Fragmented Red Cell as a Possible Favorable Prognostic Marker of Hematopoietic Stem Cell Transplantation Associated Thrombotic Microangiopathy

Dong Wook Jekarl,¹ Yonggoo Kim,^{2*} Jihyang Lim,² Myungshin Kim,² Kyungja Han,² Bin Cho,³ Hak-Ki Kim,³ Woo-Sung Min,⁴ and Chang-Ki Min⁴

¹Department of Laboratory Medicine, Incheon St. Mary's Hospital, Catholic University of Korea, Seoul, Korea

²Department of Laboratory Medicine, Seoul St. Mary's Hospital, Catholic University of Korea, Seoul, Korea

³Division of Pediatric Hematology/Oncology, Department of Pediatrics, Seoul St. Mary's Hospital, Catholic University of Korea, Seoul, Korea

⁴Division of Hematology, Department of Internal Medicine, Catholic Hematopoietic Stem Cell Transplantation Center, Seoul St. Mary's Hospital, Catholic University of Korea, Seoul, Korea

Background: Fragmented red cell (FRC) by automated hematologic analyzer is known to detect schistocyte. In this study, it is noted that FRC might be a favorable prognostic marker of hematopoietic stem cell transplantation associated thrombotic microangiopathy (TA-TMA). **Methods:** The peripheral blood samples and clinical data of 89 patients were collected. The diagnosis of TA-TMA was defined by the Blood and Marrow Transplant Clinical Trials Network's criteria and schistocyte or both schistocyte and FRC-positive cases and other parameters fulfilled are regarded as TA-TMA. **Results:** Schistocyte and FRC displayed a correlation coefficient of 0.461 ($P < 0.001$) by Spearman's method. The diagnostic concordance of TA-TMA using schistocyte and

FRC was 92.1% with kappa index of 0.531 ($P < 0.001$). The number of diagnosed patients and mean survival month were as follows: TA-TMA by schistocyte, 8 (8.9%), 13.5 month; TA-TMA by schistocyte and FRC, 7 (7.8%), 40.4 month; No TMA, 74 (83.1%), 38.3 month, respectively. Kaplan–Meier survival analysis by log-rank method of the patient with TA-TMA by schistocyte and rest of the group showed statistical significance ($P < 0.01$). **Conclusion:** As evidenced by the data, FRC might be a favorable prognostic marker for TA-TMA, but additional studies with larger patients groups are required for validation of clinical applications. J. Clin. Lab. Anal. 29:444–450, 2015. © 2014 Wiley Periodicals, Inc.

Key words: fragmented red cell; schistocyte; transplantation-associated thrombotic microangiopathy

INTRODUCTION

Thrombotic microangiopathy (TMA) is a microvascular occlusive disorder expected to be caused by endothelial injury that leads to thrombus formation associated with intimal swelling and fibrinoid necrosis of the vessel wall. The thrombus in microcirculation results in mechanical injury of red blood cells (microangiopathic hemolytic anemia), which is the primary characteristic of TMA (1). TMA can be classified as non-transplantation-associated TMA and transplantation-associated TMA (TA-TMA). The etiology of non-TA-TMA includes thrombotic thrombocytopenic purpura,

hemolytic uremic syndrome, disseminated intravascular coagulopathy, pregnancy-related disorders, and

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*Correspondence to: Yonggoo Kim, Department of Laboratory Medicine, Seoul St. Mary's Hospital, Catholic University of Korea, 505 Seocho-gu, Banpo-dong, 137-701 Seoul, Korea. E-mail: yonggoo@catholic.ac.kr

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autoimmune disease (2). TA-TMA is one of the severe complications that occur after transplantation with an incidence rate of 0.5–63.6% (1). Risk factors of TA-TMA are graft-versus-host disease (GVHD); veno-occlusive disease; infection; and immunosuppressants such as FK506, cyclosporine, cytotoxic drugs, and irradiation (3–6).

Although TA-TMA shows severe clinical features, the diagnosis of TA-TMA is difficult as there are more than 28 sets of proposed criteria (3). No standard diagnostic criteria have yet been agreed upon but, recently, two sets of diagnostic criteria have been proposed by the Blood and Marrow Transplant Clinical Trials Network Toxicity Committee (BMT-CTN) and International Working Group (IWG) in regard to the definition of TA-TMA (7, 8). Both of the criteria include schistocyte and lactate dehydrogenase (LDH). Among them, the schistocyte is the most important diagnostic parameter in diagnosing TA-TMA, as the schistocyte is specific for RBC injury caused by intravascular pathologic conditions (9).

As the importance of the schistocyte was noted for diagnosing TA-TMA, standardization and automation of schistocyte had been reported in previous literatures (10–13). Fragmented red cell (FRC) are small round cells derived from the remnants of injured RBCs. Hematology analyzers are known to detect these small cells, which are smaller than RBCs and contain less RNA in a scattergram. These features are reported to be correlated with presence of schistocyte. The reported correlation of schistocyte and FRC ranged from 0.727 to 0.902 (10–13). However, previous literatures focus on FRC as a parameter that might substitute schistocyte but not as a marker for prognosis. The current study was undertaken to evaluate the performance of an automated hematologic analyzer in detecting schistocyte and the clinical significance of FRC as a possible prognostic marker of TA-TMA.

MATERIALS AND METHODS

Patients

Eighty-nine consecutive patients treated by hematopoietic stem cell transplantation at the Catholic Hematopoietic Stem Cell Transplantation Center were enrolled from November 2006 and followed up until April 2011. Demographic characteristics and clinical data of patients were recorded at admission, and laboratory data were recorded at the time of sample arrival (Table 1). This study was approved by the Institutional Review Board of Seoul St. Mary's Hospital, Seoul, Korea.

Schistocyte Evaluation by Manual Microscopy

Schistocyte enumeration was carried out by microscopy on peripheral blood smears after conventional Wright–

TABLE 1. Characteristics and Clinical Data of the 89 Patients

Characteristics	(n = 89)
Sex, male/female	55 (61.8)/34 (38.2)
Age, year <40/≥40	61 (68.5)/28 (31.5)
Diagnosis	
AA/MDS	14 (15.7)/5 (5.6)
AML/ALL	38 (42.7)/24 (27.0)
CML/MM	2 (2.2)/4 (4.5)
Others	2 (2.2)
Donor type	
Sibling/unrelated/auto	47 (52.8)/32 (36.0)/10 (11.2)
Stem cell source	
BM/PB/CB	40 (44.9)/46 (51.7)/3 (3.4)
CD34 count (×10 ⁶ /Kg)	
<5.0/≥5.0	49 (55.1)/40 (44.9)
HLA match	
Full/1-Ag mismatch	68 (76.4)/11 (12.4)
2-Ag mismatch	10 (11.2)
ABO	
Match/unmatch	47 (52.8)/42 (47.2)
Sex pair (donor-recipient)	
Match/unmatch	26 (29.2)/63 (70.8)
Female-male/others	21 (22.4)/42 (47.2)
Conditioning regimen	
TBI + CP/TBI + BU	27 (30.3)/2 (2.2)
TBI + CY/TBI + CY + MEL	3 (3.4)/14 (15.7)
TBI + CY + CP/BU	10 (11.2)
FLU + BU + ATG/CY + MEL	1 (1.1)/2 (2.2)
CP + CY/CP + BU	5 (5.6)/13 (14.6)
Others	12 (13.5)
GVHD prophylaxis	
Cs + MTX/TL + MTX	46 (51.7)/13 (14.6)
MMF + CS/TL	8 (9.0)
Others	22 (23.5)

AA, aplastic anemia; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; MM, multiple myeloma; BM, bone marrow; PB, peripheral blood; CB, cord blood; TBI, total body irradiation; CP, cyclophosphamide; BU, busulfan; CY, cytarabine; MEL, melfalan; FLU, fludarabine; ATG, anti thymocyte globulin; Cs, cyclosporine; TL, tacrolimus; MMF, mycophenolate mofetil; GVHD, graft-versus-host disease.

Giemsa staining. A schistocyte was defined as a helmet cell, triangular-shaped RBC (triangle cell), or crescent-shaped RBC (crescent cell, (11–13)). The average RBC count on one oil immersion field examined at high power was obtained from approximately 200 cells in one high-power field, and the number of schistocytes per high-power field multiplied by 1/2 was used to calculate the percentage of schistocyte. Schistocyte counts were independently performed within the proper spreading zone by two researchers. The positive cut-off value was 1%, which was equivalent to 2/high-power field (14). The schistocyte by microscopy was regarded as reference value. The agreement of schistocyte enumeration between two observers was performed.

Automated Detection of FRCs and Performance Evaluation

Schistocytes in treated EDTA were automatically detected and enumerated using the reticulocyte channel in a model Sysmex XE 2100 hematologic analyzer (Sysmex, Kobe, Japan) with upgraded software (RET master; Sysmex). The fluorescent dye containing polymethine and oxazine stains the RNA in the red blood cells and is detected by flow cytometric method. The laser beam cast a laser resulting in forward scatter, which indicates cell volume, and side scatter fluorescence, which indicates RNA content of the stained cell. The forward scatter was plotted on *y*-axis and side scatter are plotted on *x*-axis. The algorithm of software detects small red cells with low RNA content on the scattergram of the reticulocyte channel, which is considered to be schistocyte (10, 15). The cut-off value for a positive FRC was 1.2% (14). For the precision of FRC, evaluation was carried twice each run, twice a day for 5 days with patient samples (16). The linearity of the FRC was validated using patient sample with 2.6% FRC verified by microscopy ((5, 6)/high-power field) and mixed with normal sample (17).

Diagnosis of TA-TMA

The diagnosis of TA-TMA was made according to the BMT CTN criteria (7). These criteria were as follows: (a) RBC fragmentation with ≥ 2 schistocytes per high-power field in a peripheral smear; (b) concurrent increased serum LDH activity above 500 IU/L; (c) concurrent renal and/or neurologic dysfunction without other explanations, where renal dysfunction is defined as either a doubling of serum creatinine from baseline, where baseline is the creatinine level taken before hydration and just before initiation of the conditioning regimen, or a 50% decrease in creatinine clearance from baseline; and (d) negative direct and indirect Coombs' test results (7). TA-TMA was diagnosed with schistocyte, or both schistocyte with FRC that resulted in TA-TMA by schistocyte and TA-TMA by both schistocyte and FRC and other criteria fulfilled. Disseminated intravascular coagulopathy was excluded by normal prothrombin time and activated partial thromboplastin time. The autoimmune hemolytic anemia was excluded by negative Coombs' test and haptoglobin level.

Definition and Statistical Analyses

The TA-TMA index was defined as LDH divided by platelet as previously described (18). TA-TMA grade was defined as follows: grade 0, schistocytes ≤ 1.2 , normal or increased LDH; grade 1, schistocytes ≥ 1.3 , normal LDH; grade 2 schistocytes 1.3–4.8, increased LDH; grade 3,

schistocytes 4.9–9.6, increased LDH; grade 4 schistocytes ≥ 9.7 , increased LDH, respectively (18). The degree of agreement of schistocyte enumeration between the two observers was analyzed by intraclass correlation coefficient. For the accuracy of FRC values, it was compared with schistocyte and analysis by Passing–Bablok regression was performed. For the precision of FRC values, coefficients of variation (CVs) are calculated for positive and negative sample. The correlation between schistocyte and FRC was analyzed using Spearman's correlation method. The Kaplan–Meier survival analysis by log-rank method was performed for the survival of TA-TMA by schistocyte, TA-TMA by schistocyte and FRC, and No TA-TMA. The starting point of survival was the time of diagnosis of TA-TMA and primary end point was death by any cause. The comparison among No TA-TMA, TA-TMA by schistocyte and FRC, TA-TMA by FRC, and TA-TMA by schistocyte was performed for laboratory data including complete blood count, blood chemistry data, and clinical data by chi-square test or Fisher's exact test for categorical variable and Mann–Whitney *U* test for continuous variable. The statistical analysis was performed using Medcalc software 9.0 (Medcalc, Mariakerke, Belgium).

RESULTS

Accuracy

The Passing–Bablok analysis showed schistocyte, which is the reference value plotted against FRC. They showed linear relationship, but FRC had higher values as evidenced by the degree of slope ($y = 1.5x + 0.02$; $r^2 = 0.213$; $n = 89$). There was statistically significant correlation between FRC and schistocyte by Spearman's method ($r = 0.461$, $P < 0.001$) as shown in the scatter plot (Supplementary Material Figure 1).

Precision and Linearity

Schistocyte was identified by one of the two researchers in the Department of Laboratory Medicine, and the agreement between the two observers for schistocyte enumeration analyzed by the intraclass coefficient correlation method was 0.723 ($P < 0.001$) and considered good. Specimens that were schistocyte-positive and schistocyte-negative were evaluated and the mean $\pm$ SD of schistocyte positive and schistocyte negative specimens was $1.86 \pm 0.096\%$ (CV, 1.0%) and $0.05 \pm 0.053\%$ (CV, 1.0%), respectively. The blood samples of 35 healthy donors resulted in mean $\pm$ SD of $0.069 \pm 0.085\%$ with CV of 1.23%. The ratio of high (H) and low (L) sample for linearity verification was prepared as follows: control 1, 4:0; control 2, 3:1; control 3, 2:2; control 4, 1:3; control 5, 0:4. Each sample

was tested four times and the mean value was plotted. The result showed that $y = 0.494x + 0.014$ with $R^2 = 0.9849$ (Supplementary Material Figure 2).

Diagnostic Concordance

The number of patients diagnosed with TA-TMA by schistocyte, TA-TMA by both schistocyte and FRC, and No TA-TMA were as follows: 7 (7.9%) patients, 8 (9.0%) patients, 74 (83.1%) patients, respectively. The concordance and discordance rates of diagnosis were 92.1 and 7.9%, respectively. The concordance analyzed by kappa index was 0.531 ($P < 0.001$), which was regarded as moderate concordance.

Kaplan–Meier Survival Analysis by Log-Rank Method

The mean survival month of TA-TMA patients was studied from the time of diagnosis of TA-TMA to death by any cause. The mean survival month of TA-TMA, which includes all of the schistocyte positive patients, and No TA-TMA was as follows: TA-TMA, 26.4 month ($n = 15$); No TA-TMA, 38.3 month ($n = 74$), respectively, without statistical significance ($P = 0.06$, Fig. 1A). After TA-TMA patients were divided into schistocyte-positive and both schistocyte- and FRC-positive groups, the mean survival month of TA-TMA by schistocyte, TA-TMA by schistocyte and FRC, and No TA-TMA patients was as follows: TA-TMA by schistocyte, 14.1 month ($n = 8$); TA-TMA by schistocyte and FRC, 40.4 month ($n = 7$); No TA-TMA, 38.3 month ($n = 74$), respectively. Kaplan–Meier survival analysis by log-rank method of the patient with TA-TMA by schistocyte and rest of the group showed statistical significance ($P < 0.01$, Fig. 1B).

Comparison of Patients With TA-TMA by Schistocyte, TA-TMA by Schistocyte and FRC, and No TA-TMA

The patient characteristics of TA-TMA by schistocyte, TA-TMA by schistocyte and FRC, and No TA-TMA are shown in Table 2. The comparison of laboratory data including complete blood count (Supplementary Material Table 1) and blood chemistry data (Supplementary Material Table 2) are performed by chi-square test or Fisher's exact test for categorical variable and Mann–Whitney U test for continuous variables. Their clinical data are listed in Table 3.

In the case of laboratory tests, TA-TMA by schistocyte showed that schistocyte, platelet, LDH, total bilirubin, creatinine, and albumin were statistically significant compared to No TA-TMA patients. TA-TMA by schistocyte and FRC showed that schistocyte, FRC, LDH,

Fig. 1. Kaplan–Meier survival analysis with log-rank method showing (A) No TA-TMA and TA-TMA patients ($P = 0.06$); (B) TA-TMA by schistocyte and FRC, No TA-TMA, and TA-TMA by schistocyte patients ($P < 0.001$).

total bilirubin were statistically significant compared to No TA-TMA patients. Clinical data showed that CMV infection, TMA index, and TMA grade were increased in TA-TMA by schistocyte patients. TA-TMA by schistocyte and FRC showed no statistical significance compared to No TA-TMA patients.

DISCUSSION

TA-TMA is a severe complication occurring after hematopoietic stem cell transplantation. The diagnosis of TA-TMA is hindered by the fact that exact pathophysiology is not exactly known. Absence of ADAMTS-13 deficiency and decreased response to total plasmapheresis indicates that TA-TMA might be different from classical thrombotic thrombocytopenic purpura (19, 20). The entity of TA-TMA is obscured by the association of TA-TMA with risk factors representing similar clinical

TABLE 2. Characteristics and Clinical Data of No TA-TMA, TA-TMA by Schistocyte, and TA-TMA by Schistocyte and FRC Patients

Characteristics	No TA-TMA (n = 74)	TA-TMA by Schistocyte (n = 8)	TA-TMA by Schistocyte and FRC (n = 7)
Sex, male/female	25 (33.8)/49 (66.2)	4 (50.0)/4 (50.0)	2 (28.6)/5 (71.4)
Age, year			
<40/≥40	50 (67.6)/24 (32.4)	5 (62.5)/3 (37.5)	6 (85.7)/1 (14.3)
Diagnosis			
AA/MDS	11 (14.9)/3 (4.1)	1 (7.1)/2 (25.0)	2 (28.6)/0
AML/ALL	34 (45.9)/20 (27.0)	1 (12.5)/3 (37.5)	3 (42.9)/1 (14.3)
CML/MM	2 (2.7)/3 (4.1)	0/1 (12.5)	0/0
Others	1 (1.4)	0	1 (14.3)
Donor type			
Sibling/unrelated/auto	37 (50.0)/27 (36.5)/10 (13.5)	5 (62.5)/3 (37.5)/0	5 (71.4)/2 (28.6)/0
Stem cell source			
BM/PB/CB	32 (43.2)/41 (55.4)/1 (1.4)	4 (50.0)/3 (37.5)/1 (12.5)	4 (57.1)/2 (28.6)/1 (14.3)
CD34 count (×10 ⁶ /Kg)			
<5.0/≥5.0	42 (56.8)/32 (43.2)	3 (37.5)/5 (62.5)	4 (57.1)/3 (42.9)
HLA match			
Full/1-Ag mismatch	58 (78.4)/9 (12.2)	5 (62.5)/0	5 (71.4)/2 (28.6)
2-Ag mismatch	7 (9.5)	3 (37.5)	0
ABO			
Match/unmatch	39 (52.7)/35 (47.3)	5 (62.5)/3 (37.5)	3 (42.9)/4 (57.1)
Sex pair (donor-recipient)			
Match/unmatch	20 (27.0)/54 (73.0)	3 (37.5)/5 (62.5)	4 (57.1)/3 (42.9)
Female-male/others	17 (23.0)/3 (53.0)	2 (25.0)/3 (37.5)	2 (28.6)/2 (28.6)
Conditioning regimen			
TBI + CP/TBI + BU	24 (32.4)/2 (2.7)	3 (37.5)/0	0/0
TBI + CY/TBI + CY + MEL	2 (2.7)/14 (18.9)	1 (12.5)/0	0/0
TBI + CY + CP/BU	8 (10.9)	2 (25.0)	0/0
FLU + BU + ATG/CY + MEL	0/2 (2.7)	0/0	1 (14.3)/0
CP + CY/CP + BU	4 (6.1)/10 (15.2)	0/0	0/3 (42.9)
Others	7 (9.5)	2 (25.0)	3 (42.9)
GVHD prophylaxis			
Cs + MTX/TL + MTX	37 (50.0)/12 (16.2)	4 (50.0)/1 (12.5)	5 (71.4)/0
MMF + Cs/TL	4 (5.4)	3 (37.5)	1 (14.3)
Others	21 (28.4)	0	1 (14.3)

AA, aplastic anemia; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; MM, multiple myeloma; BM, bone marrow; PB, peripheral blood; CB, cord blood; TBI, total body irradiation; CP, cyclophosphamide; BU, busulfan; CY, cytarabine; MEL, melfalan; FLU, fludarabine; ATG, anti thymocyte globulin; Cs, cyclosporine; TL, tacrolimus; MMF, mycophenolate mofetil; GVHD, graft-versus-host disease.

features (21–23). However, the most important diagnostic parameter of TA-TMA is schistocyte identification (2). Regardless of the importance of this parameter, detection of schistocyte is not standardized either by manual or automated method.

Automated detection of schistocyte is reported in previous literature and showed that FRC correlated well with schistocyte with correlation coefficient ranging from 0.727 to 0.902, with statistical significance (13, 15, 24–26). Unlike previous studies, this report showed that correlation coefficient was 0.461 by Spearman's method, which was lower than previous literatures. In addition, the concordance rate of diagnosis of TA-TMA was high, but kappa index was in moderate range. This difference was expected to be derived from the fact that our data included schistocyte of 74 patients with normal values. However, consid-

ering degree of the difference of the correlation coefficient and kappa index being too low, we searched for the explanation of difference between our data and data from previous literatures.

It was noted that the mean survival month of TA-TMA by schistocyte was shorter compared to No TA-TMA and TA-TMA by schistocyte and FRC patients with statistical significance. This result was also supported by the fact that TA-TMA by schistocyte had higher TMA index and TMA grade. This was in line with previous literature that higher TMA index and grade are associated with shorter survival (18, 21, 22). Other laboratory data also showed that TA-TMA by schistocyte had lower platelets, albumin, and higher creatinine level compared to No TMA group, which was not the case with TA-TMA by schistocyte and FRC. These laboratory data support the fact that

TABLE 3. Clinical Data of No TA-TMA, TA-TMA by Schistocyte, and TA-TMA by Schistocyte and FRC Patients

Characteristics	No TMA (<i>n</i> = 74)	TMA by Schistocyte (<i>n</i> = 8)	TMA by Schistocyte and FRC (<i>n</i> = 7)
GVHD			
Acute/none	39 (52.7)/35 (47.3)	4 (50.0)/35 (47.3)	5 (71.4)/2 (28.6)
Organ involvement			
Skin/liver	20 (27.0)/6 (8.1)	2 (25.0)/1 (12.5)	3 (42.9)/0
Gut/other	6 (8.1)/1 (1.4)	0/1 (12.5)	0/0
Skin + gut	9 (12.2)	1 (12.5)	1 (14.3)
Skin + liver	0	0	1 (14.3)
Grade			
I~II/III~IV	33 (44.6)/9 (12.2)	5 (62.5)/0	4 (57.1)/1 (14.3)
Concurrent infection			
CMV ^a /fungal	25 (33.8)/7 (9.5)	6 (75.0)/1 (12.5)	1 (14.3)/2 (28.6)
No infection/other	39 (52.7)/3 (4.1)	1 (12.5)/0	3 (42.9)/1 (14.3)
Concurrent condition			
None/other	50 (67.6)/10 (13.5)	3 (37.5)/1 (12.5)	6 (85.7)
VOD/BO	1 (1.4)/2 (2.7)	0/1 (12.5)	0/1 (14.3)
Hemorrhagic cystitis	4 (5.4)	1 (12.5)	0
Hemorrhage	3 (4.1)	2 (25.0)	0
Toxic hepatitis	4 (5.4)	0	0
Relapse/no relapse (%)	19 (25.7)/55 (74.3)	2 (25.0)/6 (75.0)	1 (14.3)/6 (85.7)
TMA index ^b			
<20/≥20	NA	3 (37.5)/5 (62.5)	7 (100)/0
TMA grade ^b			
0/1/2	NA	1 (12.5)/0/7 (87.5)	5 (71.4)/0/2 (12.5)

^aParameters with statistical significance analyzed by chi-square or Fisher's exact test between No TA-TMA and TA-TMA by schistocyte.

^bParameters with statistical significance analyzed by chi-square or Fisher's exact test between No TA-TMA and TA-TMA by schistocyte and FRC. GVHD, graft-versus-host disease; CMV, cytomegalovirus; VOD, veno-occlusive disease; BO, bronchiolitis obliterans; NA, not applicable.

TA-TMA by schistocyte has more severe clinical features compared to TA-TMA by schistocyte and FRC. In addition, if TA-TMA had been diagnosed only by FRC without schistocyte and other BMT-CTN criteria fulfilled, the mean survival month was 33.6 month, which was close to No TMA group, indicating favorable survival compared to TA-TMA by schistocyte (data not shown).

On the basis of our data, we hypothesized that FRC might be a marker associated with favorable prognosis. FRC might be a milder form of schistocyte, compared to schistocyte defined as crescent-shaped, helmet-shaped, and triangle-shaped RBC that represents marked mechanical injury of red blood cell. Another hypothesis would be that FRC might be associated with convalescence mechanism of RBC injury. FRC might be a result of activation of repairing mechanism of injured RBC that circulates in peripheral blood with normal function albeit small size. In case of TA-TMA by schistocyte, absence of FRC might indicate absence of convalescence mechanism or rapid progression of mechanical injury of RBC without having time for repairing injured RBC.

The limitation of this study is that the diagnosis of TA-TMA was performed only by BMT-CTN criteria and not IWG criteria. The IWG criteria required schistocyte to be more than 4%, which was too high to be applied in our data as well as others (27). If the schistocyte level

was dropped to 1%, and utilizing other criteria by IWG, there would be seven patients diagnosed with TA-TMA (data not shown). The mean survival was 25.6 month and it was statistically significant compared to No TMA patients by Kaplan–Meier survival analysis with generalized Wilcoxon method. However, among seven patients only two of them had positive FRC, which was too small to be analyzed.

In conclusion, TA-TMA by schistocyte had worse prognosis compared to TA-TMA by both schistocyte and FRC. FRC might be a possible favorable prognostic marker in TA-TMA patients. However, as this study was performed in one center with a small number of patients, additional studies are required with larger number of patients in various centers for validation of clinical application.

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REFERENCES

1. Moake JL. Thrombotic microangiopathies. *N Engl J Med* 2007;347:589–600.

2. Choi CM, Schmaier AH, Snell MR, Lazarus HM. Thrombotic microangiopathy in haematopoietic stem cell transplantation. *Drugs* 2009;69:183–198.
3. George JN, Li X, McMinn JR, Terrell DR, Vesely SK, Selby GB. Thrombotic thrombocytopenic purpura-hemolytic uremic syndrome following allogeneic HPC transplantation: A diagnostic dilemma. *Transfusion* 2004;44:294–304.
4. Fujino M, Kim Y, Ito M. Intestinal thrombotic microangiopathy induced by FK506 in rats. *Bone Marrow Transplant* 2006;39:367–372.
5. Pettitt AR, Clark RE. Thrombotic microangiopathy following bone marrow transplantation. *Bone Marrow Transplant* 1994;14:495–504.
6. Nakamae H, Yamane T, Hasegawa T, et al. Risk factor analysis for thrombotic microangiopathy after reduced-intensity or myeloablative allogeneic hematopoietic stem cell transplantation. *Am J Hematol* 2006;81:525–531.
7. Ho VT, Cutler C, Carter S, Martin P, Adams R, Horowitz M. Blood and marrow transplant clinical trials network toxicity committee consensus summary: Thrombotic microangiopathy after hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2005;11:571–575.
8. Ruutu T, Giovanni B, Benjamin RJ, Clark RE, George JN, Gratwohl A. Diagnostic criteria for hematopoietic stem cell transplant-associated microangiopathy: Results of a consensus process by an international working group. *Haematologica* 2007;92:95–100.
9. Bull B, Kuhn I. The production of schistocytes by fibrin stands (a scanning electron microscope study). *Blood* 1970;35:104–111.
10. Jiang M, Saigo K, Kumagai S, Imoto S, Kosaka Y, Matsumoto H. Quantification of red blood cell fragmentation by automated haematology analyzer XE-2100. *Clin Lab Haematol* 2001;23:167–172.
11. Lesesve JF, Lecompte T, Alla F, Fenneteau O, Cynober T, Siest JP. Reproducibility of the morphological identification of schistocytes and evaluation of non-observer-dependent method. *Ann Biol Clin* 2005;63:279–289.
12. Lesesve JF, Fenneteau O, Cynober T, Lecompte T, Grange MJ, Flandrin G. Role of the biologist in the study of schistocyte. *Ann Biol Clin* 2003;61:505–512.
13. Lesesve JF, Salignac S, Alla F, Defente M, Benbih M, Bordigoni P. Comparative evaluation of schistocyte counting by an automated method and by microscopic determination. *Am J Clin Pathol* 2004;121:739–745.
14. Cho BS, Min CK, Eom KS, et al. Clinical impact of thrombotic microangiopathy on the outcome of patients with acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2008;41:813–820.
15. Saigo K, Jiang M, Tanaka C, Fujimoto K, Kobayashi A, Nozu K. Usefulness of automatic detection of fragmented red cells using a hematology analyzer for diagnosis of thrombotic microangiopathy. *Clin Lab Haematol* 2002;24:324–351.
16. Clinical and Laboratory Standards Institute. Evaluation of Precision Performance of Quantitative Measurement Method; Approved Guideline EP5-A2, Wayne, PA: Clinical and Laboratory Standards Institute; 2006.
17. Clinical and Laboratory Standards Institute. Evaluation of Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline EP6-A, Wayne, PA: Clinical and Laboratory Standards Institute; 2003.
18. Zeigler ZR, Shadduck Rk, Nemunaitis J, Andrews DF, Rosenfeld CS. Bone marrow transplantation-associated thrombotic microangiopathy: A case series. *Bone Marrow Transplant* 1995;15:247–253.
19. Daly AS, Hasegawa WS, Lipton JH, Messner HA, Kiss. Transplantation-associated thrombotic microangiopathy is associated with transplantation from unrelated donors, acute graft-versus-host disease and venoocclusive disease of the liver. *Transfus Apher Sci* 2002;27:3–12.
20. Batts ED, Lazarus HM. Diagnosis and treatment of transplantation-associated thrombotic microangiopathy: Real progress or are we still waiting? *Bone Marrow Transplant* 2007;40:709–719.
21. Laskin B.L., Goebel J, Davies S.M., Jodele S. Small vessels, big trouble in the kidneys and beyond: Hematopoietic stem cell transplantation associated-thrombotic microangiopathy. *Blood* 2011; Epub ahead of print.
22. Uderzo C, Bonanomi S, Busca A, Renoldi M, Ferrari P, Iacobelli M. Risk factors and severe outcome in thrombotic microangiopathy after allogeneic hematopoietic stem cell transplantation. *Transplantation* 2006;82:638–644.
23. Fuge R, Bird JM, Fraser A, Hart D, Hunt L, Cornish JM. The clinical features, risk factors and outcome of thrombotic thrombocytopenic purpura occurring after bone marrow transplantation. *Br J Hematol* 2001;113:58–64.
24. Banno S, Ito Y, Hori T, Fujimoto K, Suzuki T, Hashimoto T. Quantification of red blood cell fragmentation by the automated hematology analyzer XE-2100 in patients with living donor liver transplantation. *Clin Lab Haematol* 2005;27:292–296.
25. Lesesve JF, Salignac S, Lecompte T, Bordigoni P. Automated measurement of schistocytes after bone marrow transplantation. *Bone Marrow Transplant* 2004;34:357–362.
26. Imoto S, Murayama T, Nagai K, Hirabayashi N, Tanaka C, Misawa M. Usefulness of sequential automated analysis of fragmented red blood cell for the differential diagnosis of thrombotic thrombocytopenic purpura-hemolytic uremic syndrome following allogeneic hematopoietic cell transplantation. *Lab Hematol* 2005;11:131–136.
27. Cho BS, Yahng SA, Lee SE, et al. Validation of recently proposed consensus criteria for thrombotic microangiopathy after allogeneic hematopoietic stem cell transplantation. *Transplantation* 2010;90:918–926.