Increased plasma thrombopoietin levels in patients with myelodysplastic syndrome: A reliable marker for a benign subset of bone marrow failure

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Abstract

Background: Although myelodysplastic syndromes are heterogeneous disorders comprising a benign subset of bone marrow failure similar to aplastic anemia, no laboratory test has been established to distinguish it from bone marrow failures that can evolve into acute myeloid leukemia.

Design and Methods: Plasma thrombopoietin levels were measured in 120 patients who had myelodysplastic syndrome with thrombocytopenia ($< 100 \times 10^9$/L) to determine any correlation to markers associated with immune pathophysiology and outcome.

Results: Thrombopoietin levels were consistently low for patients with refractory anemia with excess of blasts, while patients with other myelodysplatic syndrome subsets had more variable results. Patients with thrombopoietin levels ≥320 pg/mL had increased glycosylphosphatidylinositol-anchored protein-deficient blood cells (49.1% versus 0%), were more likely to have a low International Prognostic Scoring System (IPSS) score (≤1.0, 100% versus 65.5%), a higher response rate to immunosuppressive therapy (84.2% versus 14.3%), and a better 5-years progression-free survival rate (94.1% versus 63.6% for refractory cytopenia with unilineage dysplasia; 100.0% versus 44.4% for refractory cytopenia with multilineage

dysplasia).

Conclusions: Increased plasma thrombopoietin levels were associated with a favorable prognosis of bone marrow failure and could therefore represent a reliable marker for a benign subset of myelodysplastic syndrome.

Introduction

Myelodysplastic syndromes (MDS) are characterized by peripheral cytopenia and morphological abnormalities in mature and immature blood cells. Although MDS were originally defined as clonal hematopoietic disorders with a propensity to become acute myeloid leukemia (AML), they also comprise benign bone marrow (BM) failure which may benefit from immunosuppressive therapy (IST) like acquired aplastic anemia (AA) does.(1-3) MDS with thrombocytopenia are often difficult to differentiate from non-severe AA because diagnoses rely on a subjective judgment of blood cell morphological abnormalities, and BM cellularity is often inconsistent among the sites examined.(4, 5) Laboratory markers such as glycosylphosphatidylinositol-anchored protein-deficient (GPI-AP$^-$) cells and HLA-DRB1*1501 may differentiate between benign and preleukemic MDS subtypes.(6, 7) Diagnostic values of these markers have not been established, however, due to conflicting results in the prediction of IST response (8) and cumbersome procedures in the detection of minor GPI-AP$^-$ cell populations.(9, 10)

Thrombocytopenia is a symptom common to AA and a subset of MDS. Previous studies showed that plasma levels of thrombopoietin (TPO), a critical regulator of thrombopoiesis, were elevated in patients with AA (11) but remained low in patients

with MDS despite the presence of severe thrombocytopenia.(12-14) The low TPO levels in MDS patients have been connected to an increase in megakaryocytes or TPO receptors on megakaryocytes,(13) but no studies have focused on the possibility of different TPO levels among MDS subtypes. Patients with low-risk MDS could have high TPO levels and respond to IST like AA patients. To test these hypotheses, plasma TPO was measured in patients with various types of MDS and correlated to IST response, patient prognosis and an increase in the percentage of GPI-AP$^-$ cells.

Design and Methods

Patient characteristics

TPO plasma concentration was measured in 50 healthy volunteers (23 male, 27 female) 18-82 years old (median age, 41 years) and 191 patients diagnosed with thrombocytopenia from 2005 to 2010 at Kanazawa University Hospital, hospitals participating in the Study Group of Intractable Hematopoietic Disorders in Japan, or other affiliated institutions. There were 120 patients with MDS, including 37 with refractory cytopenia with unilineage dysplasia (RCUD), 40 with refractory cytopenia with multilineage dysplasia (RCMD), 22 with refractory anemia with excess of blasts (RAEB), and 21 with unclassified MDS (MDS-U), as well as 47 patients with AA and

24 with immune thrombocytopenia (ITP). Characteristics of these participants are summarized in Table 1. Patients dependent on platelet transfusions or with a liver dysfunction were excluded from the study. AA and MDS were classified according to diagnostic criteria from the International Agranulocytosis and Aplastic Anemia Study Group and World Health Organization 2008, respectively. Prognostic sores of MDS patients were calculated according to the International Prognostic Scoring System (IPSS). AA severity was determined by criteria from Camitta et al.(15) All patients with MDS were judged to have 30% or more marrow cellularity by pathologists. MDS-U patients with abnormal karyotypes included 15 with del(13q), two with del(1;7), one with del(11q), one with t(4;21)(q11;q11), one with trisomy 8, and one with del(20q). The patients with trisomy 8 and del(20q) were diagnosed with MDS-U by the presence of bicytopenia. The ethics committee of Kanazawa University Graduate School of Medical Science approved the study protocol, and all patients provided their informed consent prior to sampling.

Measurement of TPO levels

Ethylenediaminetetraacetic acid (EDTA)-anticoagulated whole blood was drawn from patients at diagnosis, and plasma was separated by centrifugation at 1000 *g* for 10 min

for storage at −20°C. Plasma TPO was measured with a commercially available assay kit (Quantikine™ Human TPO Immunoassay, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Corrected TPO values were calculated by dividing TPO concentration by platelet count (x 10^{10}/L).

Detection of GPI-AP$^-$ blood cells

EDTA-anticoagulated peripheral blood was drawn from each patient. To detect GPI-AP$^-$ granulocytes, erythrocytes were lysed in buffer (8.26 g/L NH_4Cl, 1.0 g/L $KHCO_3$, and 0.037 g/L EDTA-4Na). After a saline wash, 50 μL of leukocyte suspension was incubated with 4 μL of phycoerythrin (PE)-labeled anti-CD11b mAb (Becton Dickinson, Franklin Lakes, NJ, USA) and 6 μL of Alex Fluor 488-labeled inactive toxin aerolysin (FLAER; Pinewood Scientific Services, Victoria, BC, Canada). To detect GPI-AP$^-$ erythrocytes, fresh blood was diluted to 3% in phosphate buffered saline (PBS), and 50 μL was then incubated with 4 μL of PE-labeled anti-glycophorin A mAb (clone JC159; DAKO, Glostrup, Denmark), fluorescein-isothiocyanate (FITC)–labeled anti-CD55 mAbs (clone IA10, mouse IgG2a; Pharmingen, San Diego, CA), and FITC-labeled anti-CD59 mAbs (clone p282, mouse IgG2a; Pharmingen). At least 1 × 10^5 CD11b$^+$ granulocytes and glycophorin A$^+$ red blood cells (RBCs) were analyzed

within each corresponding gate of a FACSCanto II® (Becton Dickinson) flow cytometer.(9) A significant increase in the percentage of GPI-AP$^-$ cells was defined as greater than 0.003% aerolysin$^-$CD11b$^+$ granulocytes and/or greater than 0.005% glycophorin A$^+$ RBCs as determined from the peripheral blood of 50 healthy individuals. Careful handling of samples with elaborate gating could lower the cut-off values to these levels without producing false positive results.(9, 16, 17)

IST

Horse anti-thymocyte globulin (ATG, Lymphoglobulin; Genzyme, Cambridge, MA, USA, 15 mg/kg/d) was administered for 5 days in combination with cyclosporine A (CsA; Novartis, Basel, Switzerland, 6 mg/kg/d). CsA was also given at 5-6 mg/kg/d for at least 3 months as monotherapy. Hematologic improvements, partial response, and complete response were defined by the International MDS Working Group Criteria.(18)

Human androgen receptor assay (HUMARA)

The androgen receptor gene was amplified from the genomic DNA of eight female patients, as previously described by Ishiyama et al. with some modifications.(19) To

correct an inequality of amplification efficiency between the 2 alleles, we determined the ratios of both allele areas before (lower allele/higher allele: A/B) and after (lower allele/higher allele:A'/B') *Hha*I digestion using a C value calculated by (A/B)/(A'/B') as a marker of skewing in granulocytes (C_G) and T lymphocytes (C_L). Skewing was judged evident when the absolute values of log (C_G/C_L) (S value) was more than 0.4.

Statistical Analysis

Plasma TPO concentrations were compared between subgroups with different clinical characteristics using the Mann-Whitney U test. Receiver operating characteristic (ROC) curves were calculated from the TPO levels and corrected TPO values of AA patients (representing benign BM failure) and RAEB patients (representing preleukemic BM failure) to determine thresholds of plasma TPO levels and define patients with high TPO levels. ROC curves were also calculated from the TPO levels of patients with early stage MDS who responded to IST and those who did not respond, and of patients who progressed to AML/death and those who did not progress. The Kaplan-Meier method and the Cox proportional hazards model were performed to estimate time-to-event analysis. Differences between patients with TPO levels ≥320 pg/mL (TPO^{high}) and patients with TPO levels <320 pg/mL (TPO^{low}) were assessed by the

log-rank test. The differences in the percentages of patients with low International Prognostic Scoring System (IPSS) scores and of patients requiring platelet transfusions between TPO^{high} and TPO^{low} patients were assessed by Fisher's exact probability test. All statistical analyses were performed using JMP version 5.0.1J software (SAS Institute, Cary, NC, USA). A P value < 0.05 was considered significant.

Results

TPO levels for patients with thrombocytopenia

Plasma TPO levels ranged from 19.4 to 38.8 pg/mL (mean ± standard deviation, 54.5 ± 21.1 pg/mL) in healthy individuals with platelet counts higher than 150 x 10^9/L. Similar to previous reports, TPO levels for patients with AA (1254.6 ± 551.1 pg/mL) were noticeably higher than for patients with ITP (63.5 ± 8.7 pg/mL) or RAEB (44.7 ± 85.2 pg/mL) (Figure 1A). Corrected TPO values were also higher in AA patients than RAEB patients (Figure 1B). ROC curves set the threshold between benign and preleukemic BM failure at 320 pg/mL for TPO levels and 157 pg/mL/10^{10} L for corrected TPO values (Figure 2A). All patients with TPO levels ≥320 pg/mL also had corrected TPO values >157 pg/mL/10^{10} L, except one with 155 pg/mL/10^{10} L, indicating that the difference between TPO^{high} and TPO^{low} patients is not due to platelet count.

TPO levels were highly variable in patients with MDS subtypes other than RAEB, with the proportion of TPO^{high} patients at 40.5% for RCUD, 32.5% for RCMD, and 77.3% for MDS-U.

Prevalence of increased GPI-AP$^-$ cells, clonality, and IPSS scores for patients with MDS

Small populations of GPI-AP^- granulocytes and/or erythrocytes were detected in some MDS patients, at 29.7% for RCUD, 7.5% for RCMD, 62.9% for MDS-U, and 0% for RAEB. Figure 3A displays the percentage of GPI-AP^- granulocytes in 55 TPO^{high} (25 with RCUD, 14 with RCMD, and 16 with MDS-U) and 65 TPO^{low} (12 with RCUD, 26 with RCMD, 5 with MDS-U, and 22 with RAEB) patients. The prevalence of increased GPI-AP^- granulocytes and/or erythrocytes in each group was 45.8% and 0% ($P = 0.003$, Figure 3B). Eight female patients (four with RCMD and four with MDS-U) underwent the HUMARA. All four TPO^{low} patients showed S values more than 0.4 (1.23, 1.39, 1.14, and 0.71) compatible with the presence of clonal hematopoiesis while none of the four TPO^{high} patients showed such high S values (0.06, 0.16, 0.08, and 0.09, $P = 0.002$, Supplementary Figure 2). When correlation of TPO levels and IPSS scores was examined for each MDS patient, none of the TPO^{high}

patients had an IPSS score above 1.5 (Figure 3C). In fact, the percentages of 0, 0.5, or 1.0 scores in TPO^{high} and TPO^{low} patient groups were 100.0% and 65.5%, respectively ($P < 0.001$).

Response of patients with MDS to IST

Forty-five TPO^{high} MDS patients were treated with IST. Sixteen patients (seven with RCUD and nine with MDS-U) were treated with a combination of ATG and CsA, while 29 patients (nine with RCUD, 10 with RCMD, and 10 with MDS-U) were treated with CsA alone. Response rates to ATG+CsA and CsA were 81.2% and 85.3%, respectively. In contrast, seven TPO^{low} patients received CsA monotherapy which produced only one case of improved anemia (from 5.4 g/dL to 8.1 g/dL). The cumulative rate of response to IST in TPO^{high} patients was significantly higher than that in TPO^{low} patients (P=0.002, Supplementary Figure 1A), while there was no significant difference either between patients with or without increased GPI-AP^{-} granulocytes (P=0.38, Supplementary Figure 1B) or between TPO^{high} patients with or without increased GPI-AP^{-} granulocytes (P=0.28, Supplementary Figure 1C). ROC analysis revealed the threshold TPO level affecting the response to IST to be 286.7 pg/mL, with a sensitivity of 0.580 and a specificity of 0.850 (Figure 1B).

After 6 to 17 months of IST, TPO levels were measured again in four patients who achieved a platelet recovery above 100 x 10^9/L. The corrected TPO levels before and after IST were 718.7/14.2 pg/mL/10^{10} L, 266.8/10.8 pg/mL/10^{10} L, 330.6/8.75 pg/mL/10^{10} L, and 170.6 /8.8 pg/mL/10^{10} L.

Correlation of high TPO levels with the prognosis of patients with MDS

The 5-year overall survival rates for RCUD, RCMD, MDS-U, and RAEB patients were 53.6%, 50.8%, 53.1%, and 37.4%, respectively (Figure 4A). The 5-year progression-free survival (PFS) rates for RCUD, RCMD, and MDS-U patients were 60.9%, 60.6%, and 71.4%, respectively (Figure 4B). The 5-year PFS rates, defined as whichever came first among time from the first day of diagnosis until progression to AML or disease related death, was significantly higher for TPO^{high} patients than TPO^{low} patients with RCUD (94.1% versus 60.6%, *P*=0.03) or RCMD (100.0% versus 44.4%, *P*=0.006), while there was no significant difference between the two groups in patients with MDS-U (82.3% versus 80.0%, *P*=0.22, Figure 4C). An ROC analysis revealed the threshold TPO level affecting progression to AML to be 244.2 pg/mL with a sensitivity of 0.927 and a specificity of 0.636 (Figure 1C). A multivariate analysis on patients with RCUD, RCMD and MDS-U revealed TPO^{low} and patient gender to be

significant factors associated with progression to AML (Table 2). In contrast to previous analyses (20), red blood cell transfusion requirements were not identified as a significant prognostic factor in this study cohort. This may reflect the fact that we selected patients with thrombocytopenia. There was no significant difference in the percentages of patients requiring platelet transfusions among TPO^{high} (8/52, 4.3%) and TPO^{low} (2/46, 15.3%, P=0.09) patients. TPO levels were serially recorded for one patient as AA evolved into MDS-RAEB 20 months after IST. The TPO concentration was 1960 pg/mL at the onset of AA and 151 pg/mL when thrombocytopenia recurred with the emergence of atypical blasts in peripheral blood (Supplementary Figure 3).

Discussion

Consistent with previous reports, the present study revealed an increase in the TPO levels of patients with AA, a decrease in those with ITP, and relatively low TPO levels in patients with MDS.(13, 14) While patients with RAEB had consistently low TPO levels, patients with low-risk MDS (RCUD, RCMD, and MDS-U) produced highly variable results. Feng et al. recently demonstrated similar variability among patients with hypoplastic MDS and reported the usefulness of TPO in distinguishing hypoplastic MDS from AA;(21) however, the significance of high TPO levels in a subset of patients

with low-risk MDS was not focused on by the authors. Our study is the first to demonstrate that a high concentration of TPO is associated with benign BM failure in patients with MDS as seen by the higher prevalence of increased GPI-AP^{-} cells, lower incidence of clonal hematopoiesis, lower IPSS score, and better PFS in TPO^{high} patients compared to TPO^{low} patients. The negative prognostic impact of TPO levels <320 pg/mL was confirmed by a multivariate analysis, which also identified patient gender to be a poor prognostic factor as demonstrated by previous studies. (22, 23)

Plasma TPO levels are negatively regulated by platelets and megakaryocytes through their binding of TPO.(24, 25) Some studies show an inverse correlation between TPO levels and BM megakaryocyte count (26, 27), but the TPO levels of patients with RAEB were consistently low in our study despite the low megakaryocyte content (data not shown). Low TPO levels in MDS patients with a poor prognosis may be explained by an increased turnover of platelets produced by abnormal megakaryocytes (28). We previously demonstrated that the aberrant increase in the proportion of immature platelets is frequently seen in MDS patients with chromosomal abnormalities associated with poor prognosis (28). Increased production and destruction of platelets may accelerate TPO consumption in patients with RAEB, leading to a decrease in plasma TPO levels. Although one report demonstrated

increased TPO receptors on the megakaryocytes of MDS patients using flow cytometry, (14) we were unable to reproduce their results (data not shown).

Small populations of GPI-AP$^-$ cells have been detected in a subset of patients with refractory anemia as defined by French-American-British classification and are associated with a favorable response to IST and good future prognosis.(2) Patients with increased GPI-AP$^-$ cells (PNH$^+$ patients) are characterized by predominant thrombocytopenia and no increase in BM megakaryocytes,(6, 9) which agrees with our findings of high TPO levels in all PNH$^+$ patients with MDS and no PNH$^+$ patients with low TPO levels. These results show no need to examine peripheral blood for GPI-AP$^-$ cells in patients with BM failure if their TPO levels are less than 320 pg/mL.

The response rate to ATG+CsA therapy or CsA monotherapy for TPO^{high} MDS patients was as high as or higher than the response for AA patients.(29) When we previously assessed the efficacy of CsA therapy for patients with thrombocytopenia and decreased BM megakaryocytes, more than 70% improved regardless of the presence or absence of GPI-AP$^-$ cells (unpublished observation). High TPO levels may have a stronger association with the immune pathophysiology of BM failure in a subset of MDS than the presence of increased GPI-AP$^-$ cells. However, the impact of the increased percentage of GPI-AP$^-$ granulocytes on PFS could not be analyzed by our

multivariate analysis because all patients with increased GPI-AP^{-} granulocytes were sorted into the TPO^{high} group. When we conducted another multivariate analysis using increased GPI-AP^{-} granulocytes, rather than TPO levels, as an independent factor, the hazard ratio of the increased GPI-AP^{-} granulocytes was 0.64, with a *P*-value of 0.69. Thus, the impact of increased GPI-AP- granulocytes on PFS appears to be much smaller than high TPO levels.

Previous reports documented the important role of DRB1*1501 as a predictor of response to IST in patients with MDS (8). This factor may also have affected PFS in our patient cohort. Unfortunately, since only a limited number of patients underwent HLA typing, its impact on PFS could not be determined in this study. However, we previously examined various factors, including GPI-AP^{-} cells and DRB1*1501, for their association with good response to IST in 140 patients with AA using multivariate analysis and found that only the presence of increased GPI-AP^{-} cells significantly predicts favorable response to IST(30, 31). Thus, it is unlikely that DRB1*1501 affects PFS of patients with MDS more strongly than increased GPI-AP^{-} cells or high TPO levels. Cytogenetic abnormality was not identified as a poor risk factor by the multivariate analysis. This is probably because 15 of 21 patients with cytogenetic abnormalities had del(13q), which represents benign BM failure with immune

pathophysiology (32).

Although the exact mechanism for decreased TPO in MDS patients with a poor prognosis remains unclear, measurement of its concentration could play a significant role in managing MDS with thrombocytopenia. A patient with TPO levels ≥320 pg/mL is considered to have a good prognosis with a small likelihood of developing AML, and IST can be considered rather than radical treatments such as chemotherapy and stem cell transplantation. Recent National Comprehensive Cancer Network guidelines recommend hypomethylating agents as a treatment for MDS patients with thrombocytopenia,(33) but this option may be hazardous to TPO^{high} patients because their BM failure is not based on abnormal stem cells with preleukemic features. Measurement of TPO levels is therefore recommended for patients with low-risk MDS and thrombocytopenia so that the most appropriate therapy can be chosen. The exact role of TPO levels in the management of MDS will need to be evaluated with a prospective study.

Authorship and Disclosures

Contribution: Y. Seiki performed the experiments and analyzed the data; Y. Sasaki performed HUMARA; K. Hosokawa, C. Saito, N. Sugimori and H. Yamazaki contributed to collecting patient samples and clinical data; A. Takami performed statistical analyses; S. Nakao designed the study and supervised the project; Y. Seiki and S. Nakao wrote the manuscript. All authors critically reviewed the final manuscript.

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Table 1. Characteristics of study participants with thrombocytopenia and healthy volunteers.

	Number of patients	Sex, (M/F)	Age (years), (range)	Platelet count ($\times 10^9$/L), (median ± SE)
MDS	120	60/49	70 (19-91)	54.0 ± 2.7
RCUD	37	17/20	65 (46-85)	53.7 ± 4.3
RCMD	40	23/17	69 (33-91)	43.5 ± 8.2
RAEB	22	16/6	74 (43-85)	49.1 ± 2.7
MDS-U	21	10/11	72 (19-76)	62.2 ± 2.1
AA	47	25/22	58 (12-93)	24.9 ± 7.3
Severe	23	12/11	56(28-93)	11.6 ± 6.9
Moderate	24	13/11	58(12-83)	43.9 ± 5.4
ITP	24	14/10	62(35-82)	23.5± 7.9
Healthy volunteers	50	23/27	41 (18-59)	304.3 ± 8.6

MDS, myelodysplastic syndrome; RCUD, refractory cytopenia with unilineage dysplasia; RCMD, refractory cytopenia with multilineage dysplasia; RAEB, refractory anemia with excess of blasts; MDS-U, unclassified myelodysplastic syndromes; AA, aplastic anemia; ITP, immune thrombocytopenia; HV, healthy volunteers.

Table 2. Multivariate analysis of factors negatively affecting progression-free survival of patients with myelodysplastic syndrome

	hazard ratio	*P*-value
TPO (pg/mL) <320 vs ≥320	0.05	0.009
Age (years old) <60 vs ≥60	1.87	0.262
Sex female vs male	0.12	0.009
IPSS 0 vs 0.5 and 1.0	0.34	0.158
Transfusion yes vs no	1.21	0.752
Abnormal karyotype yes vs no	0.93	0.916

Figure legends

Figure 1. Plasma thrombopoietin levels in patients with thrombocytopenia and healthy volunteers. (A) Plasma thrombopoietin (TPO) levels for each patient group. (B) Corrected TPO values for each patient group. The horizontal line in each figure represents the cut-off value that potentially separates benign and preleukemic bone marrow failure. RCUD, refractory cytopenia with unilineage dysplasia; RCMD, refractory cytopenia with multilineage dysplasia; RAEB, refractory anemia with excess of blasts; MDS-U, unclassified myelodysplastic syndromes; AA, aplastic anemia; ITP, immune thrombocytopenia; HV, healthy volunteers.

Figure 2. Receiver operating characteristic (ROC) curves to determine thresholds of plasma TPO levels discriminating benign (aplastic anemia)/preleukemic BM failure (RAEB, A), response/no response to immunosuppressive therapy (IST, B), and progression/no progression to AML/death (C).

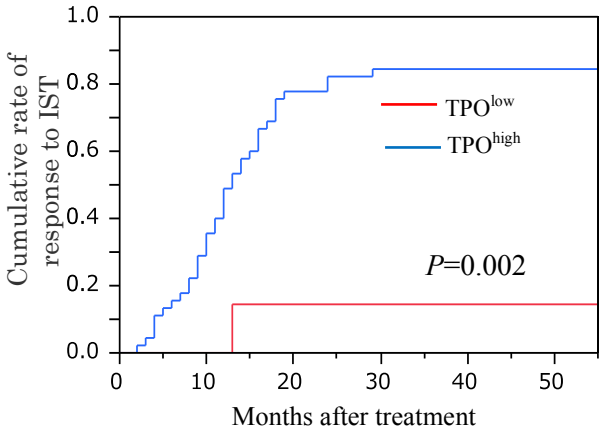
Figure 3. Relationship between thrombopoietin levels and other prognostic markers for patients with myelodysplastic syndrome. (A) Percentage of GPI-AP^{-} granulocytes in TPO^{high} (n = 55) and TPO^{low} (n = 65) patients. The horizontal line represents the cut-off value that separates significant and non-significant increases in GPI-AP^{-}

granulocyte percentage. (B) The prevalence of increased GPI-AP$^-$ granulocytes and/or erythrocytes in TPO^{high} (n = 55) and TPO^{low} (n = 65) patients. (C) TPO levels for MDS patients classified by IPSS score. GPI-AP$^-$ cells, glycosylphosphatidylinositol-anchored protein-deficient cells; TPO^{high}, thrombopoietin levels ≥320 pg/mL; TPO^{low}, thrombopoietin levels <320 pg/mL; RCMD, refractory cytopenia with multilineage dysplasia; MDS-U, unclassified MDS; AA, aplastic anemia; IPSS, International Prognostic Scoring System.

Figure 4. Influence of thrombopoietin levels at diagnosis on survival rates of patients with myelodysplastic syndrome. (A) Overall survival and (B) PFS of patients with myelodysplastic syndrome (MDS; 37 with RCUD, 40 with RCMD, 21 with MDS-U, and 20 with RAEB); (C) PFS of TPO^{high} and TPO^{low} patients by MDS subtypes. The numbers of TPO^{high}/TPO^{low} patients for each subtype were 17/22 for RCUD, 13/27 for RCMD, and 16/5 for MDS-U. RCUD, refractory cytopenia with unilineage dysplasia; RCMD, refractory cytopenia with multilineage dysplasia; MDS-U, unclassified MDS; AA, aplastic anemia; PFS, Progression free survival; TPO^{high}, thrombopoietin levels ≥320 pg/mL; TPO^{low}, thrombopoietin levels <320 pg/mL.

Supplementary Figure 1

(A)



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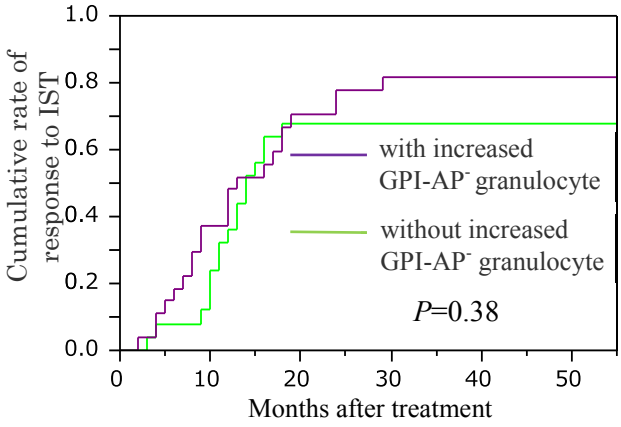
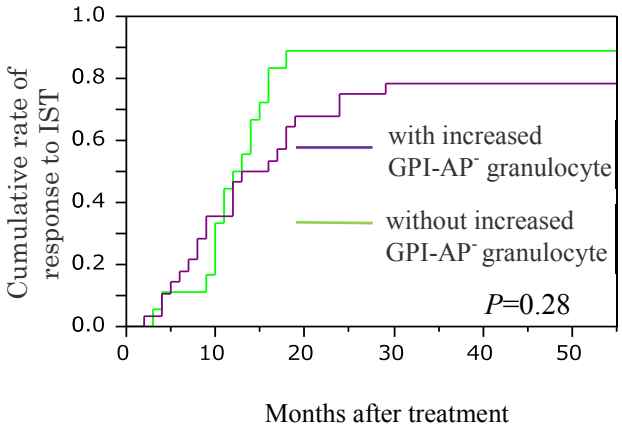
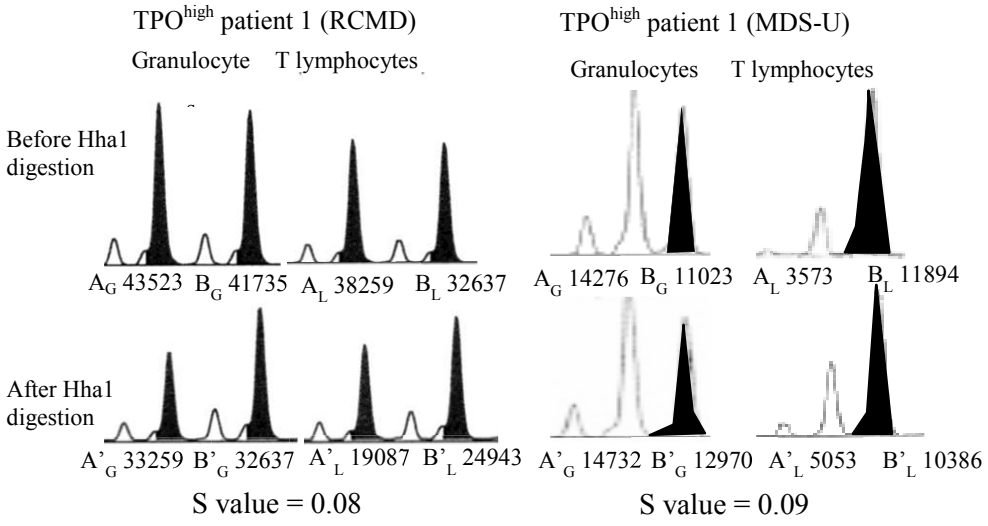
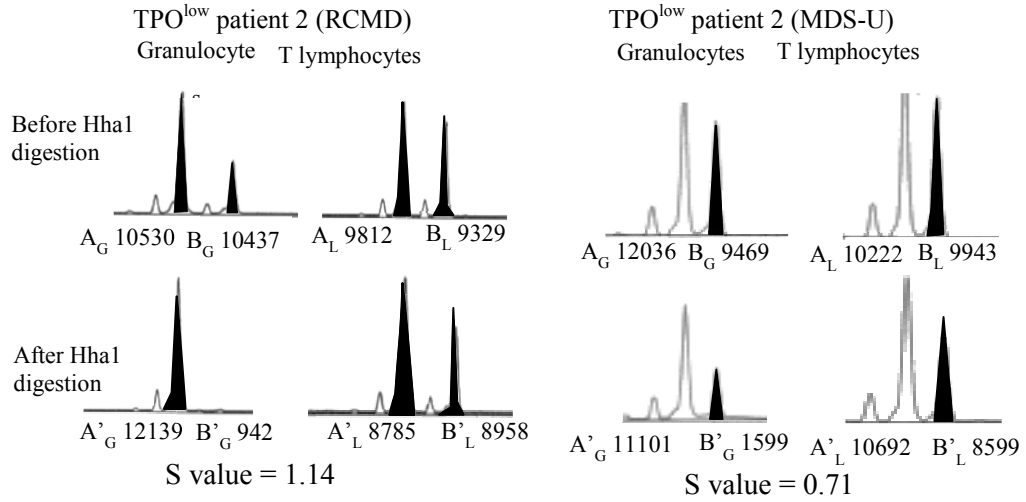


(C)

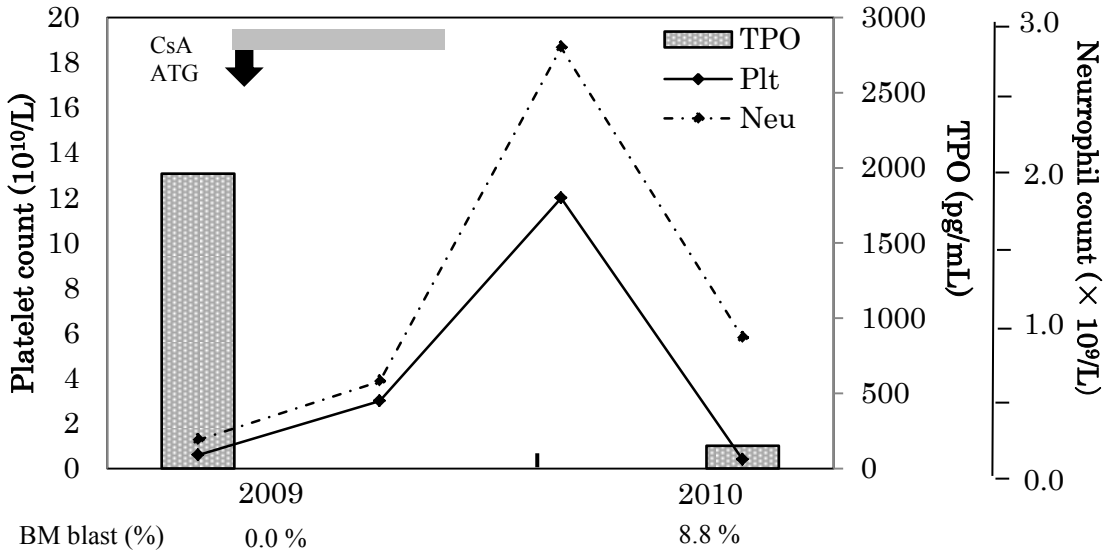


Supplementary Figure 2





Supplementary Figure 3



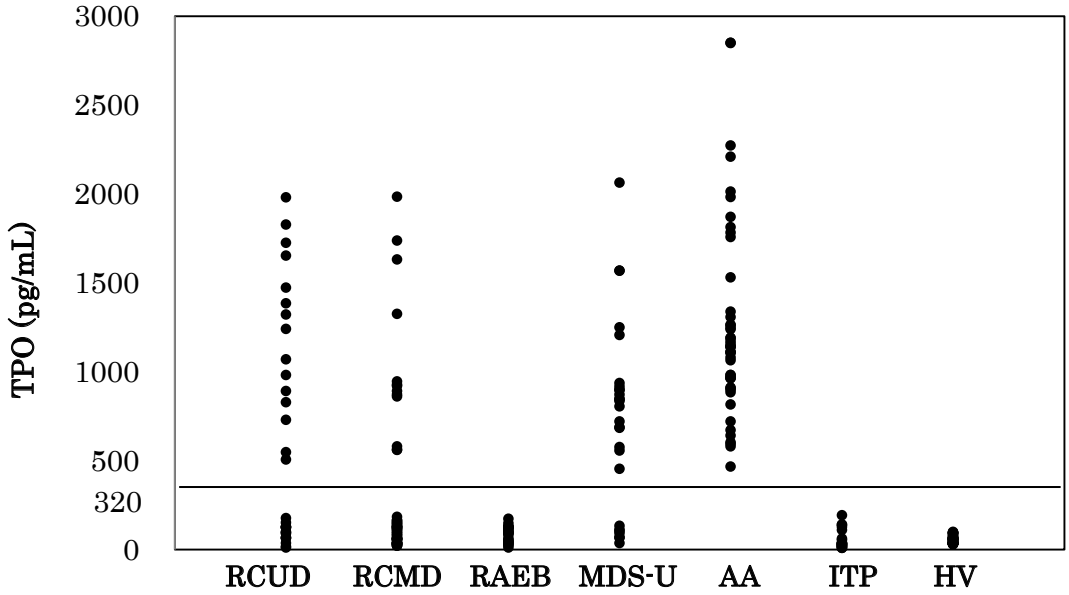
Supplementary Figure 1. Cumulative rates of response to IST in different subsets of patients defined by TPO levels and the increase in percentages of GPI-AP$^-$ granulocytes. A, TPO^{high} and TPO^{low} patients; B, patients with or without increased GPI-AP$^-$ granulocytes; C, TPO^{high} patients with or without increased GPI-AP$^-$ granulocytes.

Supplementary Figure 2. Skewing of the inactivation pattern for androgen receptor genes from the granulocytes of patients with myelodysplastic syndromes (MDS). Representative human androgen receptor assay results for two TPO^{high} patients (top; left, RCMD and right, MDS-U) and two TPO^{low} patients (bottom; left, RCMD and right, MDS-U) are shown. The S value represents the absolute values of log (C_G/C_L) (See Design and Methods)

Supplementary Figure 3. Clinical course of a patient with aplastic anemia that evolved into refractory anemia with excess of blasts as thrombopoietin levels decreased. Bone marrow examination revealed the presence of 8.8% myeloblasts and hypogranular neutrophils, as well as a pseudo-Pelger-Huet nucleus, with the recurrence of pancytopenia in October 2010. Plt, platelet count; TPO, thrombopoietin; Neu, neutrophils; BM, bone marrow; CsA, cyclosporine A; ATG, anti-thymocyte globulin.

Figure 1

(A)


3000
2500
2000
1500
1000
500
320
0
TPO (pg/mL)
RCUD
RCMD
RAEB
MDS-U
AA
ITP
HV

(B)

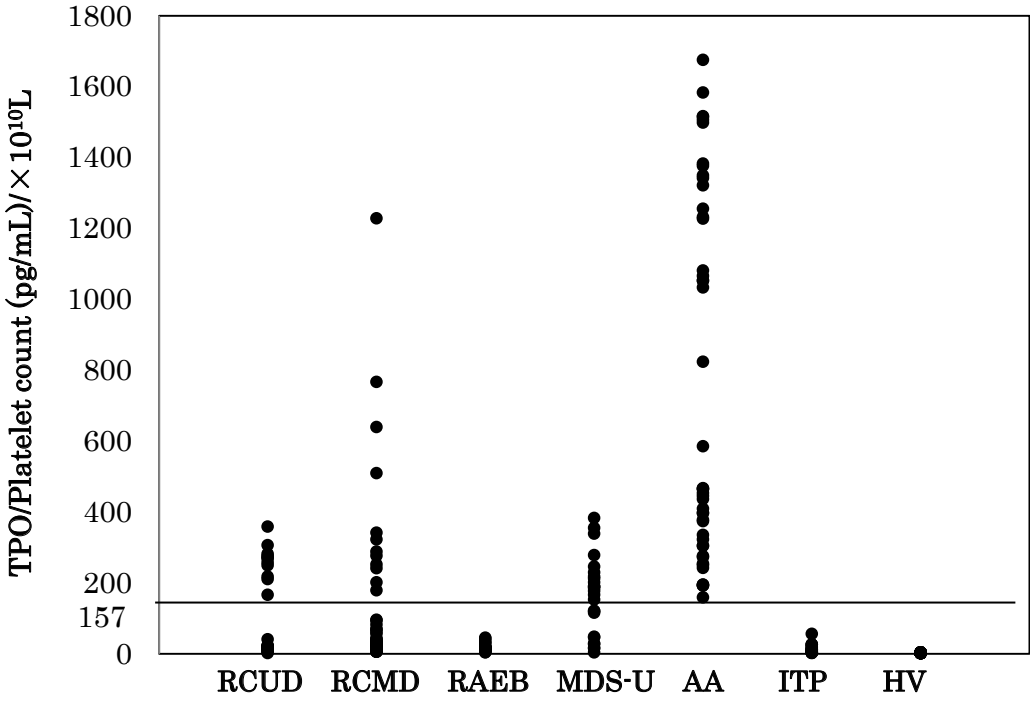
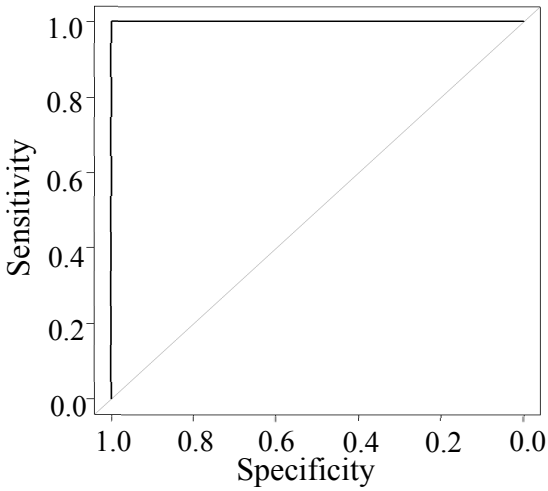
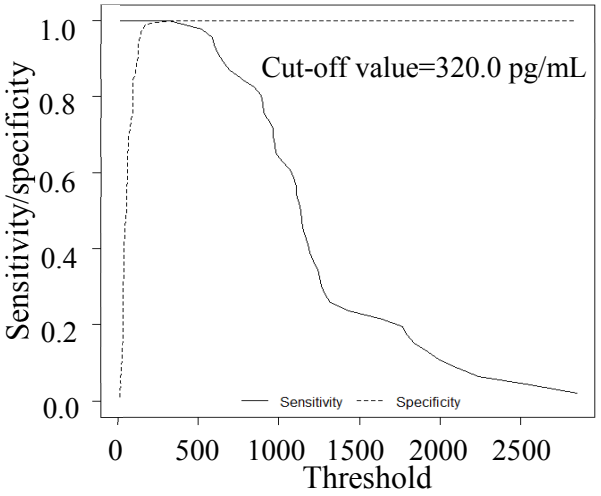

1800
1600
1400
1200
1000
800
600
400
200
157
0
TPO/Platelet count (pg/mL)/×10¹⁰L
RCUD
RCMD
RAEB
MDS-U
AA
ITP
HV

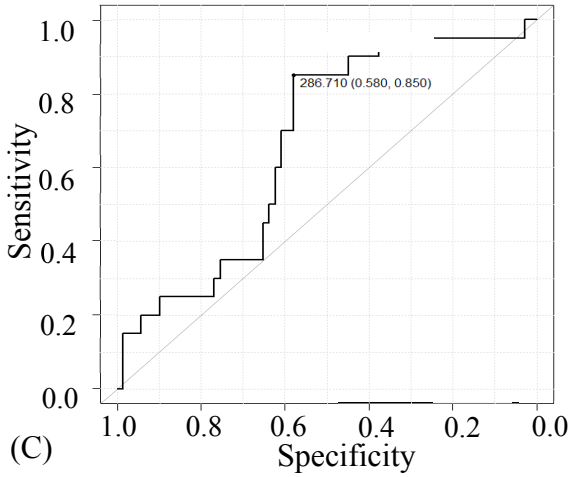
Figure 2

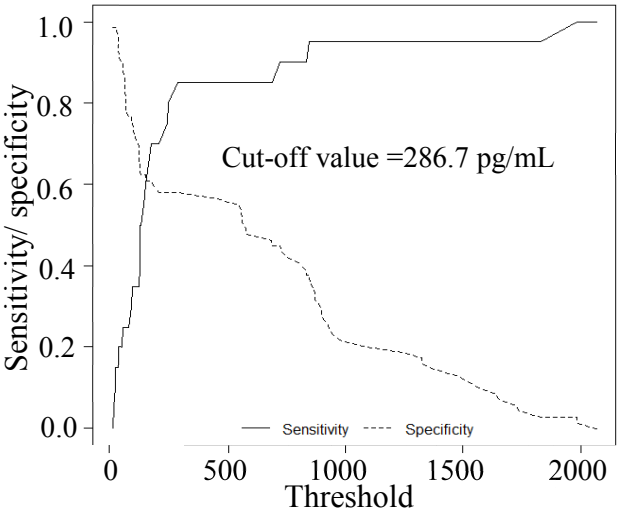
(A)


Sensitivity
Specificity
1.0
0.8
0.6
0.4
0.2
0.0

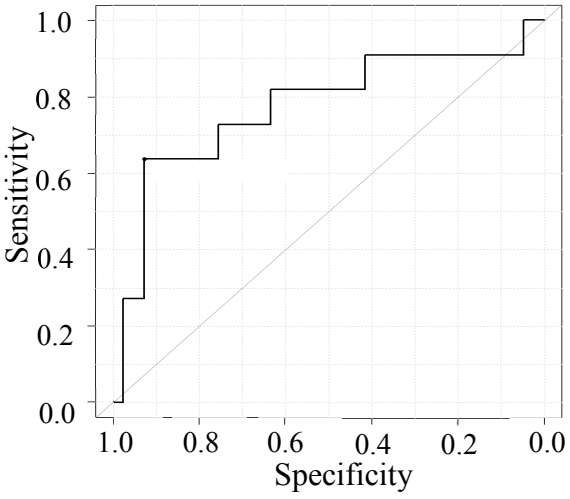

Cut-off value=320.0 pg/mL
Sensitivity/specificity
Threshold
Sensitivity
Specificity
0
500
1000
1500
2000
2500

(B)


286.710 (0.580, 0.850)
Sensitivity
Specificity


Cut-off value =286.7 pg/mL
Sensitivity/ specificity
Threshold
Sensitivity
Specificity

(C)


Sensitivity
Specificity

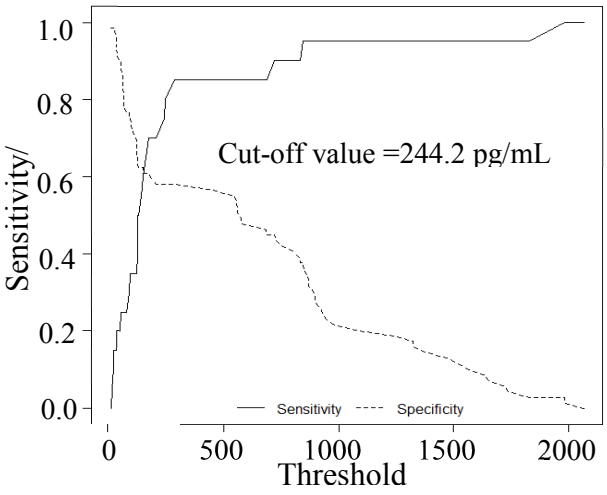
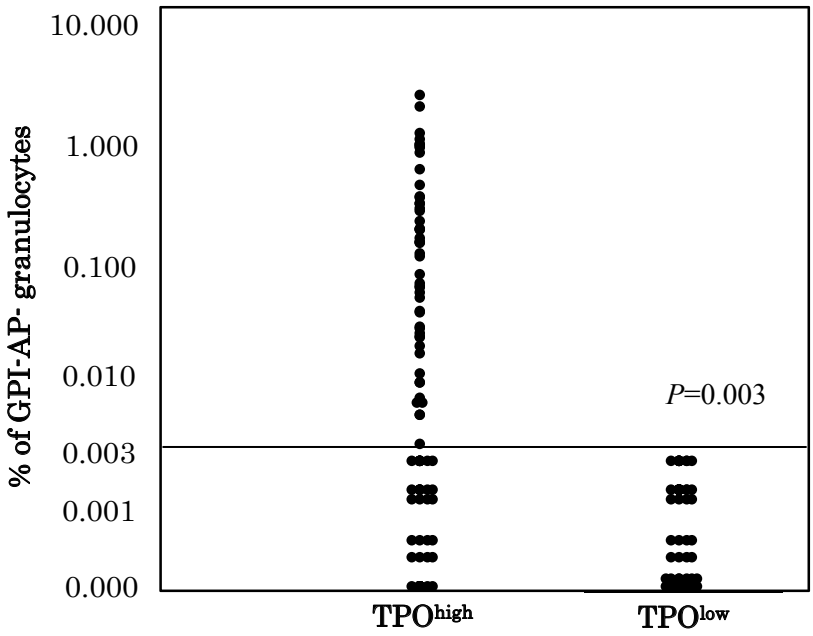
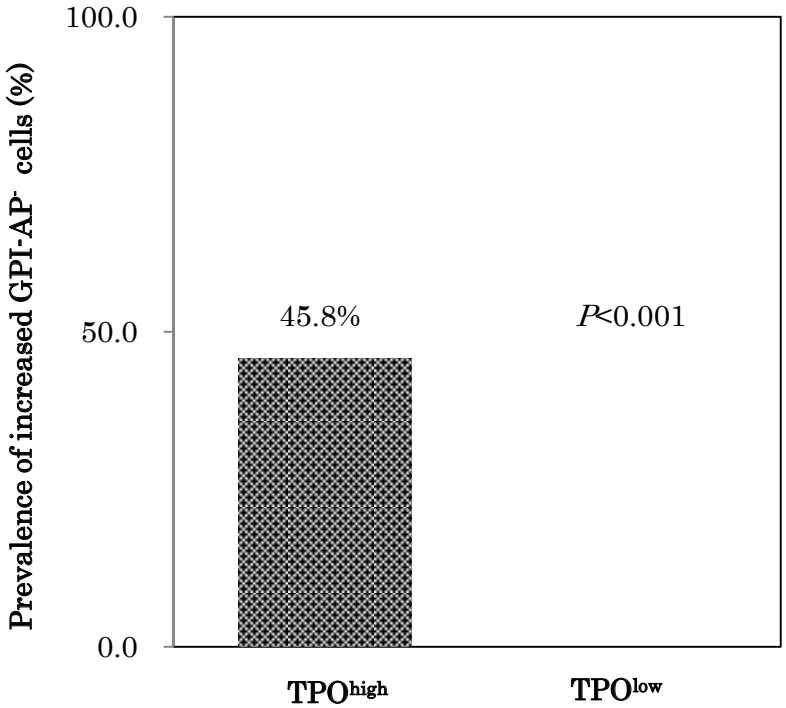

Cut-off value =244.2 pg/mL
Sensitivity/
Threshold
Sensitivity
Specificity

Figure 3

(A)


10.000
1.000
0.100
0.010
0.003
0.001
0.000
% of GPI-AP- granulocytes
P=0.003
TPOhigh
TPOlow

(B)


100.0
50.0
0.0
Prevalence of increased GPI-AP- cells (%)
45.8%
P<0.001
TPOhigh
TPOlow

(C)

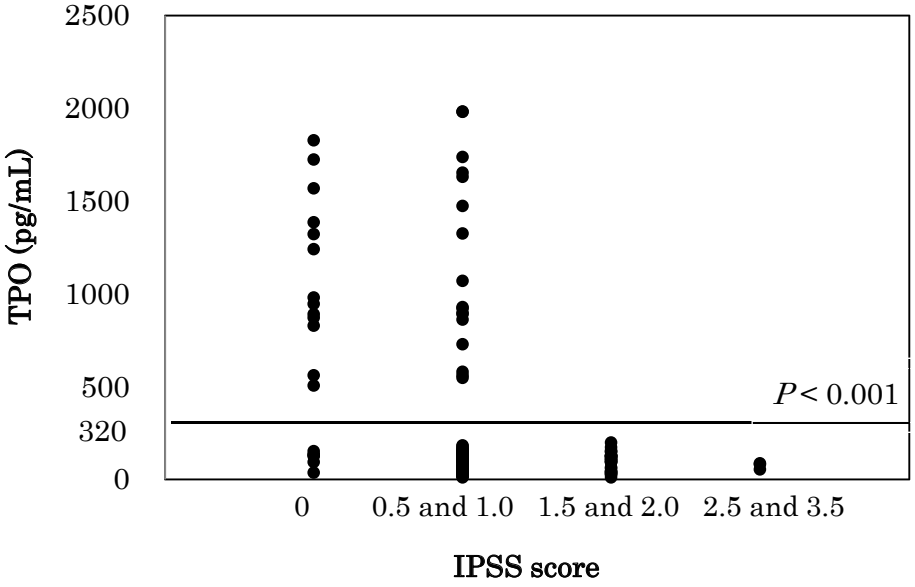
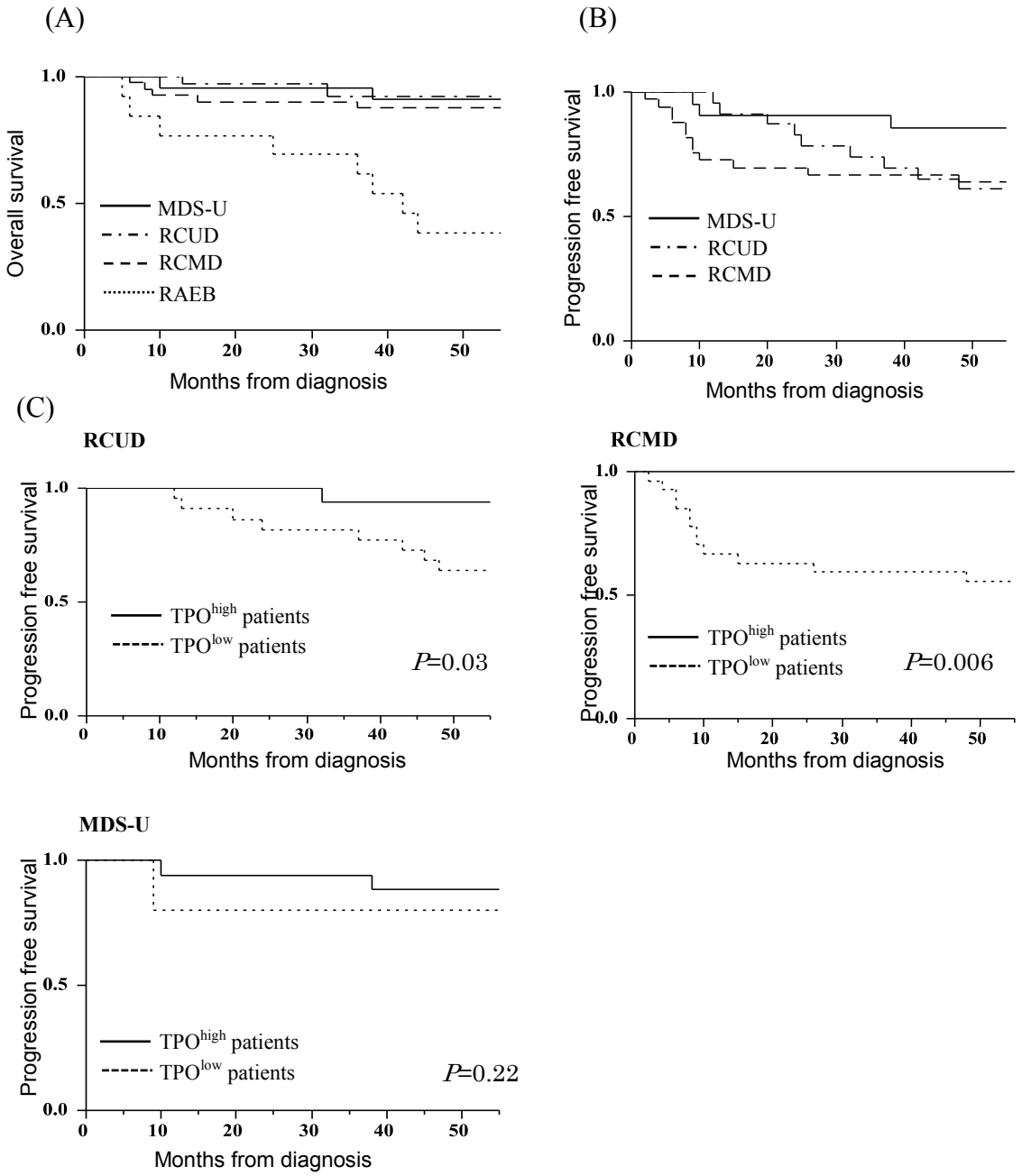

2500
2000
1500
1000
500
320
0
TPO (pg/mL)
P < 0.001
0
0.5 and 1.0
1.5 and 2.0
2.5 and 3.5
IPSS score

Figure 4


(A)
Overall survival
1.0
0.5
0.0
0
10
20
30
40
50
Months from diagnosis
MDS-U
RCUD
RCMD
RAEB
(B)
Progression free survival
1.0
0.5
0.0
0
10
20
30
40
50
Months from diagnosis
MDS-U
RCUD
RCMD
(C)
RCUD
Progression free survival
1.0
0.5
0.0
0
10
20
30
40
50
Months from diagnosis
TPO^high patients
TPO^low patients
P=0.03
RCMD
Progression free survival
1.0
0.5
0.0
0
10
20
30
40
50
Months from diagnosis
TPO^high patients
TPO^low patients
P=0.006
MDS-U
Progression free survival
1.0
0.5
0.0
0
10
20
30
40
50
Months from diagnosis
TPO^high patients
TPO^low patients
P=0.22