Int J Hematol (2013) 97:558–563
DOI 10.1007/s12185-013-1324-x



# Border between aplastic anemia and myelodysplastic syndrome

**Hirohito Yamazaki · Shinji Nakao**

Received: 1 April 2013 / Revised: 3 April 2013 / Accepted: 3 April 2013 / Published online: 24 April 2013
© The Japanese Society of Hematology 2013

**Abstract** Distinguishing between acquired aplastic anemia (AA) and myelodysplastic syndrome (MDS) with a low blast cell percentage is often difficult and problematic, as both diseases are syndromes primarily defined by morphological findings, and their diagnostic criteria do not necessarily reflect the pathophysiology of their bone marrow (BM) failure. As a result, many patients with benign BM failure that should be managed as AA are diagnosed as having MDS, due to the absence of BM hypocellularity and the presence of dysplastic signs in the BM, and are treated inappropriately with toxic therapies, such as hypomethylating agents, and stem cell transplantation from unrelated donors. BM failure syndromes need to be managed in ways appropriate to their pathophysiology, which is more accurately determined by using markers such as the presence of glycosylphosphatidylinositol-anchored protein-deficient cells and HLA-A lacking leukocytes. We recently found that plasma thromobopoietin level is one of the most useful markers for distinguishing benign and pre-leukemic BM failure syndromes.

**Keywords** Acquired aplastic anemia · Myelodysplastic syndrome · GPI-AP-deficient cells · Plasma thrombopoietin level · Immunosuppressive therapy

H. Yamazaki · S. Nakao (✉)
Cellular Transplantation Biology, Kanazawa University Graduate School of Medical Science, 13-1, Takaramachi, Kanazawa, Ishikawa 920-8641, Japan
e-mail: snakao8205@staff.kanazawa-u.ac.jp

## Introduction

The differentiation of aplastic anemia (AA) from low-risk myelodysplastic syndrome (MDS) has long been a matter of clinical importance to hematologists. Although several markers have been shown to be useful for differentiating between these conditions, none has been widely accepted due to their low reliability.

One reason for the difficulty in differentiating these two diseases is that their diagnostic criteria were established separately, without considering the distinctions between them. Thus, AA is diagnosed based on the presence of pancytopenia and bone marrow (BM) hypoplasia without apparent dysplasia in immature and mature blood cells [1], while MDS is diagnosed when cytopenias and dysplastic signs are present [2]. Not surprisingly, patients who are diagnosed with AA by a physician may be diagnosed with MDS by other physicians, due to the presence of equivocal dysplasia [3]. Despite the fact that the diagnosis of both syndromes relies primarily on morphological findings, AA, and MDS are defined as benign and premalignant BM failure, respectively, and this mismatch between diagnostic criteria and disease concepts may further confound correct diagnosis. Since the diagnoses of the two syndromes are not mutually exclusive, attempts to differentiate between them by following conventional diagnostic criteria are of limited value. Instead, it is clinically relevant to distinguish a benign subset of BM failure, which is likely to respond to immunosuppressive therapy (IST), from other types of BM failure, including those with pre-leukemic features and inherited BM failure syndromes [4]. In this review article, we discuss the adverse outcomes of a misdiagnoses of AA and low-risk MDS, citing two representative cases, and introduce markers that can help to differentiate immune pathophysiology from non-immune pathophysiology in

BM failure. In this review, we refer to benign and pre-leukemic BM failure syndromes, respectively, as AA and MDS.

## Case presentation 1

A 37-year-old man was found to have mild pancytopenia with a white blood cell count (WBC) of 3.5 × $10^9$/L, a hemoglobin (Hb) level of 13.9 g/dL and a platelet count of 131 × $10^9$/L in March 2009, when he visited a local hospital for a routine checkup. He did not undergo a closer examination at that time. He visited the same hospital due to dyspnea on exertion in March 2010. A blood test revealed a WBC of 2.9 × $10^9$/L, an absolute neutrophil count of 0.73 × $10^9$/L, an Hb level of 6.4 g/dL, and a platelet count of 18 × $10^9$/L. Blood chemistry data were all normal, except for an increase (332 IU/L) in LDH. BM aspiration showed hypercellularity, with a nuclear cell count of 492 × $10^9$/L and erythroid hyperplasia (Fig. 1). The percentage of myeloblasts was 1.8 %, and there were signs of dysplasia, such as nuclear deformities in neutrophils and erythroblasts. The karyotype was 46 XY in all 20 dividing cells. The patient was diagnosed with refractory cytopenia with multilineage dysplasia (RCMD) and was referred to our clinic for BM transplantation from an unrelated donor.

T1-weighted sagittal magnetic resonance images (MRI) of the thoracolumbar spine and the iliac bones showed fatty marrow with some hematopoietic nests (Fig. 2a, b). Flow cytometry detected glycosylphosphatidylinositol-anchored protein (GPI-AP)-deficient blood cells (Fig. 2c). As the examination of BM from a different site from the one examined at the referring hospital revealed marked hypocellularity, the patient's diagnosis was changed to non-severe AA. He received antithymocyte globulin (ATG) plus cyclosporine, and achieved a complete hematological recovery in 1 year.

## Reasons for the difficulty in diagnosing Case 1

### BM distribution is inconsistent in patients with non-severe AA

Bone marrow aspiration and trephine biopsy are essential for the differential diagnosis of BM failure syndromes, and hypocellular BM is a prerequisite for diagnosing AA. However, assessing BM cellularity in patients with BM failure is often difficult, particularly when the patient's cytopenia is not severe. Even when the BM failure of a patient is caused by a decrease in hematopoietic stem cells and the BM is grossly replaced with fat tissue, some hematopoietic nests remain and may show hypercellularity due to increased BM activity that compensates for the decreased hematopoiesis in other BM sites. BM aspiration or biopsy from the hot spots can produce erroneous results, as shown in Case 1. When the pathological reports of the BM examination show hyper- or normocellularity, the attending physicians do not generally consider a

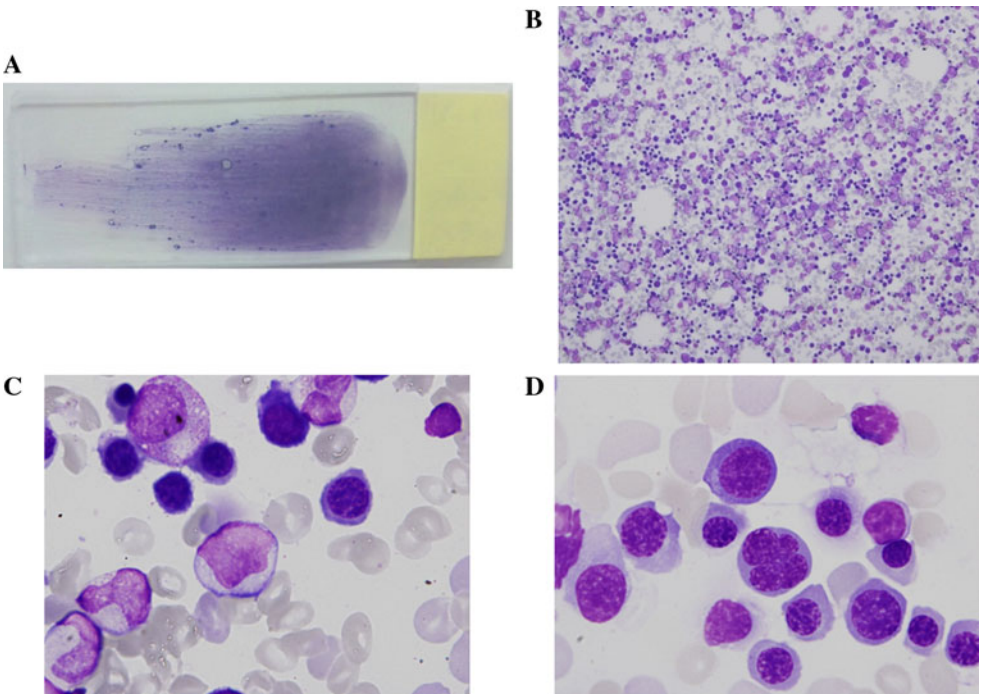


**Fig. 1** Bone marrow morphology of Case 1. **a** Bone marrow smear slide, **b–d** bone marrow pictures

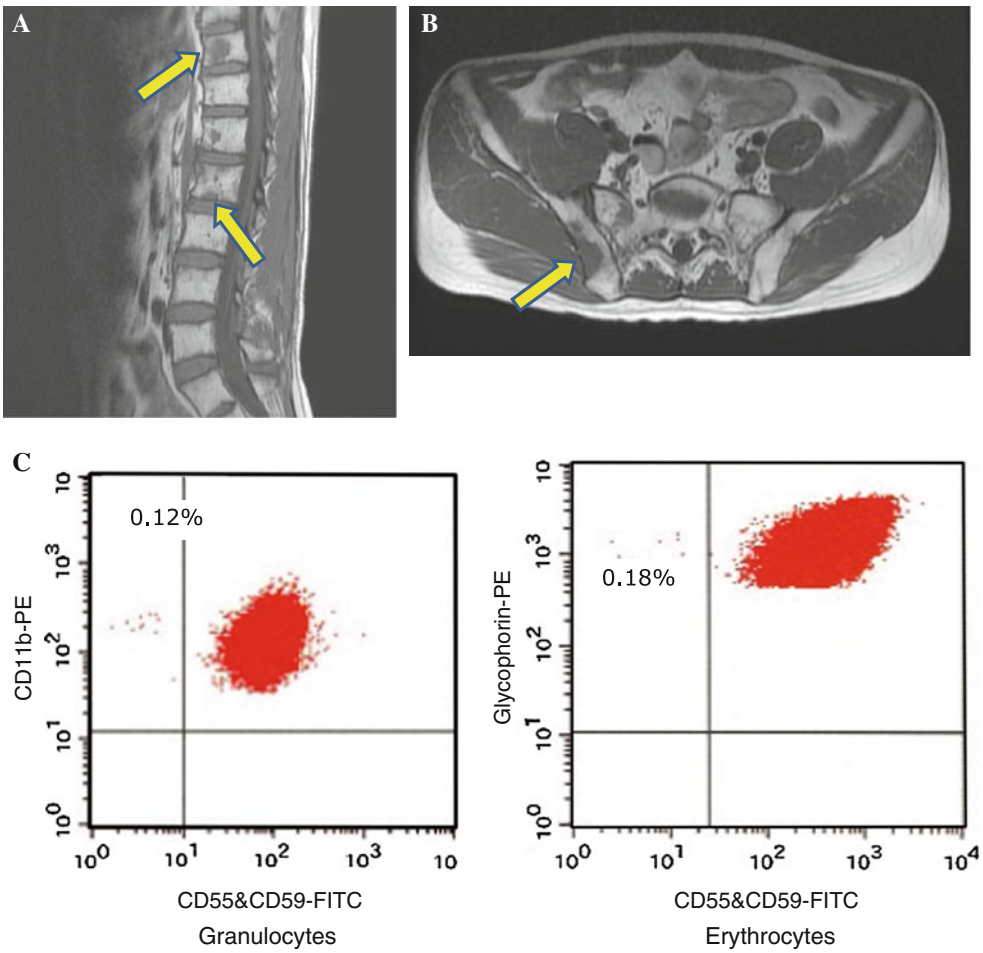


**Fig. 2** T1-weighted MRI images and flow cytometry results of Case 1. **a** Thoracolumbar spine; **b** iliac bone. *Arrows* indicate hematopoietic nests; **c** detection of glycosylphosphatidylinositol-anchored protein-deficient granulocytes and erythrocytes. 0.12 % $CD55^{-}CD59^{-}CD11b^{+}$ granulocytes and 0.18 % $CD55^{-}CD59^{-}glycophorin^{+}$ erythrocytes were revealed by flow cytometry

differential diagnosis of AA. Care should thus be taken with respect to cellularity when interpreting results of BM examinations. It is prudent to consider that BM cellularity cannot be accurately determined by BM biopsies from limited sites in the iliac bone. MRI of the thoracolumbar spine can be used to supplement the cellularity assessment by BM biopsies. However, MRI of elderly patients often produces equivocal results, due to age-related fatty changes in the BM. Thus, hematologists should keep it in mind that BM hypercellularity does not necessarily preclude a diagnosis of AA.

## Dysplasia is common in the BM of patients with AA

The presence of dysplastic signs in the BM is thought to be a hallmark of MDS. However, dysplastic signs in erythroblasts, such as nuclear deformities and karyorrhexis, are often detectable in the BM of patients with non-severe AA [5]. These dysplastic signs are detectable even in the BM of healthy individuals [6, 7]. Physicians nonetheless tend to interpret these dysplastic signs as significant, and make a diagnosis of refractory cytopenia with unilineage dysplasia (RCUD) or RCMD, particularly when BM examination does not reveal hypocellularity. In the end, many of the patients with non-severe AA are currently being diagnosed with MDS, as seen in Case 1. The pathophysiology of patients with increased GPI-AP-deficient blood cells diagnosed with RCUD or RCMD is essentially the same as that of AA [8]. The therapeutic plan should therefore be based on the pathophysiology of BM failure deduced from markers, rather than the degree of dysplasia.

## Signs of inefficient erythropoiesis are common in chronic AA

Ineffective erythropoiesis characterized by macrocytosis, erythroid hyperplasia in the BM, and intramedullary hemolysis is commonly seen in patients with MDS, but is also observed in patients with chronic AA, where the pancytopenia gradually progresses. This is particularly common in patients possessing increased GPA-AP-deficient blood cells [9]. Although it is unclear how ineffective erythropoiesis occurs in these patients based on immune pathophysiology, inflammatory cytokines produced by

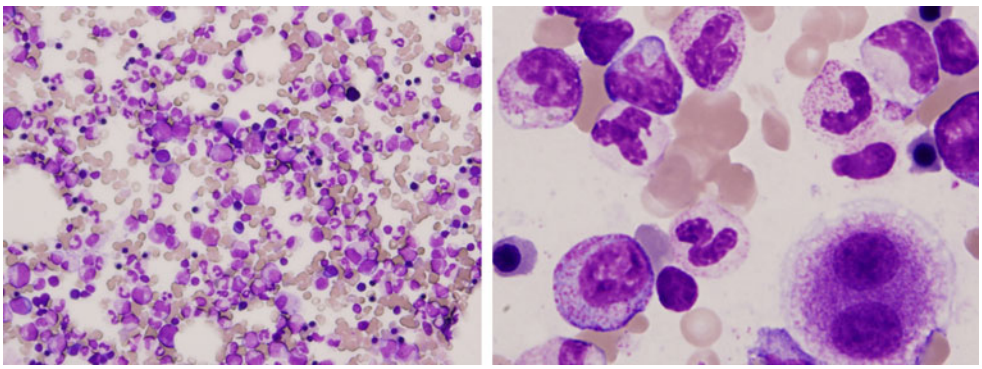

**Fig. 3** Bone marrow morphology of Case 2. Bone marrow was normocellular with decreased megakarytocytes, and dysplastic signs in granulocytes, erythroblasts and megakaryocytes were seen

pathogenic T cells may cause reversible dyserythopoiesis [10]. In fact, the signs of ineffective erythropoiesis usually disappear when patients respond to IST and achieve remission [5]. Thus, it is essential for physicians to avoid making a premature diagnosis of MDS based on the presence of ineffective erythropoiesis.

## Case presentation 2

A 35-year-old man was found to have pancytopenia during an annual checkup in 2007. The WBC count was $2.8 \times 10^9$/L, Hb was 12.5 g/dL, and the platelet count was $76 \times 10^9$/L. Idiopathic thrombocytopenic purpura (ITP) was suspected at first, but the pancytopenia gradually progressed and he became dependent on red blood cell transfusions. A BM biopsy performed in July 2010 revealed hypercellular BM. He was diagnosed with RCMD at the local hospital, and was referred to our clinic for further therapy. The patient's BM was normocellular and showed some dysplastic signs, such as nuclear deformities in the neutrophils and small megakaryocytes (Fig. 3). The BM karyotype was 46 XY in all 20 dividing cells. Flow cytometry failed to detect increased GPI-AP-deficient blood cells. Although the BM findings were compatible with RCMD, we reasoned that the patient had a benign BM failure that was likely to respond to IST, as his plasma thrombopoietin (TPO) level was high (1,070 pg/mL). Treatment with ATG plus cyclosporine induced complete remission in the patient. He has not shown any signs of relapse of pancytopenia or progression to MDS for 2.5 years after IST.

## Reasons for the choice of IST for Case 2

### Significance of TPO measurement in the management of MDS

We previously demonstrated that increased GPI-AP-deficient blood cells are often detectable in patients with thrombocytopenia and decreased megakaryocytes in the BM [9]. A subsequent analysis of response to IST in patients with BM failure revealed that the presence of thrombocytopenia with a decreased number of megakaryocytes predicts a good response to IST, irrespective of the presence of GPI-AP-deficient cells (unpublished observation). However, it is difficult to determine the cellularity of megakaryocytes in patients with BM failure, as a BM biopsy specimen taken from an iliac bone site does not accurately represent the number of megakaryocytes in the BM of whole body. We hypothesized that plasma TPO levels may serve as a surrogate marker for the number of megakaryocytes [11], and measured TPO levels in a large number of patients with thrombocytopenia [12]. Similar to the findings from previous studies [13, 14], TPO levels in patients with AA were noticeably higher than those in patients with ITP or refractory anemia with excess of blasts (RAEB). Receiver operating characteristic curves set the threshold for the TPO level between benign BM failure (AA) and preleukemic BM failure (RAEB) BM at 320 pg/mL. MDS patients with TPO levels $\geq$320 pg/mL had increased GPI-AP-deficient blood cells were more likely to have a low International Prognostic Scoring System (IPSS) score ($\leq$1.0), a higher response rate to IST, and a better 5-year progression-free survival rate in patients with RCUD and RCMD (Fig. 4). These findings indicate that MDS with TPO levels $\geq$320 pg/mL is actually AA.

### Classification of BM failure according to the plasma TPO levels

Figure 5 shows interrelationships among the various types of BM failure. BM failure with thrombocytopenia can be divided into two ($TPO^{high}$ and $TPO^{low}$) groups according to the plasma TPO values ($\geq$320 or <320 pg/mL). All patients having increased $GPI\text{-}AP^-$ cells fall into the $TPO^{high}$ group. Accordingly, physicians do not need to examine the peripheral blood in these patients for the presence of $GPI\text{-}AP^-$ cells to determine the pathophysiology of BM failure when the TPO levels of these patients

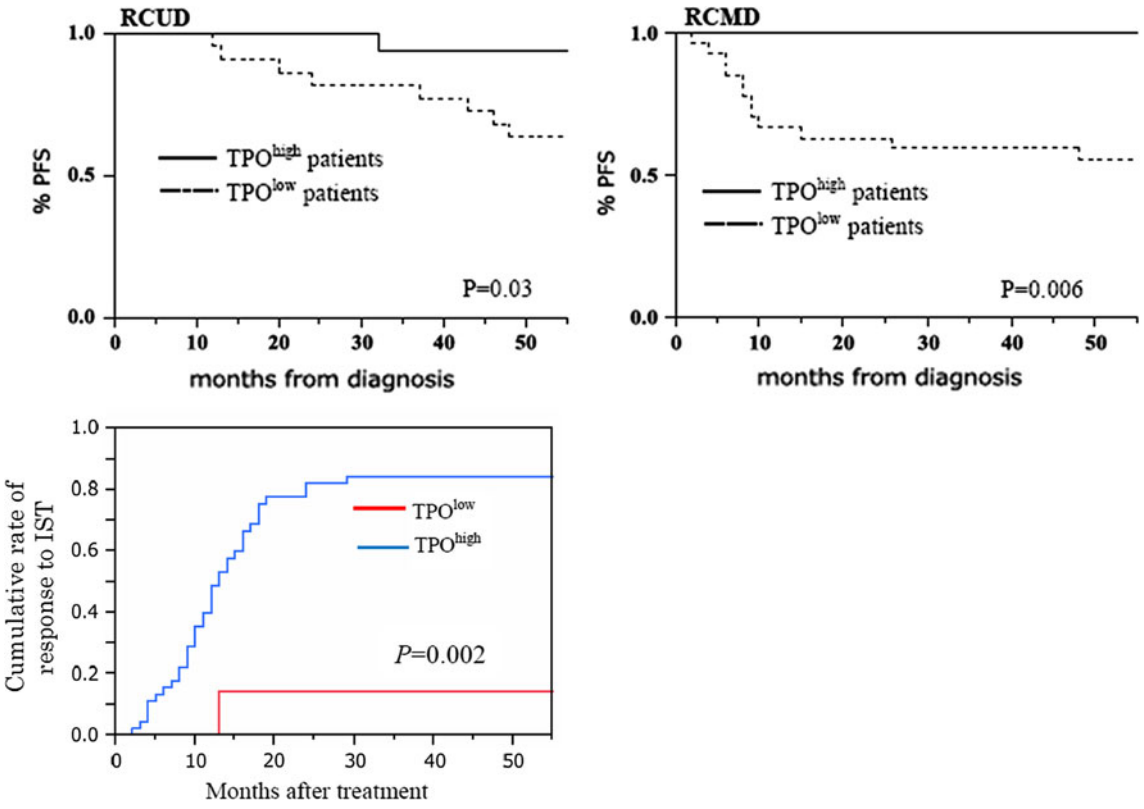


**Fig. 4** Progression-free survival (PFS) of patients with MDS and response to immunosuppressive therapy. RCUD, refractory cytopenia with unilineage dysplasia; RCMD, refractory cytopenia with multilineage dysplasia; $TPO^{high}$, TPO values ≥ 320; $TPO^{low}$, TPO values <320 pg/mL

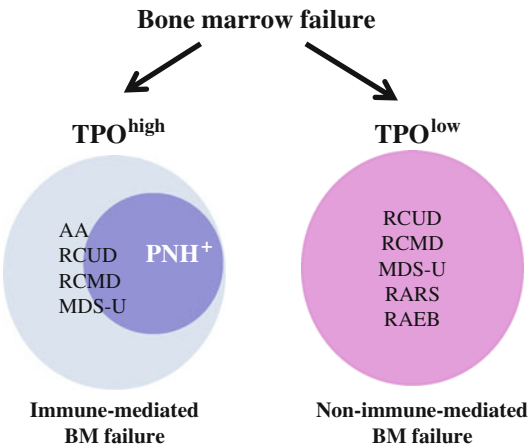


**Fig. 5** Interrelationships between different bone marrow failure syndromes. AA, aplastic anemia; RCUD, refractory cytopenia with unilineage dysiplasia; RCMD, refractory cytopenia with unilineage dysplasia; MDS-U, myelodysplastic syndrome-unclassifiable; RAEB, refractory anemia with excess of blasts; $TPO^{high}$, TPO values ≥ 320 pg/mL; $TPO^{low}$, TPO values <320 pg/mL; $PNH^{+}$, positive for increased GPI-AP-deficient blood cells

are available. The recent National Comprehensive Cancer Network guidelines recommend using hypomethylating agents for the treatment of MDS patients with thrombocytopenia [15], but this option may be hazardous to $TPO^{high}$ patients, as their BM failure is not based on abnormal stem cells with preleukemic features.

## Conclusion

Measurement of the TPO levels is recommended for all BM failure patients with thrombocytopenia as an aid in selecting the most appropriate therapeutic approach. The precise role of TPO levels in the management of MDS will need to be evaluated by a large prospective study.

## References

1. International Agranulocytosis and Aplastic Anemia Study. Incidence of aplastic anemia: the relevance of diagnostic criteria. By the International Agranulocytosis and Aplastic Anemia Study. Blood. 1987;70:1718–21.
2. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol. 1982;51:189–99.
3. Barrett J, Saunthararajah Y, Molldrem J. Myelodysplastic syndrome and aplastic anemia: distinct entities or diseases linked by a common pathophysiology? Semin Hematol. 2000;37:15–29.
4. Sakaguchi H, Nakanishi K, Kojima S. Inherited bone marrow failure syndromes in 2012. Int J Hematol. 2013;97:20–9.

5. Nakao S, Sugimori C, Yamazaki H. Clinical significance of a small population of paroxysmal nocturnal hemoglobinuria-type cells in the management of bone marrow failure. Int J Hematol. 2006;84:118–22.
6. Bain BJ. The bone marrow aspirate of healthy subjects. Br J Haematol. 1996;94:206–9.
7. Parmentier S, Schetelig J, Lorenz K, Kramer M, Ireland R, Schuler U, et al. Assessment of dysplastic hematopoiesis: lessons from healthy bone marrow donors. Haematologica. 2012;97: 723–30.
8. Wang H, Chuhjo T, Yasue S, Omine M, Nakao S. Clinical significance of a minor population of paroxysmal nocturnal hemoglobinuria-type cells in bone marrow failure syndrome. Blood. 2002;100:3897–902.
9. Sugimori C, Mochizuki K, Qi Z, Sugimori N, Ishiyama K, Kondo Y, et al. Origin and fate of blood cells deficient in glycosylphosphatidylinositol-anchored protein among patients with bone marrow failure. Br J Haematol. 2009;147:102–12.
10. Miescher PA, Favre H, Beris P. Autoimmune myelodysplasias. Semin Hematol. 1991;28:322–30.
11. Emmons RV, Reid DM, Cohen RL, Meng G, Young NS, Dunbar CE, et al. Human thrombopoietin levels are high when thrombocytopenia is due to megakaryocyte deficiency and low when due to increased platelet destruction. Blood. 1996;87:4068–71.
12. Seiki Y, Sasaki Y, Hosokawa K, Saito C, Sugimori N, Yamazaki H, et al. Increased plasma thrombopoietin levels in patients with myelodysplastic syndrome: a reliable marker for a benign subset of bone marrow failure. Haematologica. 2013.
13. Hou M, Andersson PO, Stockelberg D, Mellqvist UH, Ridell B, Wadenvik H. Plasma thrombopoietin levels in thrombocytopenic states: implication for a regulatory role of bone marrow megakaryocytes. Br J Haematol. 1998;101:420–4.
14. Feng X, Scheinberg P, Wu CO, Samsel L, Nunez O, Prince C, et al. Cytokine signature profiles in acquired aplastic anemia and myelodysplastic syndromes. Haematologica. 2011;96:602–6.
15. Greenberg PL, Attar E, Battiwalla M, Bennett JM, Bloomfield CD, DeCastro CM, et al. Myelodysplastic syndromes. J Natl Compr Cancer Network: JNCCN. 2008;6:902–26.