Case Report

Schistocytosis in acute myeloid leukemia with myelodysplasia-related changes, showing predominant erythroid proliferation

Futoshi Iioka¹*, Katsuyo Tsuda², Daiki Shimomura², Miho Nakagawa², Atsuko Okumura³, Masahiko Hayashida¹, Kiyotaka Izumi¹, Yoshimasa Kamoda¹, Takashi Akasaka¹, Hitoshi Ohno¹,³

¹Department of Hematology, Tenri Hospital; ²Department of Laboratory Medicine, Tenri Hospital; ³Tenri Institute of Medical Research

A 37-year-old man was referred to our department with pancytopenia. His hemoglobin level was 5.6 g/dL, white blood cell count was 2.41 × 10³/μL, and platelet count was 32.0 × 10³/μL. A blood smear examination revealed significantly large numbers of schistocytes and marked anisopoikilocytosis. His bone marrow contained 37.0% blasts with the morphological and cytochemical characteristics of proerythroblasts, and showed trilineage dysplasia. Flow cytometric and electron microscopic analyses provided persuasive evidence for the immature erythroid features of blasts. Metaphases obtained from the bone marrow carried complex cytogenetic abnormalities, including del(5q) and −7, both of which were detected in approximately 70% of bone marrow cell nuclei by fluorescence in situ hybridization. The patient was treated with induction chemotherapy consisting of cytarabine and idarubicin, leading to a complete hematological response and the normalization of erythrocyte morphology. Schistocytosis as well as anisopoikilocytosis in the blood may reflect defective erythrocyte development in the bone marrow. Despite the erythroid features of the blasts, the presence of del(5q) and −7, and the trilineage dysplasia indicated that the leukemia of this case was acute myeloid leukemia with myelodysplasia-related changes, according to the current WHO classification.

Keywords: Schistocyte, acute myeloid leukemia with myelodysplasia-related changes, erythroid hyperplasia, del(5q), −7

INTRODUCTION

A morphological examination of peripheral blood smears is a crucial diagnostic test for patients with anemia, thrombocytopenia, or leukemia.¹ It may immediately provide a specific diagnosis or indicate a narrow range of diagnostic possibilities.¹,² Red blood cells (referred to as erythrocytes in this report) are uniform in shape, size, and color, appearing round with smooth contours and a central pallor under normal conditions. In contrast, morphological modifications have been reported under pathological conditions.¹,²

Schistocytes, a term derived from Greek the schisto, meaning broken or cleft, originate from erythrocyte fragments or amputated erythrocytes, and the detection of such cells is an important morphological indicator of primary thrombotic microangiopathy (TMA) syndromes, including thrombotic thrombocytopenic purpura (TTP) and hemolytic-uremic syndrome (HUS).¹,⁴ The Schisto-
cyte Working Group of the International Council for Standardization in Haematology (ICSH) has described the morphology of schistocytes as triangles, microcre- scents, helmet cells, keratocytes (cells with horns), or microspherocytes. The formation of these fragmented erythrocytes has been attributed to ‘extrinsic’ mechanical damage to the erythrocyte membrane, caused by fibrin strands on the endothelial surface of microvessels.

Schistocytes have been detected not only in TMA/TTP-HUS, but also various inherited or acquired erythrocyte disorders that may cause ‘intrinsic’ defects in erythrocytes (e.g. erythrocyte membrane defects, thalassemia, megaloblastic anemia, primary myelofibrosis, and myelodysplastic syndrome [MDS]). We herein described a male patient who presented with severe anemia and thrombocytopenia. TTP was initially suggested because his peripheral blood smears contained significantly large numbers of schistocytes; however, his bone marrow was subsequently found to be replaced by leukemia cells with erythroid characteristics.

**CASE REPORT**

**Case Presentation**

A 37-year-old man was admitted to the Hematology Department with pancytopenia. He had been burned ten months earlier, and his laboratory test at the other hospital had been normal. However, he developed general fatigue and faintness when standing up or walking 5 months prior to being admitted to our hospital. Laboratory tests at this time showed pancytopenia, and he was referred to our hospital. His past medical history and family history were unremarkable, and he was not taking any medication. On admission, he was alert with a normal mental status. His body temperature was 36.5°C, blood pressure was 121/68 mmHg, heart rate was 62 beats per minute, and oxygen saturation was 99% while he was breathing ambient air. Although his palpebral conjunctiva was anemic, the results of a physical examination were otherwise normal.

Complete blood counts revealed normocytic, normochromic anemia, thrombocytopenia, and leukopenia (Table 1). The white cell differential was 51.0% lymphocytes, 17.0% monocytes, 30.0% segmented neutrophils, and 2.0% banded neutrophils; no blasts were identified. There was a significantly large number of schistocytes that matched the morphological criteria of ICSH (Figure 1). On the other hand, non-fragmented erythrocytes, which appeared to maintain intact cell contours, showed anisocytosis (unequal size) and poikilocytosis (variable shape) (Figure 1). The high levels of serum iron and ferritin and low unsaturated iron binding capacity suggested iron overload (Table 1). Vitamin B₁₂ and folic acid values were within normal ranges. Other blood chemistry values included: creatinine, 0.6 mg/dL; total bilirubin, 0.9 mg/dL; total serum protein, 5.6 g/dL; and albumin, 3.8 g/dL. Routine blood coagulation tests excluded disseminated intravascular coagulation. The activity of ADAMTS13 was 63% and its inhibitor was not detected. A urine occult blood test was negative.

| Table 1. Laboratory test results at initial presentation |
|------------------------------------------|---------|------------------------------------------|
| Variable                                 | Value   | Normal range                           |
| Red cell count (x10⁶/μL)                 | 2.03    | 3.90-5.60                               |
| Hemoglobin (g/dL)                        | 5.6     | 13.1-17.0                               |
| Hematocrit (%)                           | 17.4    | 38.0-50.0                               |
| Mean corpuscular volume (fL)             | 86      | 84-99                                   |
| Mean hemoglobin concentration (pg)       | 27.6    | 27.0-34.0                               |
| Mean corpuscular hemoglobin concentration (%) | 32.2 | 31.0-36.0                              |
| Reticulocytes (%)                        | 0.6     | 0.7-1.9                                  |
| Platelet count (x10⁹/μL)                 | 32      | 150-350                                 |
| White cell count (x10⁹/μL)               | 2.41    | 3.50-8.50                               |
| Lactate dehydrogenase (IU/L)             | 288     | 100-225                                 |
| Haptoglobin (mg/dL)                      | 1.0     | 19.0-170.0                              |
| Serum iron (μg/dL)                       | 215     | 80-160                                  |
| Total iron binding capacity (μg/dL)      | 233     | 290-400                                 |
| Unsaturated iron binding capacity (μg/dL) | 18  | 180-270                                 |
| Transferrin saturation (%)               | 92.3    | 28.0-41.0                               |
| Serum ferritin (ng/mL)                   | 469     | 10-260                                  |
| Vitamin B₁₂ (pg/ml)                      | 496     | 233-914                                 |
| Folic acid (ng/mL)                       | 5.1     | 3.6-12.9                                 |
| C-reactive protein (mg/dL)               | <0.2    | <0.2                                    |
| ADAMTS13 activity (%)                    | 63      | 100                                     |
| ADAMTS13 inhibitor titer (BU/mL)         | 0.0     | Negative                                |
Figure 1. Peripheral blood pictures, showing schistocytes in the setting of anisopoikilocytosis and thrombocytopenia (Wright stain; original magnification, ×100 objective). An elliptocyte (arrows in B), codocyte (arrow in A), dacrococyte (arrow in C), leptocyte (asterisk in D), crescent-shaped erythrocyte (arrow in D), and a large-sized platelet (asterisk in B) are indicated. Some schistocytes had a central pallor (arrowheads in A, C, and D).

while hemosiderin granules were found in the urinary sediment. Imaging studies revealed no vascular tumors within the body.

Appearance of the Bone Marrow

The bone marrow showed normocellularity comprised of 37.0% blasts, 10.5% rubricytes (polychromatic erythroblasts), and 12.3% metarubricytes (orthochromat erythroblasts). Blasts were characterized by round nuclei, fine chromatin with often prominent nucleoli, and deeply basophilic cytoplasm (Figure 2A), which was consistent with the features of rubriblasts (pro-erythroblasts). Some cells had vacuoles and fine azurophilic granules in the cytoplasm. Myeloperoxidase and non-specific esterase staining were negative (Figure 2B and C). On the other hand, erythroid precursor cells showed a dot-like pattern for acid phosphatase staining and the micro-granular pattern of positivity for periodic acid-Schiff staining (Figure 2D and E). Mature erythroblasts and megakaryocytes showed marked dysplastic morphologies (Figure 2F and G), and ringed sideroblasts comprised of 20% mature erythroblasts (Figure 2H). Neutrophils displayed a hypogranular cytoplasm and pseudo-Pelger nuclear abnormality.

Flow Cytometry (FCM)

Single-color FCM of the mononuclear cell fraction revealed that the cells were CD36+, glycophorin A (GPA)+/−, CD34+/−, HLADR+/−, CD13+/−, CD33+/−, CD117+/−, CD41+, CD11c+, CD11b+/−, CD2+, and CD7+ (Figure 3A). We next employed multi-color FCM and found that the cells appeared to be composed of 4 populations, i.e.
CD34⁺GPA⁻, CD34⁺GPA⁺, CD34⁻GPA⁺, and CD34⁻ GPA CD13⁺CD33⁻. The former two populations represent immature erythroblasts, the third mature erythroblasts, and the last myeloid precursors with monocytic differentiation, respectively (Figure 3B).

**Electron Microscopy**

Blasts were 9 to 17 µm in diameter and the nuclear-cytoplasmic ratio ranged from 0.6 to 0.9. Nuclei were round with indentation and had dispersed chromatin, and nucleoli were small to medium. Cytoplasmic organelles varied in abundance. Some blasts included θ granules in...
their cytoplasm, the sizes of which ranged between 0.3 and 0.6 µm in diameter (Figure 4). Myeloperoxidase and platelet peroxidase reactions were negative.

**Figure 3.** Single-color (A) and multi-color (B) flow cytometry (FCM) of bone marrow cells. Side scatter (SSC) and forward scatter (FSC) characteristics of the cells are shown at the *left end*. Gated mononuclear cells were subjected to FCM using the antibodies indicated in each panel. In B, CD34⁺ GPA⁻ immature erythroblasts are colored in blue, CD34⁺ GPA⁺ immature erythroblasts in purple, CD34⁻ GPA⁺ mature erythroblasts in red, and CD34⁻ GPA⁻ CD13⁺ CD33⁺ myeloid precursors with monocytic differentiation in green. Fluorochrome-conjugated monoclonal antibodies were CD34-PC7 (Beckman Coulter, Marseille, France), CD235a (GPA)-PE (DAKO Glostrup, Denmark), CD13-APC (Biolegend San Diego, USA), CD33-APC Alexa750 (Beckman Coulter), CD36-FITC (Beckman Coulter), HLADR-ECD (Beckman Coulter), and CD14-PerCP (BD Biosciences, Oxford, UK).

**Figure 4.** Electron microscopy. (A) Myeloperoxidase and (B) platelet peroxidase. A θ granule was present in the Golgi area (red arrow in A and *inset* under higher magnification).

**Cytogenetic Studies**

Metaphase cells obtained from the bone marrow carried numerical and structural abnormalities, including the deletion of 5q and loss of chromosome...
7 (Figure 5), both of which were confirmed by fluorescence in situ hybridization (FISH) using probes to detect del(5q)/−5 and del(7q)/−7 (Figure 6). Del(5q) and −7 cells comprised approximately 70% of the interphase nuclei counted (Figure 6). Because an unknown chromosomal material was added to the 5q11 band of del(5q), the abnormality was correctly designated add(5)(q11). The karyotype was:

![G-banded karyotype](image)

Figure 5. G-banded karyotype obtained from the bone marrow. The loss of chromosomes and structural abnormalities are indicated by arrows. The karyotype of this figure was: 44,X,−Y,−4,add(5)(q11),−7,−9,add(19)(p13),+2mar.

![Metaphase and interphase FISH](image)

Figure 6. Metaphase and interphase FISH confirming del(5q) (A and B) and −7 (C and D), using the Vysis LSI EGR1/D5S23, D5S721 Dual Color Probe and Vysis D7S486/CEP7 FISH Probe, both of which were purchased from Abbott Laboratories, Abbott Park, IL, USA.
Treatment Course
The patient was treated with ‘7 + 3’ induction chemotherapy consisting of cytarabine and idarubicin, leading to a hematological response with the disappearance of schistocytes in the peripheral blood smear. The percentage of marrow blasts decreased to 0.2% on day 38 of the treatment, and hematopoietic cellular morphology became normal. FISH of the bone marrow smear slide confirmed a response at the cytogenetic level. He underwent two cycles of consolidation chemotherapy, and then received allogeneic stem cell transplantation from a HLA-identical sibling donor with myeloablative preconditioning.

DISCUSSION
In contrast to TTP, in which schistocytes are observed as the dominant morphological abnormality in association with polychromasia, reflecting the stimulation of erythropoiesis to compensate for erythrocyte destruction (Supplementary Figure S1), peripheral blood pictures of the present case were characterized not only by schistocytosis, but also a wide range of additional changes in both the size and shape of erythrocytes (i.e. anisopoikilocytosis), which were not specific for a diagnosis of TTP. Because erythroid precursors in the bone marrow showed a prominent dysplastic morphology, schistocytosis as well as anisopoikilocytosis in the blood may have reflected defective erythrocyte development in the bone marrow. This was supported by the morphology of erythrocytes in the normalizing blood when the patient achieved a complete response and normal hematopoiesis recovered.

The erythrocyte skeleton plays an essential role in determining the shape of the cell, and defects of erythrocyte membrane proteins may cause loss of membrane structural integrity. By analogy with the findings of erythrocyte membrane protein defects identified in hereditary erythrocyte disorders, molecular abnormalities of these proteins in MDS have been studied. In a study of 50 cases of MDS exhibiting some abnormalities of erythrocyte morphology, showed a partial deficiency of membrane proteins, including band 3 deficiency in 3 cases, P4.1 deficiency in 1 case, P4.2 deficiency in 3 cases, and band 7 deficiency in 2 cases. More importantly, of the 3 band 3-deficient patients, 2 carried mutations within the band 3 gene. Thus, it is possible that in the present case, abnormalities of the erythrocyte membrane proteins associated with the development of MDS may have accounted in part for the schistocytosis and anisopoikilocytosis.

Acute myeloid leukemia (AML) associated with predominant erythroid proliferation (50% or more of erythroid precursors) has been described as M6 in the French-American-British classification or as acute erythroid leukemia (AEL) in the 2001/2008 WHO classification, which is further subdivided into erythroleukemia and pure erythroid leukemia (erythroid precursors ≥80%). However, erythroid hyperplasia may be observed in MDS (blasts <20% of all nucleated cells and also <20% of non-erythroid cells) and AML with myelodysplasia-related changes (AML-MRC) (blasts ≥20% and ≥50% dysplastic cells in ≥2 cell lineages) proposed in the 2008 WHO classification, and these conditions may overlap with AEL. Because the majority of immature cells in the bone marrow of the present case appeared to be committed to the erythroid lineage, which was confirmed by FCM and electron microscopy, leukemia of this case may be included in the pure erythroid leukemia category, even though the percentage (59.8%) did not reach the level of the criteria. However, the presence of del(5q) and −7 and trilineage dysplasia suggested that the classification of this leukemia as AML-MRC was more appropriate than AEL, according to the current WHO classification. Hasserjian et al. previously reported that there was no significant difference in overall survival (OS) among AEL, erythroid hyperplasia-associated MDS, and AML-MRC, and that OS in the

37~45,XY,4,add(5)(q11),−7,−9,−15,−16,−19,add(19) (p13),−22,+1~3mar[cp12].

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three categories was not associated with blast percentage, but strongly with the cytogenetic risk group. As this case had unfavorable or adverse cytogenetic abnormalities according to both the United Kingdom Medical Research Council criteria and International Prognostic Scoring System, allogeneic stem cell transplantation at first remission was warranted.

This case was presented at the monthly Hematology Grand Conference, November 11, 2014.

REFERENCES


Supplementary Figure

**Figure S1.** Peripheral blood pictures of a 61-year-old female with TTP showing a severe deficiency in ADAMTS13 activity and anti-ADAMTS13 inhibitors. Numerous schistocytes as well as microspherocytes and polychromatophilic erythrocytes are shown. Arrows indicate triangle schistocytes, in which a central pallor is absent. Asterisks indicate keratocytes and helmet cells. The platelet count was $4.0 \times 10^7/\mu L$. 
症例：37歳男性。汎血球減少を認めため血液内科を紹介受診した。

検査所見：ヘモグロビン 5.6 g/dL、白血球 2.41×10^3/µL（芽球 0%）、血小板 32.0×10^3/µL、LDH 288 IU/L、ハプトグロビン 1.0 mg/dL。末梢血塚末標本では、大小不同赤血球や奇形赤血球とともに多数の破砕赤血球を認めた。骨髄は正形成で、芽球を 37.0%、多染性赤芽球を 10.5%、正染性赤芽球を 12.3%、三系統血球の異形性を認めた。芽球は大型で、類円形の核・繊細な核網・明瞭な核小体と、好塩基性の細胞質を有し、ペルオキシダーゼ染色と非特異的エステラーゼ染色は陰性であった。PAS 染色で赤芽球の細胞質が顆粒状に陽性を示した。電子顕微鏡検査では、一部の芽球にθ顆粒を認め、電顕 MPO と PPO 反応は陰性であった。フローサイトメトリー解析では、芽球は CD36^+、glycophorin A^−/−、CD34^+、CD33^+、HLADR^−/−、CD13^−/+. 核型は 37~45, XY, −4, add(5)(q11), −7, −9, −15, −16, −19, add(19)(p13), −22, 1~3mar[cp10]. 骨髄塚末標本の FISH 解析で del(5q) と −7 を約 70%の細胞で認めた。

経過：イダルビシンとシタラビンを用いた寛解導入療法によって血液学的・細胞遺伝学的寛解に至った。診断時に末梢血塚末標本で認めた破砕赤血球も消失した。

考案：本症例の芽球の多くは幼若赤芽球であったが、芽球比率や異形成と染色体異常から 2008 年 WHO 分類の AML with myelodysplasia-related changes に該当した。大小不同・奇形赤血球とともに認められた破砕赤血球は、骨髄異形成による形態異常と考えられた。

キーワード：AML with myelodysplasia-related changes, 破砕赤血球, 骨髄異形成