Refractory Thrombocytopenia with Multilineage Dysplasia: A Rare Type of Myelodysplastic Syndrome


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ABSTRACT

Thrombocytopenia may be the presenting cytopenia of myelodysplastic syndrome (MDS) and is named as refractory thrombocytopenia (RT) and categorized in the refractory cytopenia with multilineage dysplasia (RCMD) group according to the recent World Health Organization (WHO) classification of the acute leukemias and MDS. Abnormal cytogenetics can be found in 60% to 80% of patients with MDS. Most common cytogenetic abnormalities include monosomy 5, 5q-, monosomy 7, trisomy 8, deletion 20q and loss of X or Y chromosome. Here we report clinical features and outcomes of nine patients with RT. Cytogenetic abnormalities were detected in seven. Among two patients who have a normal karyotype at diagnosis, one of them transformed to acute myeloid leukemia (AML). During a median follow-up of 29 months, two patients died of hemorrhage and one of AML. The features and prognosis of patients with RT needs to be determined by larger series.

Key Words: Myelodysplastic syndrome, Refractory thrombocytopenia, Refractory cytopenia with multilineage dysplasia, Cytogenetics.

ÖZET

Çok Dizinli Displaziye Eşlik Eden Refrakter Trombositopeni: Seyrek Rastlanan Bir Myelodisplastik Sendrom Tipi


Anahtar Kelimeler: Myelodisplastik, Refrakter trombositopeni, Refrakter sitopeni, Sitogenetik.


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INTRODUCTION

Myelodysplastic syndromes (MDS) are acquired clonal stem cell disorders characterized by ineffective hemopoiesis leading to blood cytopenias as well as dysplasia of one or more hematopoietic lineages. The diagnosis is most secure in patients with cytopenia, tri-lineage dysplasia, a high percentage of blast cells, or cytogenetic anomalies. Anemia is the most common cytopenia and only refractory anemias are considered in the French-British-American (FAB) classification; however, thrombocytopenia may be the presenting cytopenia of MDS and is categorized as refractory thrombocytopenia (RT)[2-6]. This group of patients may be interpreted as refractory cytopenia with multilineage dysplasia (RCMD) according to the recent World Health Organization (WHO) classification of acute leukemias and MDS[7]. Misdiagnosis as immune thrombocytopenic purpura (ITP) is possible if megaloblastic hyperplasia is present and morphologic features of dysplasia are not identified. In these patients in order to establish a diagnosis of MDS a clonal chromosomal abnormality should be obtained at presentation or in the follow up.

Here we report clinical features and outcome of 9 patients whose initial presentations were thrombocytopenia with bone marrow dysplasia.

MATERIALS and METHODS

We evaluated nine patients with refractory thrombocytopenia out of 130 MDS patients who have been diagnosed at our clinic and in our follow-up between 1990-2001 whose peripheral blood smears, bone marrow biopsy and aspirate specimens, and clinical information are available. The complete blood cell counts at initial examination have been reviewed.

Wright stained peripheral blood and bone marrow differential counts were performed on 100 and 500 cells, respectively. Peripheral blood smears were studied for the presence of oval macrocytic erythrocytes and a variety of misshapen cells, basophilic stippling, Howell-Jolly bodies, nucleated red blood cells, Pelger-Huet neutrophils, agranular/hypogranular neutrophils, large platelets and hypogranular platelets. In bone marrow aspirates; erythroid dysplasia was described as megaloblastoid change and/or nuclear indentation, budding, bridging and/or multiple nuclei and/or karyorrhexis and/or cytoplasmic vacuolization in erythroid precursors. Granulocytic dysplasia was considered when maturation defects such as hypo- or hypersegmentation of neutrophils, giant metamyelocytes and bands or cytoplasmic hypogranularity or agranularity of neutrophils, bands or metamyelocytes or nuclear changes such as pseudo Pelger-Huet, ring nuclei seen in neutrophils were observed in 10% or more of this lineage. A minimum of 25 megakaryocytes was examined in each case. Megakaryocytic dysplasia was considered in the presence of at least 10 micromegakaryocytes or megakaryocytes carrying other nuclear segmentation changes such as mononuclear or abnormally segmented forms (with a multiple separate nuclei) with a fully mature cytoplasm or cytoplasmic vacuoles and granular abnormalities. Platelet shedding were also determined. The percentage of blasts or sideroblasts in bone marrow aspirates was noted. In order to confirm our impressions in bone marrow aspirates (such as cellularity) and to diagnose fibrotic changes by hematoxylin-eosin and reticulum staining, bone marrow biopsies were also evaluated.

Recorded laboratory parameters included platelet counts, mean corpuscular volume (MCV), mean platelet volume, erythrocyte distribution width (RDW), serum vitamin B12 and folate values, total iron binding capacity, ferritin, bleeding time, viral markers for hepatitis viruses, Epstein-Barr virus (EBV), citomegalovirus (CMV), HIV and blood chemistry.

Cytogenetic analyses were performed with a trypsin-Giemsa banding technique from bone marrow aspirates. Chromosomal abnormalities were described according to the International System for Human Cyto-
genetic Nomenclature[8]. When needed as a method for molecular analysis, nested reverse transcriptase-polymerase chain reaction (nested RT-PCR) was done.

Statistical analyses for overall survival estimation (OS) was done by Kaplan Meier’s method.

RESULTS

The median age was 59 (35-78) and male: female ratio was 2:1. Mean platelet value was 57.3 x 10^9/L (10-118). Median follow-up of the patients were 29 months (10-56 months). Overall survival was 33 ± 4.5 months (24-42 months). Clinical and laboratory features of the patients were summarized in Table 1. At presentation, none of the patients had splenomegaly and lymphadenopathy. Vitamin B12 and ferritin levels were within normal limits in all patients. In clinical follow-up, two patients developed anemia with a Hb value below 10g/dL. Two patients died of cerebral hemorrhagia; one of them at 10th month and the other who developed pancytopenia at 17th month after the diagnosis. One patient evolved to acute myeloid leukemia (AML) and died.

All patients had an hemoglobin value over 12 g/dL. Three of the nine patients had a MCV higher than 100 fl. Dyserythropoiesis was present with varying degrees in all patients. In peripheral blood smear macrocytes and ovalocytes were observed in all patients. There were also tear drop forms, nucleated red blood cells in the first patient whose bone marrow biopsy disclosed reticular fibrosis of grade II. Pelger-Huet neutrophils were observed in three cases and hypogranular neutrophils in four cases. Large platelets were seen in seven patients. None of the patients revealed monocytosis in WBC differential count of peripheral blood smear. The bone marrow was normocellular in three cases and hypercellular in six cases. Megakaryocytes were quan-

Table 1. The features of patients with refractory thrombocytopenia

<table>
<thead>
<tr>
<th>Patient no:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
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<tr>
<td>Age</td>
<td>35</td>
<td>68</td>
<td>61</td>
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<td>53</td>
<td>58</td>
<td>53</td>
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<tr>
<td>Sex</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Platelet (x 10^9/L)</td>
<td>10</td>
<td>95</td>
<td>55</td>
<td>64</td>
<td>25</td>
<td>118</td>
<td>74</td>
<td>15</td>
<td>60</td>
</tr>
<tr>
<td>Hemoglobin(g/dL)</td>
<td>12.3</td>
<td>12.4</td>
<td>12.4</td>
<td>14.5</td>
<td>12.5</td>
<td>13.3</td>
<td>13.7</td>
<td>12.4</td>
<td>12.5</td>
</tr>
<tr>
<td>WBC (x 10^9/L)</td>
<td>13</td>
<td>4.7</td>
<td>6.2</td>
<td>4.1</td>
<td>8.1</td>
<td>6.2</td>
<td>6.3</td>
<td>14</td>
<td>12</td>
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<td>MCV (fl)</td>
<td>103</td>
<td>83</td>
<td>110</td>
<td>95</td>
<td>105</td>
<td>100</td>
<td>86</td>
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<td>95</td>
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<td>Bone marrow cellularity</td>
<td>H</td>
<td>H</td>
<td>N</td>
<td>H</td>
<td>N</td>
<td>H</td>
<td>H</td>
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<td>Bone marrow fibrosis</td>
<td>+</td>
<td>-</td>
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<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Follow-up (months)</td>
<td>10</td>
<td>31</td>
<td>56</td>
<td>55</td>
<td>29</td>
<td>30</td>
<td>28</td>
<td>17</td>
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<tr>
<td>Additional cytopenias in follow up (months)</td>
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<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
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<tr>
<td>Outcome</td>
<td>Died of Hemorrhagia</td>
<td>Died of AML</td>
<td>Alive</td>
<td>Alive</td>
<td>Alive</td>
<td>Alive</td>
<td>Died of AML</td>
<td>Alive</td>
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</table>

H: Hypercellular, N: Normocellular.
titatively normal in 6, decreased in 1, and increased in 2 patients. Micromegakaryocytes were observed in 2, small mononuclear megakaryocytes in 7, large mononuclear megakaryocytes in 3 and large abnormally segmented forms in 7, hypogranular forms in 4, vacuolated forms in 2 patients (Figure 1). Abnormal platelet shedding was observed in 3 patients. Dysgranulopoiesis was observed in all patients. Type I blasts not exceeding 5% was observed at initial examination in 3, and during follow-up period in 1 patient. One patient who had 3% type I blasts at initial diagnosis was transformed to acute myeloblastic leukemia (FAB-M1) 29 months later after the diagnosis. Pathologic ringed sideroblasts were observed in none of the patients and iron stores were sufficient with iron staining. A reticulin fibrosis was present in 2 patients (1st and 4th cases). The screening tests for viral markers including HIV were all negative. Serum iron binding capacity, serum ferritin, vitamin B12 and folate levels were found to be within normal limits in all patients.

Of 9 patients, two (3rd and 7th cases) of them were first diagnosed as ITP who had purpura and ecchymoses at their admission to hospital. These patients were treated with steroids with no benefit. Splenectomy was performed in the 3rd case who also benefited from immunoglobulin treatment, but the platelet count gradually lowered in months after reaching a peak level of 100 x 10^9/L after the operation.

Karyotypic clonal abnormalities were detected in 7 patients, 20 q deletion in 2 cases, trisomy 8 in 1, trisomies of chromosomes 1 and 12 and deletions of Y and 7 in 1, a composite karyotype in 1, hyperdiploidy/tetraploidy in 1 and marker chromosome abnormality in 1 case (Table 2). Two patients disclosed a normal karyotype. One of them evolved to AML (2nd patient) in follow-up and this patient exhibited a normal karyotype at this time again. The other patient (3rd patient) also lacked any karyotypic abnormality at presentation. He developed anemia in his follow-up and at this time his bone marrow examination revealed 3.5% blasts of

![Figure 1](image)

**Figure 1.** Small mononuclear megakaryocyte with vacuoles (x 100).

<table>
<thead>
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<th>Patient no</th>
<th>Cytogenetic analysis</th>
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<td>1</td>
<td>47, XY, del(20)(q11.2), + del(20)(q11.2) [20]</td>
</tr>
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<td>46, XY [20]</td>
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<tr>
<td>3</td>
<td>46, XY [20]</td>
</tr>
<tr>
<td>4</td>
<td>45, X, -Y, del(20)(q11)[10]</td>
</tr>
<tr>
<td>5</td>
<td>46, X, -Y, +1,-7, +12[5]/45, X, -Y, +1, -3, -6, +12, del(6)(q23), del(1;7)(q10;p10)[1]</td>
</tr>
<tr>
<td>6</td>
<td>46, XX [20]/46, X-C, +2mar[2]</td>
</tr>
<tr>
<td>7</td>
<td>46, XX [8]/42~44, X, -X, -14, -17, -19, -20[cp10]</td>
</tr>
<tr>
<td>8</td>
<td>46, XX [22]/hyperdiploidy, tetraploidy[15]</td>
</tr>
<tr>
<td>9</td>
<td>47, XY, +8[20]</td>
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</table>
lymphoid nature. As cytogenetic investigation revealed no karyotypic abnormality again we also looked for TEL-AML, MLL-AF4, p190 bcr-abl and p210 bcr-abl transcripts by nested RT/PCR. This study revealed TEL-AML t(12;21). In the 1st patient in addition to del(20)q karyotypic abnormality, p210 bcr-abl(b3a2) chimeric transcript was also found to be positive by peripheral blood nested RT-PCR DNA analysis (Table 2).

**DISCUSSION**

RT is a rare type of MDS described by various authors previously[2-6]. Presence of isolated thrombocytopenia, absence of other cytopenias, insufficient interpretation of dysplastic features of peripheral smear and bone marrow and the presence of megakaryocytic hyperplasia in some cases often leaded to the misdiagnosis of ITP. Similarly, we had 2 patients whose initial diagnosis were ITP. Review of bone marrow smears of our patients disclosed dysplastic granulocytic and megakaryocytic elements in addition to dyserythropoiesis. Dysmegakaryopoiesis was prominent in all cases with variable morphologic features. Rosati et al emphasized that presence of multilineage dysplasia carries a worse prognosis in the setting of RA + RARS[9]. In new WHO proposal, recognition of isolated cytopenias other than anemia together with significant dysplasia has made it possible to identify these cases as a subgroup of MDS, namely RCMD who otherwise were unclassifiable according to the FAB[7].

Except for one, all of our patients were over 50 years old. In previous reports, most of the patients were also over 50 years old although younger patients were also identified in other series.

All nine patients who have been in our follow-up presented with thrombocytopenia and trilineage dysplasia of the bone marrow. To confirm the diagnosis of MDS in those patients we needed to demonstrate clonal karyotypic changes. Seven patients met this criteria with conventional cytogenetics. In one patient a chromosomal abnormality was detected by molecular analysis in his follow-up. One patient who did not disclose a chromosomal abnormality transformed to AML. We could not make any further molecular analysis for the detection of a probable clonal disorder in this patient.

In MDS, the karyotype is normal in 30 to 50 percent of cases[10]. The karyotypic abnormality can also appear and disappear over time[11,12]. RA, RARS have a much lower probability of a detectable karyotypic abnormality (< 30%) compared with RAEB and RAEB-T (50 to 70 %)[10]. Two of our patients revealed del 20q abnormality in association with other abnormalities. The del (20q) marker appears to be primarily associated with myeloid disorders. It is not a marker of any specific disease but rather of certain disease entities: Polycythemia vera, agnogenic myeloid metaplasia, MDS and AML. It is detected in 5% of patients with primary MDS[13]. One patient possesed loss of Y chromosome in addition to del 20 q. The other patient demonstrated p210 bcr-abl chimeric transcript by molecular analysis. In this patient, although in presentation there was slight leukocytosis and bone marrow myeloid hyperplasia, the presence of thrombocytopenia, multilineage dysplasia of bone marrow and absence of splenomegaly forwarded us to the diagnosis of MDS rather than CML. The Ph chromosome is rare in myelodysplastic syndromes. In most cases acquisition of the Ph chromosome was concomitant with transformation and poor prognosis[13-18]. Our patient had also a short survival and died of bleeding. We observed Y chromosome loss in 2 patients in association with other abnormalities. The prognostic association of Y chromosome loss for survival, appears to be favorable[19]. In 1 patient we detected trisomy 8. This is the single most frequent acquired aberration in MDS[13]. Poliploidy which has been the sole abnormality in one of our patients could also be observed in MDS[20]. Trisomy 12 which could be frequently found in RAEB and RAEB-T has been found in one patient in association with a complex karyotype[21]. We did not observe any clinical
evolution in this patient. Molecular analysis of bone marrow aspiration by nested RT-PCR revealed TEL/AML (t12;21) abnormality in the 3rd patient which was the most frequent molecular defect seen in childhood ALL[22]. We observed a few lymphoid blasts in the bone marrow (below 5%) of this patient.

In conclusion, in patients presenting with isolated thrombocytopenia and multilineage dysplasia any clonal disorder should be investigated by conventional cytogenetics and molecular techniques for diagnosis of MDS. More cases need to be identified in the future in order to determine the features and prognosis of the patients with RT.

REFERENCES

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