy	1. Total RBC >40 units[a] (in pediatric patients, RBCs >100 mL/kg body weight) *and/or* 2. Serum ferritin >1,000 ng/mL Decisions should be made taking both criteria into consideration, especially for patients: –with chronic bleeding or hemolysis; –who no longer need RBC transfusions; –with complications that chronically raise serum ferritin levels independently of transfusion; e.g., Still's disease, hemophagocytic syndrome and malignancies
Target serum ferritin maintenance level	Serum ferritin 500–1,000 ng/mL

Classified severity of iron overload

Serum ferritin (ng/mL)	With normal organ function	With organ dysfunction
>500	Stage 1A	Stage 1B
>1,000	Stage 2A	Stage 2B
>2,500	Stage 3A	Stage 3B
>5,000	Stage 4A	Stage 4B

The severity of iron overload is defined by serum ferritin level and organ dysfunction (cardiac, liver and pancreatic endocrine dysfunction). The dysfunction must be considered to be related to iron overload; i.e., the organ dysfunction progresses as serum ferritin or transfusion burden increase

The criteria for specific organ dysfunction are as follows

–Cardiac dysfunction: LVEF <50%

–Hepatic dysfunction: abnormal transaminase levels, fibrosis and cirrhosis of the liver

–Pancreatic endocrine dysfunction: impaired glucose tolerance

[a] 20 and 40 units of the Japanese RBC transfusion correspond to 10 and 20 Western RBC units, respectively

month. Furthermore, if drug-induced hepatic injury is suspected, withdrawal of the drug with appropriate treatments is needed. It has been reported that iron chelators can cause hearing loss and cataracts. Therefore, if any signs of dysfunction are noticed a dose reduction or discontinuation of the drug is necessary and prompt consultation by an ophthalmologist or otorhinolaryngologist is required. In pediatric patients, annual monitoring of height, weight and state of secondary sex characteristics are needed for an early diagnosis of abnormal development.

5 Conclusions

The retrospective survey of transfusion-dependent patients revealed that the mortality rate is raised in heavily iron-overloaded patients, with liver and cardiac dysfunction being the primary cause of death [3]. Daily or continuous chelation therapy is effective in reducing iron burden and improving organ function, but practically, daily or continuous administration through parenteral injection is difficult. In Japan, a novel oral chelator, deferasirox, has recently been approved. Oral iron chelators can improve compliance of treatment and many more patients who need iron chelation may benefit from a reduction in iron burden and improvement of organ function, which ultimately may lead to the improvement of patients' prognosis and quality of life.

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Proposals for a Grading System for Diagnostic Accuracy of Myelodysplastic Syndromes

Akira Matsuda,[1] Itsuro Jinnai,[1] Yasushi Miyazaki,[2] Masao Tomonaga[2]

Abstract

Despite recent advances in cytogenetics and molecular research, universal biomarkers for the diagnosis of the myelodysplastic syndromes (MDS) are still lacking. It is not easy to diagnose MDS by morphology alone, particularly in patients with < 5% blasts in the bone marrow (BM) and normal karyotype. Therefore, the possibility of misdiagnosis and discordance among observers can occur. In order to resolve these problems, we propose a grading system for diagnostic accuracy of MDS. The diagnostic accuracy of MDS is graded into "definite," "probable," or "possible" in addition to "idiopathic cytopenia(s) of uncertain significance (ICUS)." The criteria of grading for diagnostic accuracy are a combination of (1) the frequency of blasts in BM, (2) grade of dysplasia (high, intermediate, or low), and (3) division of cytogenetics (abnormal, normal, or unknown). For quantitative morphologic evaluation of dysplasias, we classified morphologic dysplastic changes into highly specific category A (pseudo–Pelger-Huet anomaly, degranulation of neutrophils, micromegakaryocytes, and ringed sideroblasts) and less specific category B (dysplasias other than those in category A). We believe that diagnostic problems would be reduced by using our grading system and repeating BM examination at suitable intervals for patients who are allocated into the "possible" or "ICUS" categories, and this will make the vague margin of MDS category clearer.

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Key words: Cytogenetics, Dyserythropoiesis, Idiopathic cytopenia of uncertain significance, Pelger-Huet

Introduction

Myelodysplastic syndromes (MDS) are acquired clonal stem-cell disorders characterized by ineffective hematopoiesis with myelodysplasia[1] and are associated with a high risk of progression to acute leukemias.[2] Despite recent advances in cytogenetics and molecular research, universal biomarkers for the diagnosis of MDS are still lacking. It is not easy to diagnose MDS, particularly in patients with < 5% blasts in the bone marrow (BM) and normal karyotype. In such patients, the diagnosis mainly depends on morphologic examinations. Minimal morphologic requirements to diagnose MDS are well established but might not be accurate or leave too much room for subjectivity. Herein, we propose a grading system for the diagnostic accuracy in an attempt to reduce misdiagnosis and improve concordance among observers.

Background for Proposals

Exclusion of nonclonal disorders[3-6] with some myelodysplasia is crucial to the diagnosis of MDS. However, in patients with < 5% blasts in the BM and normal karyotype, it is not easy to distinguish MDS from such nonclonal disorders by morphology alone. In addition, judgments of dysplasia are subjective to a certain extent. Therefore, misdiagnosis and discordance among observers are likely to occur. In patients with hypoplastic BM, it is important to distinguish hypoplastic MDS from aplastic anemia (AA). Dyserythropoiesis (Dys E) is often found in patients with AA and cannot be used alone to distinguish MDS from AA.[7]

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We previously reported that the presence of hyposegmented mature neutrophils (Pelger), degranulation of neutrophils (agranular or hypogranular neutrophils; Hypo-Gr), and micromegakaryocytes (mMgk) in BM or peripheral blood (PB) were found in 76%, 30%, and 74% of MDS cases, respectively, whereas there was no AA case with these dysplasias.[8] We confirmed the specificity of these dysplasias in a different case series showing that Pelger ≥ 10% or mMgk ≥ 10% in BM was not found in the AA group (presented at the Ninth International Symposium on MDS). We also showed that, among patients with refractory anemia (RA) according to the French-American-British (FAB) classification[9] (FAB-RA), excluding MDS associated with isolated deletion of chromosome 5q (5q-syndrome), the presence of Pelger ≥ 10% or mMgk ≥ 10% in BM (15% and 14% of RA cases, respectively) were significantly related to the shorter overall survival (OS) and leukemia-free survival (LFS). The median OS and LFS of cases with Pelger ≥ 10% were 29 months and 36 months, respectively, and were significantly shorter than those without Pelger ≥ 10% (158 months and not reached, respectively; $P < .001$ in both). Micromegakaryocytes ≥ 10% showed similar effect on OS and LFS (23 months vs. 153 months for OS [$P < .001$] and 51 months vs. not reached for LFS [$P < .001$]).[10] The concordance rates of Pelger and mMgk were reasonably high among observers.[11] These dysplasias are much easier to detect, not only for expert morphologists but also for clinical hematologists in general. We considered that misdiagnosis and discordance would be avoided by enumerating these MDS-specific dysplasias.

Idiopathic cytopenia of uncertain significance (ICUS) was first proposed by Mufti et al at the Eighth International Symposium on MDS in Nagasaki, Japan, in 2005. If patients with normal karyotype and < 5% BM blasts do not show morphologic dysplasia (ie, < 10% of any cell lineage) and all other diseases have been ruled out as a cause of cytopenia, the patients are diagnosed with ICUS. The cytopenia(s) should persist for ≥ 6 months without any other cause identified. The criteria for ICUS was proposed in a recent publication by Valent et al.[12] Idiopathic cytopenia of uncertain significance might be a useful category for patients with unexplained cytopenia who do not fulfill the criteria of MDS (either of the FAB classification or the World Health Organization [WHO] classification[13]). Extensive study for this category in terms of MDS pathophysiology, particularly a molecular aspect, will clarify the clinical and pathophysiologic features of the ICUS category.

We previously compared the morphologic features between FAB-RA, excluding 5q-syndrome AA at the Ninth International Symposium on MDS, held in Florence, Italy, in 2007. One hundred patients with FAB-RA, excluding 5q-syndrome, were diagnosed by a joint review of a Japanese and German collaboratory study.[10,14] Forty patients with AA who registered to the Japanese AA and MDS Registration System of the National Research Group on Idiopathic Bone Marrow Failure Syndromes, Japan were diagnosed by the Central Review Working Group. In all patients with FAB-RA, the frequency of dysplasia was ≥ 10% in ≥ 1 lineage. Some (17%) patients with AA showed Dys E ≥ 10% in BM; Hypo-Gr ≥ 10%, Pelger ≥ 10%, or mMgk ≥ 10% were found only in the FAB-RA group. In addition, dysplasia ≥ 10% in ≥ 2 lineages was found only in the FAB-RA group. The number of megakaryocytes was markedly decreased in all patients with AA. The presence (≥ 5%) of blasts in BM was never found in the patients with AA.

Table 1 Prerequisite Criteria

Criteria
A. Constant cytopenia (≥ 6 months) in ≥ 1 of the following lineages:
Hemoglobin < 11 g/dL
Absolute neutrophil count < 1.5×10^9/L
Platelet count < 100×10^9/L
B. Less than 20% blasts in PB or BM and absence of cytogenetic findings related with acute myeloid leukemia with recurrent cytogenetic abnormalities*
C. Less than 1×10^9/L monocytes in PB
D. Exclusion of all other hematopoietic or nonhematopoietic disorders as primary reason for cytopenia
E. Exclusion of aplastic anemia. In case of hypoplastic BM, exclusion of aplastic anemia needs to be considered using morphologic findings and cytogenetic data.

A-E must be fulfilled.
*t(8;21)(q22;q22); (AML1/ETO), t(15;17)(q22;q12); (PML/RARα), and inv(16)(p13;q22) or (t16;16)(p13;q22); (CBFβ/MYH11).

Recently, minimal diagnostic criteria for MDS have been proposed by Valent et al.[12] They did not show a list of dysplastic cells in their criteria. We think a clear and definite list of dysplastic cells is necessary for diagnostic criteria. We propose a grading system for diagnostic accuracy of MDS by combining the results of our morphologic study presented at the Ninth International Symposium on MDS with the criteria proposed by Valent et al.

A Grading System for Diagnostic Accuracy of Myelodysplastic Syndrome

Exclusion of disorders with constant cytopenia(s) and some morphologic dysplasia(s) other than MDS is a prerequisite for diagnosing MDS. We propose that dysplasia(s) be divided into category A (high specificity) and category B (low specificity) for assessment of the frequency of dysplasia(s). A quantitative morphologic evaluation of category A or A + B is essential to start diagnosis of MDS. We then suggest a grading of dysplasia based on the enumeration and a division of cytogenetic findings. The criteria for grading of diagnostic accuracy are a combination of the frequency of blasts in BM, grade of dysplasia, and divisions of cytogenetics. The grades of diagnostic accuracy are divided into "definite," "probable," or "possible" in addition to "ICUS." Patients who are diagnosed as "definite," "probable," or "possible" should be classified according to the WHO classification for MDS.

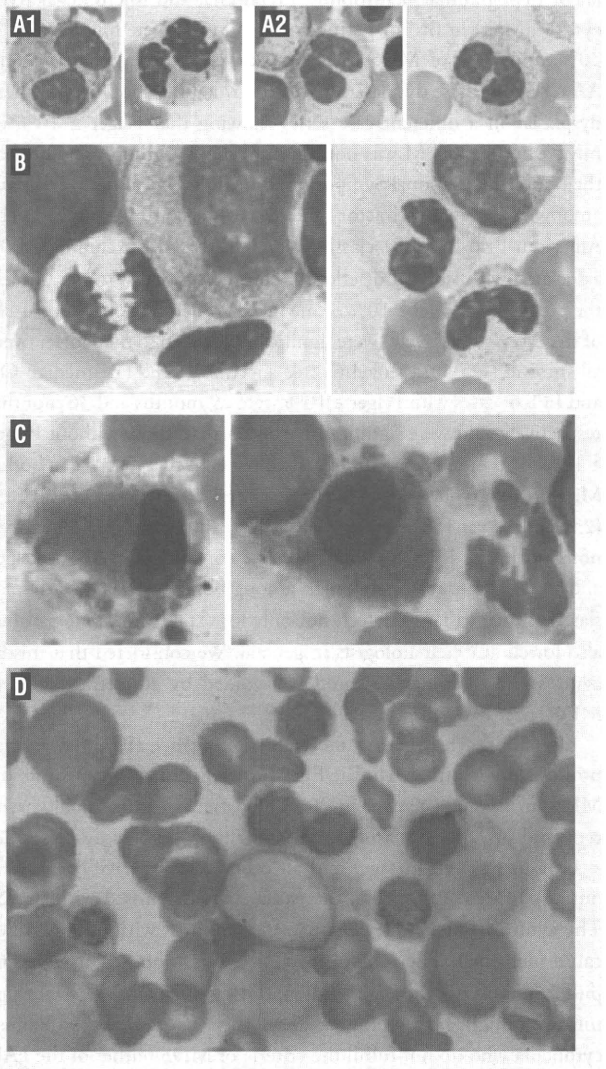
Step I: Exclusion Diagnosis of Disorders Other Than Myelodysplastic Syndrome

We modified the excellent prerequisite criteria proposed by Valent et al.[12] Table 1 shows our prerequisite criteria, consisting of the definition of constant cytopenias (≥ 6 months) and exclusion of disorders with constant cytopenias or some myelodysplasia. Acute myeloid leukemia (AML) should be excluded by frequency of blasts and cytogenetic findings. Bone marrow differential counts should be performed on 500 cells. Counting the number of monocytes in PB is necessary for the exclusion of chronic myelomonocytic

Table 2 Classification of Dysplasia

Category A
Granulocytic series
Hyposegmented mature neutrophils (Pelger)
Degranulation (agranular or hypogranular neutrophils; Hypo-Gr)
Megakaryocytic series
Micromegakaryocytes
Erythroid series
Ringed sideroblasts
Category B
Granulocytic series
Small size
Hypersegmentation
Pseudo–Chediak-Higashi granule
Megakaryocytic series
Nonlobulated nuclei
Multiple, widely separated nuclei
Erythroid series
Nucleus
Budding
Internuclear bridging
Karyorrhexis
Multinuclearity
Megaloblastoid change
Cytoplasm
Vacuolization

Figure 1 Dysplasia of Category A



(A) Hyposegmented mature neutrophils (Pelger). Two lobes are connected with a fine (1) or thin (2) filament. **(B)** Degranulation of neutrophils (agranular or hypogranular neutrophils; Hypo-Gr). **(C)** Micromegakaryocytes. **(D)** Ringed sideroblasts. **(A-D)** Provided by the National Research Group on Idiopathic Bone Marrow Failure Syndromes, Japan.

leukemia. To exclude nonclonal disorders (Table 1D), laboratory studies (eg, serum iron, ferritin, cobalamin, and folic acid levels; Coombs test; anti-nuclear antibody; thyroid function tests; etc) and abdominal ultrasonography are necessary.

For evaluations of BM cellularity, specimens of BM trephine biopsy must be examined. A BM biopsy of good quality and adequate length (≥ 1.5 cm) is necessary. Often, repeat BM examination is required to confirm the diagnosis when there is doubt about initial BM examination or if an inadequate sample was taken.[15] Because BM cellularity is highly age-dependent, hypocellularity is defined as < 30% in patients aged < 60 years or < 20% in patients aged ≥ 60 years.[16,17] In hypocellular BM, microscopic examinations for the assessment of dysplasias should be performed with ≥ 2 BM films, if necessary.

In patients with hypocellular BM, it is absolutely necessary to exclude AA to diagnose hypoplastic MDS (Table 1E). Dysplasia of BM cells, the percentage of blasts in BM, and abnormal localization of immature precursors (ALIP) are useful markers for this differentiation. As mentioned earlier, significant dysplasia in ≥ 2 lineages and > 10% of Hypo-G, Pelger, or mMgk strongly suggest MDS rather than AA. Abnormal localization of immature precursors is usually not found in AA but is found in MDS, and blasts in BM are usually < 5% in AA cases. In this regard, it is very difficult to diagnose hypoplastic RA cases that show dysplasia only in erythroid lineage. Morphologic evaluation alone might not be enough for the diagnosis, and other data such as cytogenetics will provide further useful information when they show MDS-specific karyotype (see Step VI). However, in some cases with hypoplastic BM, in particular those having dysplasia in a single lineage or at low percentage, careful course observation is necessary to make a diagnosis. On the other hand, the presence of paroxysmal nocturnal hemoglobinuria–type cells[18] or dysplasia in erythroid lineage alone does not support the diagnosis of MDS alone.

Step II: Classification of Dysplasia

Table 2 shows a classification of dysplasias into category A (highly specific) and category B (less specific), which is the thrust of our proposal. Dysplasias in Table 2 are modified from those described in the WHO classification, except for the periodic acid-Schiff (PAS) reaction for erythroid cells because the PAS reaction is no longer used routinely

in hematologic laboratories. As described earlier, Pelger, Hypo-Gr, and mMgk are highly specific to MDS when present at levels ≥ 10%. In addition, we think that the diagnostic value of ringed sideroblasts (RS) is similarly specific when present at a level of ≥ 15%. Dysplasias other than Pelger, Hypo-Gr, mMgk, and RS are less specific for MDS but, if present at ≥ 10%, are sufficient to suggest a diagnosis of MDS. Therefore, we think that the classification of dysplasias for the diagnosis of MDS is necessary and helpful for clinical hematologists in general. Quantitative assessment of category A or category B dysplasias is a basis for grading the accuracy of diagnosis of MDS. Four types of category A dysplasias are shown in Figure 1 (A-D) and Table 2. Category B dysplasias are shown in Table 2.

Pelger are hyposegmented (dumbbell-shaped) mature neutrophils. Two lobes are connected with a fine or thin filament (Figure 1A), and their chromatin structure is abnormally coarse. Hypo-Gr are neutrophils with a total or > 80% loss of neutrophilic granules in the cytoplasm (Figure 1B). Micromegakaryocytes are mono- or bi-nulceated megakaryocytes with a size less than that of normal promyelocytes and abundant platelet granule formation (Figure 1C). Ringed sideroblasts are erythroid cells with perinuclear siderotic granules occupying > one third of the nuclear margin or > 5 distinct siderotic granules in the perinuclear region (Figure 1D).

Step III: Assessment of Category-A Dysplasias

For the assessment of Pelger and Hypo-Gr, ≥ 100 mature neutrophils should be examined on BM films. The frequencies of Pelger and Hypo-Gr should be evaluated individually, not the sum of Pelger or Hypo-Gr. Because BM films frequently fail to stain optimally for neutrophil granules, observation of PB films is very helpful in confirming degranulation. In particular, when Hypo-Gr is the sole dysplasia in the absence of other dysplastic features, the assessment of Hypo-Gr should not be evaluated as a positive finding unless confirmed as mentioned earlier. Concerning the frequency of mMgk, ≥ 25 megakaryocytes should be examined on multiple BM films. When the megakaryocyte number is markedly reduced, detection of ≥ 3 mMgks is sufficient to regard this category-A dysplasia as ≥ 10%. In almost all patients with AA, megakaryocytes are absent or very few in number. For RS, ≥ 100 erythroblasts of all stages should be examined. Independent assessment of category-A dysplasias is necessary for grading of diagnostic accuracy of MDS.

Step IV: Assessment of Dysplasia A + B in Each Lineage

Concerning the frequencies of dysplasia A + B in each lineage, we suggest the microscopic methods as follows: ≥ 100 mature neutrophils, ≥ 25 megakaryocytes, and ≥ 100 erythroblasts in BM should be examined. The frequency of dysplasia in each lineage is evaluated by total dysplastic cells (%) showing category A or B. The frequency of Dys E is evaluated by the sum of frequency of RS on iron-stained films and that of category B on May-Giemsa–stained films. For example, when the frequency of RS and that of category B in erythroid lineage are 5% and 10%, respectively, the frequency of Dys E is calculated as 15%. The microscopic examinations for the assessment of dysplasia should be performed with multiple BM films if necessary. If the megakaryocyte number is markedly reduced, detection of ≥ 3 dysplastic megakaryocytes is sufficient to regard dysplasia A + B as ≥ 10%.

Table 3 Grade of Dysplasia

Dysplasia Grade
High (Defined as 1 or 2)
1. Pelger ≥ 10% or Hypo-Gr ≥ 10% plus mMgk ≥ 10%
2. RS ≥ 15%
Intermediate
Dysplasia (category A or B) ≥ 10% in 2-3 lineages
Low
Dysplasia (category A or B) ≥ 10% in 1 lineage
Minimal
Dysplasia (category A or B) 1%-9% in 1-3 lineages

Step V: Grade of Dysplasia

As shown in Table 3, the grade of dysplasia is divided into high, intermediate, low, or minimal. High is defined as follows: (1) when Pelger ≥ 10% or Hypo-Gr ≥ 10% plus mMgk ≥ 10% in granulocytic and megakaryocytic lineages or (2) when RS ≥ 15% in erythroid series. In order to classify a case as high by the existence of RS ≥ 15% alone, other sideroblastic anemias such as alcoholic anemia must be excluded. Intermediate is defined as dysplasia A + B ≥ 10% in 2-3 lineages. Low is defined as dysplasia A + B ≥ 10% in a single lineage. Minimal is defined as 1%-9% of dysplasia A + B in 1-3 lineages.

Step VI: Division of Cytogenetic Findings

The divisions of cytogenetic findings are abnormal, normal, or unknown. Abnormal is defined as typical clonal abnormal karyotypes recurrently found in MDS (del[5q], –7/7q–, +8, del[20q], complex, and others) with high frequency as reported by Haase et al.[19] This definition is similar to that of typical chromosome abnormalities proposed by Valent et al.[12] t(8;21)(q22;q22), t(15;17)(q22;q12), inv(16)(p13;q22), and t(16;16)(p13;q22) are not included in the abnormal division even when the blast percentage is < 20%. Patients with these cytogenetic abnormalities are diagnosed with AML with recurrent cytogenetic abnormalities according to the WHO classification. Normal is defined as normal karyotype by analyzing > 10 metaphases. When cytogenetic findings are not available because of poor samples or an absence of metaphases, cases are labeled unknown.

Step VII: Grade of Diagnostic Accuracy

Table 4 shows the criteria for grading the diagnostic accuracy. These criteria are a combination of the frequency of blasts in BM, grade of dysplasia, and division of cytogenetics. The grade of diagnostic accuracy is divided into definite, probable, possible, and ICUS. The reliability of the diagnosis as MDS is high in the following order: definite, probable, and possible. In patients diagnosed as possible or ICUS, the diagnostic accuracy is low; thus, re-examination at suitable intervals is required to confirm the diagnosis. In such cases, the diagnosis might become more accurate when re-examination provides a result of definite or probable or remains possible or ICUS for a long period. The observation of the clinical course of patients with possible or ICUS will provide important information on the pathophysiologic similarity or dissimilarity between these diagnostic groups based on diagnostic grading.

Table 4 Grade of Diagnostic Accuracy for Myelodysplastic Syndromes

Grade	Blasts in BM (%)	Grade of Dysplasia	Division of Cytogenetics
	5-19	High, intermediate, low	Any
MDS Definite	0-4	High, intermediate, low	Abnormal
	0-4	High	Any
MDS Probable	0-4	Intermediate	Normal or unknown
MDS Possible	0-4	Low	Normal or unknown
ICUS	0-4	Minimal or none	Normal or unknown

Step VIII: Subtyping According to the World Health Organization Classification

Patients who are diagnosed as definite, probable, or possible should be classified according to the WHO classification. Patients classified in the possible category in our system are diagnosed as RA- or MDS-unclassified (refractory neutropenia or refractory thrombocytopenia) according to the WHO classification. However, the diagnosis of these patients should be tentative. The diagnosis according to the WHO classification of these patients must be decided by re-examination of BM at suitable intervals.

Discussion

Diagnosis of MDS must be as accurate and consistent as that of acute leukemia. However, the judgment of morphologic dysplasias has the inherent problem of the subjective nature of the morphology, and the objectivity of the evaluation has long been problematic. For the elimination or reduction of these problems, we propose a grading system for diagnostic accuracy of MDS. Category-A dysplasias are much easier for clinical hematologists to detect on routine BM diagnosis. Category-B dysplasias are sufficiently reliable when observed along with category A. Therefore, quantitative, morphologic evaluation by using this system will facilitate the routine diagnosis of MDS.

Exclusion of nonclonal disorders with minimal or no morphologic dysplasia is extremely important for the differential diagnosis of MDS as described in Step I. We believe our system is also useful in this respect. If there is no certain evidence for this exclusion diagnosis of non-MDS disorders despite the careful performance of other laboratory examinations, the possibility of misdiagnosis would likely be markedly reduced by using this grading system for diagnosis. Repeat BM examination at suitable intervals for patients graded as possible or ICUS will make clearer the still-vague margin of MDS as a clinical entity. It is also important to identify differences if present in responses to new drugs such as lenalidomide and hypomethylating agents.

Of course, our diagnostic schema still requires validation and demonstration of reliability, hopefully in 2 populations or a split-sample cohort. Long-term observation of MDS cases diagnosed with our proposal is also necessary for the evaluation of this proposal. Recent techniques in the detection of genetic abnormalities such as fluorescence in situ hybridization and single nucleotide polymorphism arrays[20] expand cytogenetic data of MDS. Although universal biomarkers for the diagnosis of MDS are still lacking, new data on genetic abnormality of MDS will be quite useful for accurate diagnosis and understanding the biology of MDS. In conclusion, until the discovery of universal biomarkers for entire MDS or subtypes of MDS, this diagnostic grading system could be useful for clinical routine work.

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bjh review

Acquired pure red cell aplasia: updated review of treatment

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Summary

Pure red cell aplasia (PRCA) is a syndrome characterized by a severe normocytic anaemia, reticulocytopenia, and absence of erythroblasts from an otherwise normal bone marrow. Primary PRCA, or secondary PRCA which has not responded to treatment of the underlying disease, is treated as an immunologically-mediated disease. Although vigorous immunosuppressive treatments induce and maintain remissions in a majority of patients, they carry an increased risk of serious complications. Corticosteroids were used in the treatment of PRCA and this has been considered the treatment of first choice although relapse is not uncommon. Cyclosporine A (CsA) has become established as one of the leading drugs for treatment of PRCA. However, common concerns have been the number of patients treated with CsA who achieve sustained remissions and the number that relapse. This article reviews the current status of CsA therapy and compares it to other treatments for diverse PRCAs.

Keywords: pure red cell aplasia, corticosteroids, cyclosporine A, cyclophosphamide, alemtuzumab, rituximab.

Pure red cell aplasia (PRCA), a disorder first characterized in 1922 (Kaznelson, 1922), is a syndrome characterized by severe normochromic, normocytic anaemia associated with reticulocytopenia and absence of erythroblasts from an otherwise normal bone marrow. PRCA may appear as a congenital disorder or occur as an acquired syndrome. The acquired form of PRCA presents either as an acute self-limited disease, predominantly seen in children, or as a chronic illness that is more frequently seen in adults. It may present as a primary haematological disorder in the absence of any other disease, or secondary to parvovirus infection, collagen vascular disease, leukaemia, lymphoma, thymoma, solid tumors, treatment with recombinant human erythropoietin (EPO) or other drugs, ABO-incompatible haematopoietic stem cell transplantation and pregnancy. Depending on the cause, the course can be acute and self-limiting or chronic with rare spontaneous remissions (Dessypris, 1988; Dessypris & Lipton, 2004).

Primary, or secondary PRCA not responding to treatment of the underlying diseases, is treated as an immunologically-mediated disease, based on a number of studies implicating a pathological role of serum auto-antibodies, natural killer (NK) cell-mediated or T lymphocyte-mediated effects impairing various stages and mechanisms of erythropoiesis as extensively reviewed by Fisch *et al* (2000). The major objective in the treatment of PRCA is to induce a remission with the recovery of erythropoiesis, thus providing relief from transfusions and avoiding transfusion-associated problems. The therapeutic plan usually focuses on the sequential use of various immunosuppressive therapies until a remission is obtained. Remissions have been achieved by treatment with corticosteroids (CS), cyclophosphamide (CY), cyclosporine A (CsA), antithymocyte globulin (ATG), splenectomy, and plasmapheresis (Dessypris & Lipton, 2004). More recently, the efficacies of the anti-CD20 monoclonal antibody, rituximab (Zecca *et al*, 2001), and anti-CD52 monoclonal antibody, alemtuzumab (Willis *et al*, 2001), to induce remissions of therapy-resistant PRCA have also been reported.

In general, remission can be easily achieved in the majority of patients. To date, the efficacy of CS, CY and CsA for patients with primary or secondary PRCA has been reported to be between 30–62%, 7–20% and 65–87%, respectively (Clark *et al*, 1984; Dessypris, 1988; Raghavachar, 1990; Marmont, 1991; Lacy *et al*, 1996; Mamiya *et al*, 1997). The efficacy of a combination of CY and CS for refractory patients has been reported to be between 40–60% (Clark *et al*, 1984; Dessypris, 1988, Mamiya *et al*, 1997). Since the initial cases were successfully treated by Totterman *et al* (1984), CsA has established itself as one of the leading drugs for the treatment

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of PRCA. However, concern has centred around the precise number of patients treated with CsA who achieve a sustained remission and the number who relapse. In 1988, Dessypris pointed out that treatment of PRCA with CsA appeared to be very promising, but that such treatment should be considered still experimental, and that further studies were necessary to determine the effectiveness of this drug, the optimal and least toxic dosage, the minimum duration of therapy for induction of remission, and whether or not there was a need for maintenance treatment (Dessypris, 1988). An advantage of CsA therapy for PRCA has long remained unclear, as comparing one therapeutic approach to another has been almost impossible because the disease is so rare that controlled studies could not be performed. However, the number of patients treated with CsA has accumulated over two decades, which made it possible to conduct an analytical study. The present paper reviews the current status of CsA therapy, comparing it to other treatments for the diverse types of acquired PRCA except for transient erythroblastopenia of childhood.

Diagnosis and initial evaluation

Pure red cell aplasia in adults can be easily diagnosed when isolated anaemia, in the presence of normal white cell and platelet counts, is associated with a marrow of normal cellularity in which there is an almost complete absence of erythroblasts but normal myeloid cells and megakaryocytes (Dessypris & Lipton, 2004). The classification of the clinical course (acute or chronic) and pathogenesis, such as secondary or idiopathic (no definite underlying disease) is essential to select the optimal therapeutic modality. Evaluations for the possible causes of PRCA should include a previous history of drug use and toxins or infections, liver and kidney functions, immunological examination including auto-antibodies, a bone marrow examination including morphology, chromosome and rearrangement of T cell receptor (TCR) analysis, peripheral-blood flow cytometry, virological examination including parvovirus B19 DNA, and computed tomography and/or magnetic resonance imaging examinations to rule out the presence of thymoma and neoplasms.

Today, a careful assessment of the increase of large granular lymphocytes (LGLs) is especially critical and an analysis of immunophenotype and TCR rearrangement of lymphocytes may be essential for ruling out LGL leukaemia, also referred to as granular lymphocyte proliferative disorders (GLPD) (Oshimi *et al*, 1993) or lymphoproliferative disease of granular lymphocytes (LDGL) (Go *et al*, 2001). LGL leukaemia was the most common underlying disease of secondary PRCA in a single institutional study from the United States, and the second most common cause in Japan (Lacy *et al*, 1996; Mamiya *et al*, 1997; Sawada *et al*, 2007). Since the diagnosis of LGL leukemia is somewhat difficult in patients without lymphocytosis, this group of patients can be misdiagnosed as idiopathic PRCA although LGL leukemia-associated PRCA may require a different treatment for the primary disease. It is a heterogeneous disorder characterized by a persistent increase in the number of peripheral blood LGLs, and the majority of patients have a clonal rearrangement of T-cell receptors (Oshimi *et al*, 1993; Semenzato *et al*, 1997; Chan *et al*, 2001). Clonal disorders of LGLs arise from either mature T lymphocytes or NK cells, and may be indolent or behave as an aggressive disease. T-cell LGL leukaemia is the most common form of clonal LGL disorders and most cases behave in an indolent fashion. Neutropenia is the most frequent cytopenia in T-cell LGL leukaemia, and anaemia occurs in 48% of the patients (Loughran, 1993; Lamy & Loughran, 1998, 2003).

The evidence of a granular lymphocytosis greater than 2×10^9/l lasting for more than 6 months has been regarded as the criteria for defining the disease (Loughran & Starkebaum, 1987; Semenzato *et al*, 1987; Oshimi, 1988). However, the normal range for peripheral blood LGL counts is $0{\cdot}223 \pm 0{\cdot}099 \times 10^9$/l (Loughran *et al*, 1987) and clonal disease has been documented in 8% of patients when absolute LGL counts are between 0·6 to $1{\cdot}0 \times 10^9$/l (Loughran, 1993). Thus, an expansion of a restricted LGL subset demonstrates the diagnosis of LGL-leukaemia and a 6-month follow-up criterion is not necessary when clonality is established (Semenzato *et al*, 1997). The characteristic finding is the presence of increased numbers of LGL, usually identified by a greater size than normal lymphocytes, abundant pale cytoplasm, and prominent azurophilic granules. However, these features may vary, even among cells from the same patient (Loughran, 1993). The granulation can range from fine to coarse, and some cells may have otherwise characteristic features but lack granules (sometimes called large agranular lymphocytes) (Bassan *et al*, 1986). Occasionally, clonally expanded lymphocytes with a characteristic $CD3^+$, $CD57^+$ phenotype may not have LGL morphology on a peripheral smear (Ahern *et al*, 1990) but may represent *in vivo* antigen-activated cytotoxic effector T cells. An increase of $CD3^+/CD56^-$ or $CD3^-/CD56^+$ cells by peripheral-blood flow cytometry and/or an inverted $CD4^+/CD8^+$ cell ratio (<1·0) suggests the existence of LGL leukaemia (Gonzales-Chambers *et al*, 1992).

Initial management

During the initial evaluation, red cell transfusions can be given as necessary. In cases supposed to be primary idiopathic PRCA, it would be preferable to wait for at least a month before instituting specific treatment, with the rationale that 10–12% of PRCA patients run a short and self-limited course (Dessypris, 1988). If, after such a waiting period, no signs of recovery of erythropoiesis appear, specific treatment should be instituted. Many of the secondary PRCAs are due to drugs and disappear when the drug is stopped. Those secondary to parvovirus B19 can be treated with intravenous immunoglobulin. Secondary PRCA not responding to treatment of the underlying diseases and primary PRCA are treated as immunologically-mediated diseases.

Immunosuppressive therapy

Corticosteroids

Corticosteroids (CS) were the first immunosuppressive drugs used in the treatment of PRCA and so far have been considered the treatment of first choice, especially in young adults (Clark *et al*, 1984; Dessypris, 1988; Charles *et al*, 1996; Dessypris & Lipton, 2004). The details of CS therapy are described elsewhere (Dessypris, 1988; Dessypris & Lipton, 2004). In an era when CsA was not yet available, Clark *et al* (1984) reported the largest series of PRCA patients receiving immunosuppressive therapy and showed that 10/27 (37%) patients with acquired PRCA responded to CS within a mean period of 2·5 weeks. Comparable results of CS treatment were obtained at other institutions, ranging from 30–62% (Dessypris, 1988; Raghavachar, 1990; Marmont, 1991; Lacy *et al*, 1996; Mamiya *et al*, 1997). One of the important drawbacks of CS is that relapse is not uncommon: 80% of patients relapsed, as the dosage was tapered, during the 24 months after remission (Clark *et al*, 1984). The principal reason for discontinuing the drug, despite subsequent recurrence of anaemia, was the presence of unacceptable side effects, such as myopathy, infection, hyperglycemia, and compression fractures at the dose required to maintain remission. Treatment of relapses was successful, with 10/13 (77%) patients entering a second or third remission, and the median survival in patients with primary idiopathic PRCA was 14 years (Clark *et al*, 1984). Cytotoxic drugs administered in combination with CS were the most effective form of treatment in this study, producing 18/32 (56%) remissions. Although such vigorous immunosuppressive treatment is capable of inducing and maintaining remission in a majority of patients, it carries increased risks of serious infections, malignancy, and sterility (Clark *et al*, 1984). Thus, an individualized approach to management of PRCA has been widely accepted, i.e. escalating therapy in proportion to the severity of the disease for those patients who have failed CS therapy.

Cyclosporin A

Raghavachar (1990) reviewed the treatment of PRCA, focusing on the results of cyclosporin A (CsA) therapy, in 43 patients. He showed that the overall response rate to CsA is excellent (65%) and proposed that CsA should be the first drug to be given in acquired PRCA. Of note is that a high dosage was used in order to obtain these results (12 mg/kg per day). Comparable results of CsA treatment have been obtained at other institutions, ranging from 65–87% (Dessypris, 1988; Marmont, 1991; Means *et al*, 1991; Lacy *et al*, 1996; Mamiya *et al*, 1997; Sawada *et al*, 2007). Mamiya *et al* (1997) reviewed the clinical features of 150 patients with acquired PRCA in Japan. In their surveillance, CsA was given to 38 patients in a daily dose of 200–600 mg (most often 200–300 mg) and 31 (82%) showed haematological recovery. The response rate to CsA was 87% in the patients with primary PRCA and 73% in those with secondary PRCA, which encouraged them to recommend CsA therapy as first-line therapy for this disease.

Recently, The Japan PRCA Collaborative Study Group conducted a nationwide survey in Japan between 1990 and 2006 (Sawada *et al*, 2007). From a total of 185 patients, consisting of 73 primary idiopathic and 112 secondary PRCA cases, 62 patients with primary idiopathic PRCA were evaluated, which is the largest and the longest follow-up study so far. Although a retrospective one, this study, for the first time, answered many of the unknown questions concerning CsA therapy. The remission induction therapies for these patients by CsA and CS produced remissions in 74% and 60% of patients, respectively. The initial dose of CsA for the responding patients was 4·8 ± 1·2 mg/kg (mean ± SD, $n = 23$) with a range of 2·9–7·6 mg/kg body weight. Patients treated with CsA alone ($n = 23$) became transfusion-independent by 82 ± 200 d, with a range up to 910 d, after the start of therapy. Fifteen patients (65%) achieved transfusion-independence within 2 weeks, 17 patients (74%) within 1 month and 18 patients (78%) within 3 months. Salvage immunosuppressive treatment achieved remissions in 58 patients (94%). Forty-one and 15 patients were maintained on CsA ± CS (CsA-containing group) or CS alone (CS-group), respectively. The median relapse-free survival (RFS; estimated as transfusion-free survival) in the CsA-containing group was 103 months, significantly longer ($P < 0·01$) than that seen in the CS-group (33 months). Thus, combined CsA therapy can sustain a longer duration of initial remission than CS, however, discontinuation of maintenance therapy was strongly correlated with relapse ($P < 0·001$) and caused relapses with a median of 3 months with a range of 1·5–40 months. In contrast, 88% of relapses in the CS-group occurred during maintenance prednisolone (PSL) therapy (Sawada *et al*, 2007).

Tötterman *et al* (1989) also reported that PRCA patients did not remain in remission after CsA was stopped. An important question is whether or not the maintenance of patients in remission may have a beneficial influence on survival. One study (Sawada *et al*, 2007) reported an estimated median OS of the CsA-containing group of 12 years, which was not significantly different than the CS-group, while the 10-year OS in all patients was 95% and the median OS had not yet been reached in all patients. Of importance is the fact that the CsA is required to maintain remissions (Sawada *et al*, 2007) and the decreased probability of relapse and requirement of red cell transfusions reduces the dangers of hemolysis, infections and iron overload with possible superoxide damage to body tissues. CsA is more expensive than CS and requires renal function to be monitored, but it seems to be important to prevent relapses and to sustain remissions in primary idiopathic PRCA. Although higher doses of CsA, such as 12 mg/kg per day have been used for patients with PRCA (Raghavachar, 1990), this dosage has been toxic for Japanese PRCA patients. Since cytochrome P-450 isoenzymes, involved in CsA metabolism, have a variable frequency of a reduced function allele depending on race and each individual (Bladford, 2004), the

most important therapeutic index should be trough CsA levels. Caucasian patients with anti-EPO antibody-related PRCA have been successfully treated with CsA alone at a dose of 200 mg/d (or 100 mg twice daily) (Verhelst *et al*, 2004; Rossert *et al*, 2005). Since organ transplantations have shown that long-term immunosuppression is associated with post-transplant malignancies (Cattran *et al*, 1995; Young *et al*, 2006), continuous and careful follow-up is required for patients receiving long-term CsA therapy.

Cytotoxic immunosuppressive drugs

Patients with an absolute contraindication for CsA or patients refractory to CsA may be treated with CS or a combination of CS and other immunosuppressives. Cyclophosphamide (CY) has been the principal alkylating agent utilized as an immunosuppressive drug in PRCA. The details of CY therapy are described elsewhere (Dessypris, 1988; Dessypris & Lipton, 2004). The initial dosage of CY is 50 mg/d p.o. and PSL at a dose of 20–30 mg/d is added in the absence of any contraindication. If the white blood cell and platelet counts allow, it is increased by 50 mg weekly or biweekly to a maximum of 150 mg daily until remission occurs or bone marrow suppression develops. The mean time to response is approximately 11 to 12 weeks with an overall response rate of 40 to 60%. When response occurs, the dose of PSL is tapered, and then the dose of CY is progressively decreased and eventually discontinued after 3–4 months from the time of normalization of haematocrit.

The duration of remission induced by CY seems to be prolonged as compared to remissions induced by CS (Clark *et al*, 1984; Firkin & Maher, 1988; Go *et al*, 2001). LGL leukaemia-associated PRCA has been primarily treated with chemotherapy, such as CY with or without CS, CsA, CS or methotrexate (Dhodapkar *et al*, 1994; Loughran *et al*, 1994; Lacy *et al*, 1996; Yamada *et al*, 1997; Sood *et al*, 1998; Hamidou *et al*, 2000; Go *et al*, 2001; Battiwalla *et al*, 2003; Osuji *et al*, 2006). The combination of CY plus CS is associated with a longer duration of response than CS alone (Dhodapkar *et al*, 1994; Lacy *et al*, 1996; Go *et al*, 2001). The overall response to initial CY ± CS therapy has been reported to be 66 to 100 % (Yamada *et al*, 1997; Go *et al*, 2003) and the median duration of response is 32 months (Go *et al*, 2003).

In one study, none of the patients with a response to cytotoxic agents had relapses (Lacy *et al*, 1996), but the other studies reported that a substantial number of patients relapsed when the CY was withdrawn (Zaentz *et al*, 1976; Clark *et al*, 1984). Maintenance CY therapy may prevent relapse, however, recognition of a variety of toxicities, particularly concerns about the long-term risk of malignancy and gonadal toxicity, often lead clinicians to consider less toxic alternative medications whenever possible (Csuka *et al*, 1986; Hoffman *et al*, 1992). These toxicity risks from alkylating agents are related to the cumulative dose of the medication (Reinhold-Keller *et al*, 2000) and the duration of therapy (Radis *et al*, 1995). Thus, the best role of CY therapy for PRCA might be to induce remissions using oral treatment lasting not longer than six months, with a switch to less toxic medication, such as CsA, for maintenance, but no controlled studies exist and this is purely speculative. It has been reported that crossover to azathioprine was effective in patients initially unresponsive to CY and vice versa (Firkin & Maher, 1988).

Anti-thymocyte globulin

In the largest series of nine PRCA patients treated with anti-thymocyte globulin (ATG) at a dose of 15 mg/kg per day for 10 d, six responded to therapy, five with normal haematocrits and one with a stable haematocrit of 32% (Abkowitz *et al*, 1986). Three remained in complete remission and two relapsed, which suggests that ATG is an effective form of treatment. However, this is an expensive and confining therapy that requires hospitalization because of a possible anaphylactic reaction.

Alemtuzumab

The anti-CD52 monoclonal antibody alemtuzumab (Campath-1H) is a humanized $IgG_{1\kappa}$ monoclonal antibody directed against the CD52 antigen, present on B and T lymphocytes, NK cells, monocytes/macrophages, dendritic cells and eosinophils but not on human haemopoietic stem cells (Hale, 2001). The rationale behind the use of alemtuzumab in refractory cytopenias is that T-lymphocytes are thought to play an important role in the pathogenesis of autoimmune cytopenias, as they are involved in the control of expansion of immunoglobulin-producing, auto-reactive B-lymphocyte clones (Willis *et al*, 2001). Willis *et al* (2001) reported the effect of treatment with alemtuzumab followed by CsA administration in 21 patients with severe and life-threatening autoimmune cytopenias including four patients with PRCA. A response was seen in 2/4 patients with PRCA, although one patient relapsed at 7 months when the CsA blood level was suboptimal. One patient with PRCA in association with low-grade non-Hodgkin lymphoma died from high-grade transformation at 16 months while still in remission from the PRCA. Ru and Liebman (2003) reported two patients with chronic lymphocytic leukaemia (CLL) and a CD8 T-LGL leukaemia who were refractory to multiple treatments for PRCA. When both patients were treated with alemtuzumab, there was a rapid increase in the reticulocyte count that occurred as early as the third infusion. At the time reporting, both patients had been in PRCA remission for 9 and 5 months, respectively. These results indicated that alemtuzumab is an alternative option in the treatment of patients with refractory PRCA, however, relapse can occur and a significant number of patients need maintenance therapy with CsA after alemtuzumab treatment. Therefore, it remains uncertain whether alemtuzumab can induce a maintenance-free haematological response in PRCA. The use of alemutuzumab should be limited to patients with PRCA who are refractory to conventional immunosuppressive

therapy because of the limited information and high risk of infections.

Rituximab

Rituximab is a genetically engineered chimeric mouse/human monoclonal antibody that targets the CD20 molecule present on mature B cells, which are the precursors of autoantibody-producing plasma cells. Rituximab can selectively deplete B-cells by mechanisms including antibody-dependent cell-mediated cytotoxicity, complement-mediated cytotoxicity and inhibition of cell proliferation with direct induction of B-cell apoptosis (Smith, 2003). Rituximab has been found to be useful in treating primary autoimmune hemolytic anaemia and thrombocytopenia (Quartier *et al*, 2001; Stasi *et al*, 2001) and several reports have shown that PRCA was successfully treated with rituximab in patients with B-cell lymphoproliferative disorders mainly consisting of CLL (Batlle *et al*, 2002; Ghazal, 2002; Gupta *et al*, 2002; Hegde *et al*, 2002; Ru & Liebman, 2003; Pantelidou *et al*, 2004; Narra *et al*, 2006). The maximum time for onset of response was 4 weeks. In two cases, where PRCA and the primary disorder were simultaneously diagnosed, rituximab led to response in both PRCA and the primary disorder (Narra *et al*, 2006). One patient had a response to rituximab lasting only 4 weeks, but then responded to alemtuzumab (Ru & Liebman, 2003). No significant side effects were reported in any of these patients treated with rituximab. Dungarwalla *et al* (2007) have shown that the results of rituximab therapy in patients with severe, resistant and life-threatening PRCA refractory to conventional immunosuppression are disappointing. In their pilot study, all three of these patients with idiopathic PRCA did not respond to a conventional dose of rituximab (375 mg/m^2) weekly for 4 weeks.

Other therapeutic options

Intravenous immunoglobulin

In immunocompromised hosts, such as recipients of organ transplantation (Wong *et al*, 1999), patients infected with human immunodeficiency virus (HIV) (Frickhofen *et al*, 1990) or receiving chemotherapy (Song *et al*, 2002; Isobe *et al*, 2004), acute or chronic anaemia can develop following parvovirus B19 infection due to the lack of the production of specific antibodies. Chronic B19 infection-related PRCA is a treatable anaemia and demonstration of the virus DNA in blood by the polymerase chain reaction or dot-blot hybridization assays is essential. Intravenous immunoglobulin (IVIG) contains neutralizing antibody against parvovirus B19 and has been reported to be effective for chronic B19 infection-related anaemia in immunocompromised hosts. Recurrence of anaemia is common in HIV-infected patients with low CD4$^+$ T-cell counts ($<0{\cdot}08 \sim 0{\cdot}1 \times 10^9$/l) and requires additional IVIG (Ramratnam *et al*, 1995). Anti-retroviral therapy may resolve chronic anaemia in HIV-infected patients (Mylonakis *et al*, 1999). IVIG may also be effective for PRCA due to parvovirus B19 in patients treated with rituximab (Sharma *et al*, 2000; Song *et al*, 2002). Alemtuzumab causes prolonged, severe CD4 and CD8 lymphopenia (Keating *et al*, 2002) and PRCA due to parvovirus B19 infection in a patient with cutaneous T-cell lymphoma treated with alemtuzumab has been reported (Herbert *et al*, 2003). Tacrolimus (FK506) is often associated with chronic B19 infection-associated anaemia in organ transplant recipients and cessation of tacrolimus or replacement with other immunosuppressants results in an improvement of anaemia (Wong *et al*, 1999).

Thymectomy

Surgical resection of thymomas has been recommended as the initial treatment of thymoma-associated PRCA, with an expected haematological response rate of 25–30% (Zeok *et al*, 1979). In recent reports, resection of the thymoma by itself was effective in remitting the anaemia in only a small percentage of patients (Mamiya *et al*, 1997; Thompson & Steensma, 2006) and a significant fraction of patients developed PRCA after thymectomy (Suzuki *et al*, 2003; Thompson & Steensma, 2006; Hirokawa *et al*, 2008). Although the pathogenesis of thymoma-associated PRCA remains to be elucidated, there exist two potential mechanisms. Thymoma itself alters the subset and/or the repertoires of T lymphocytes, leading to the production of autoaggressive T-cell clones (Hoffacker *et al*, 2000). Another intriguing possibility is that thymectomy may represent a risk for the development of systemic autoimmune disorders over years (Gerli *et al*, 1999).

Thompson and Steensma (2006) reported that surgical resection of thymoma was insufficient for normalization of erythropoiesis in all 13 patients so treated, but immunosuppressive therapy was effective as an adjuvant treatment. Immunosuppressive therapy including CS, CY and CsA has been reported to be useful in cases with thymoma-associated PRCA (Marmont *et al*, 1975; Garcia Vela *et al*, 1993; Charles *et al*, 1996; Thompson & Steensma, 2006), but optimal management of this disorder has remained unclear. Recently, it has been reported that thymoma-associated PRCA showed an excellent response to CsA and CsA-containing regiments were effective in preventing relapse (Hirokawa *et al*, 2008).

Splenectomy, plasmapheresis, and bone marrow transplantation have been used on rare occasions and can be tried if all else fails.

Peptide-based EPO receptor agonist

Krantz and Kao (1967), for the first time, reported that plasma from a patient with PRCA inhibited haem synthesis by the patient's own bone marrow cells *in vitro*. The serum of patients with anti- EPO antibody-related PRCA also inhibited the growth of erythroid progenitor cells *in vitro* (Casadevall *et al*, 2002). PRCA due to autoantibodies against endogenous EPO occurs but is rare in patients who have never been treated with

recombinant human EPO (rhEPO). rhEPO-related PRCA reached a peak incidence mainly in Europe in 2001 to 2002, largely related to a change of formulation, and of uncoated rubber stoppers (leachates present), in a particular rhEPO product, Eprex, and subcutaneous administration (Casadevall *et al*, 2002; Rossert *et al*, 2004).

The two most important initial steps in the management of anti-EPO antibody-mediated PRCA are transfusions for symptomatic anaemia and stopping the administration of rhEPO (Rossert *et al*, 2004). Since PRCA in this setting is immune-mediated, and since spontaneous remissions after cessation of rhEPO therapy are rare, immunosuppressive therapy should be provided in most cases (Verhelst *et al*, 2004; Rossert *et al*, 2005). Anti-rhEPO antibodies cross-react not only with the endogenous hormone, but also with all rhEPO molecules, including darbepoetin alfa (Casadevall *et al*, 2002). Rechallenge with rhEPO preparations may cause an anamnestic antibody response, making it less possible for the antibody to either spontaneously disappear or return to clinically unimportant levels, and may induce the formation of allergic skin and systemic reactions (Weber *et al*, 2002).

Recently, a novel peptide-based EPO receptor agonist called Hematide, which does not cross-react with anti-EPO antibodies, has been developed (Stead *et al*, 2006). Hematide is a synthetic, dimeric peptidic erythropoiesis-stimulating agent covalently linked to polyethylene glycol and is being developed for the treatment of anaemia associated with chronic renal failure and cancer. Because its primary amino acid sequence is unrelated to that of rhEPO, Hematide is unlikely to induce a cross-reactive immune response against endogenous EPO and is reported to correct anaemia induced by anti-rhEPO antibodies in a rat PRCA model (Woodburn *et al*, 2007).

Table I. Treatment of pure red cell aplasia (PRCA).

Agent	Response rate (CR + PR)*	Mean time to response	Need for maintenance therapy	Feasibility of long-term maintenance§
Corticosteroids; CS (methyl-prednisolone/ prednisone/prednisolone)	30–62%	2·5 weeks† 9 weeks in patients with primary idiopathic PRCA‡ (33% of patients achieved remission within 2 weeks)	Required† (Most patients relapsed during the taper of CS) Required in patients with primary idiopathic PRCA‡	Unacceptable for the dose to maintain remission
Cyclosporine A; CsA	65–87%	12 weeks in patients with primary idiopathic PRCA‡ (65% of responders achieved remission within 2 weeks)	Required in patients with primary idiopathic PRCA‡ (86% relapsed after discontinuation of CsA while 11% relapsed during the maintenance of CsA)	May be durable but needs careful monitoring
Cyclophosphamide; CY (CY + CS)	7–20% (40–60%)	11 weeks†	Unknown: required in some patients	Unacceptable

Agent	Relapse free survival (RFS)	Median overall survival (OS)
Corticosteroids; CS (methyl-prednisolone/ prednisone/prednisolone)	80% of patients relapse within 24 months after remission during dose-reduction† Median RFS: 33 months in patients with primary idiopathic PRCA‡ (88% of patients relapsed during CS maintenance)	14-year OS in patients with primary idiopathic PRCA treated with CS or with various combinations except for CsA†
Cyclosporine A; CsA	Median RFS: 103 months in patients with primary idiopathic PRCA‡ (Including patients who relapsed after the discontinuation of CsA)	12-year OS in patients with primary idiopathic PRCA responding to remission induction therapy with CsA‡ 10-year OS was 95% and the median OS has not yet been reached in all patients‡
Cyclophosphamide; CY (CY + CS)	Unknown: the duration of remission induced by CY seems to be prolonged as compared to patients induced by CS*	Unknown

Patients with primary acquired and secondary PRCA are included if not otherwise indicated.

*References are indicated in the text.

†Referenced by Clark *et al* (1984).

‡Referenced by Sawada *et al* (2007).

§Referenced by Clark *et al* (1984), Sawada *et al* (2007), Radis *et al* (1995) and Reinhold-Keller *et al* (2000).

Thus, a recent animal study suggested that a possible alternative strategy might be to administer Hematide to patients with PRCA due to anti-rhEPO antibodies, which should enable ongoing stimulation of erythropoiesis.

Future prospects: proposal for a first-line therapy in primary acquired PRCA

There are several options for inducing remission of PRCA, but many patients with acquired PRCA require immunosuppressive therapy to maintain remissions. As summarized in Table I, CS, CsA and CY plus CS are almost equally effective for inducing remissions of PRCA, but the most important difference between these agents is the feasibility of long-term maintenance. Although the relapsed patients can be re-treated with the same agents, such as CS or CS plus CY, the cumulative side effects and toxicity become unacceptable. Considering the recurrent nature of acquired PRCA, we suggest CsA as first-line therapy for these patients at a dose of 2·5–3 mg/kg twice daily to achieve trough CsA levels of 150–250 ng/ml for a maximum of three to four months. This trough CsA level has been empirically determined according to a consensus from a multicentre randomized study in Japan for aplastic anaemia (Teramura *et al*, 2007). Maintenance therapy with CsA is a requisite for most patients to prevent relapse. Since nephrotoxicity constitutes the major limiting side effect of CsA, careful and progressive decrease of the dosage to the minimum required for maintenance of remission is appropriate. The mean maintenance dose of CsA in Japanese patients who were continuing their first remission for more than 24 months was 2·2 ± 0·8 mg/kg per day with a range of 1·1–3·8 mg/kg per day, 40% of the initial dose (Sawada *et al*, 2007), which suggests difficulty in reducing CsA under this dosage to maintain remissions. Adequate prevention and treatment of infections secondary to immunosuppression are also necessary for successful management of these patients.

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