Cytogenetic Abnormalities in a Series of 1029 Patients With Primary Myelodysplastic Syndromes

A Report From the US With a Focus on Some Undefined Single Chromosomal Abnormalities

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BACKGROUND. Conventional karyotype has an established role in myelodysplastic syndrome (MDS) and is included in the International Prognostic Scoring System (IPSS) for patient risk stratification and treatment selection. Although some chromosomal abnormalities have been well characterized, the significance of several miscellaneous, infrequent, single chromosomal abnormalities remains to be defined. In addition, the emerging therapeutic agents may change the natural course of disease in patients with MDS and the cytogenetic impact on risk stratification.

METHODS. Clinicopathologic data were retrieved on 1029 patients who had a diagnosis of primary MDS and had available cytogenetic data (karyotype) on file.

RESULTS. Cytogenetic abnormalities were identified in 458 patients (45%) and occurred most frequently in patients who had refractory anemia with excess blasts (62%). Overall, the 3 cytogenetic risk groups defined by the IPSS—good, intermediate, and poor—effectively stratified the patients’ overall survival (OS) (64 months, 31 months, and 12 months, respectively; \(P < .001\)). With the exception of gain of chromosome 8, single cytogenetic abnormalities within the intermediate group were extremely infrequent in the series but demonstrated variable OS ranging from 10 months for patients who had isochromosome (17q) to 69 months for patients who had deletion of 12p \([\text{del}(12p)]\), suggesting different prognostic significance. In the poor cytogenetic risk group, patients with isolated \(\text{del}(7q)\) and derivative \((1;7)(q10;p10)\) had a significantly better median OS than patients who had either loss of chromosome 7 or a complex karyotype \((P < .05)\).

CONCLUSIONS. The current data generated from a large cohort of patients with primary MDS indicated that some specific cytogenetic abnormalities carry different risk than their IPSS cytogenetic risk-group assignment, especially in the new treatment era. Because of the extreme low frequency, additional combined studies are needed to better categorize some rare single cytogenetic abnormalities within the intermediate cytogenetic risk group.


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KEYWORDS: myelodysplastic syndrome, karyotype, cytogenetics, survival, International Prognostic Scoring System.

Myelodysplastic syndrome (MDS) constitutes a heterogeneous group of clonal stem cell disorders characterized by ineffective hematopoiesis and an increased propensity for transformation to acute leukemia. For hematologists who treat patients with MDS, many new treatment modalities have emerged that can go beyond supportive care and change the natural course of the disease.1–9
information that was available in our files; however, these data may be underestimates; thus, this information was not used for the outcome analysis. Survival data were obtained on 998 patients (97%).

**Morphologic Evaluation**

All patients who were included in the final analysis had representative bone marrow trephine biopsy specimens and smears readily available for evaluation. The Perls reaction for iron was performed on bone marrow aspirates. For the diagnosis of morphologic dysplasia in bone marrow samples, features of dyserythropoiesis, dysgranulopoiesis, and dysmegakaryopoiesis had to be present in ≥10% of cells from the respective lineage. Unilineage dysplasia was defined by dysplasia that involved ≥10% of cells from a single cell lineage, whereas multilineage dysplasia involved ≥10% of cells from ≥2 lineages. The bone marrow differential was based a 500-cell count from multiple fields of the smears.

**Cytogenetic Analysis**

Conventional Giemsa banding analysis was performed on 24-hour and 48-hour bone marrow cultures. At least 20 metaphases were examined. Criteria defined by the International System for Human Cytogenetic Nomenclature were used to describe abnormal clones.\(^{17}\)

A karyotype was considered complex when ≥3 cytogenetic abnormalities were identified within the same clone. When ≥2 clones with ≥2 aberrations were present, patients were categorized into the complex aberration group; whereas patients who had 2 cytogenetically independent, single-anomaly clones were categorized into the 2-aberrations group.

**Statistical Analysis**

Patient survival was estimated by using the Kaplan-Meier method from the date of MDS diagnosis until death from any cause or until the last patient follow-up. Survival curves were compared statistically using the log-rank test. Differences between 2 groups were considered statistically significant if \(P\) values were <.05 in a 2-tailed test.

**RESULTS**

**Patient Characteristics, World Health Organization Classification, and Cytogenetic Risk Categories**

**Patient characteristics**

Patient characteristics are shown in Table 1. The median age of the patients was 67 years, and the ratio of men to women was 1.9:1. The WHO subgroups were as follows: 47 patients (4.5%) had del(5q) syndrome, 185 patients (18%) had RA, 86 patients (8%) had RA with ringed sideroblasts, 331 patients (32%) had refractory anemia with excess blasts; MDS-U, myelodysplastic syndrome, unclassifiable; IPSS, International Prognostic Scoring System.
into different disease categories according to the WHO classification, were not included.

**Cytogenetic results in relation to World Health Organization categories**

Of 1029 patients, 458 (44.5%) had clonal cytogenetic abnormalities identified at the time of diagnosis. The frequencies of different chromosomal abnormalities in relation to the WHO groups are shown in Tables 2 and 3.

Single cytogenetic abnormalities included the following: del(5q), del(20q) and \(^2\)Y (the IPSS good cytogenetic risk group); \(18, 3q\) rearrangement, \(25, 19, \text{ del}(11q), 11, \text{ del}(12p), 13q\) rearrangement, \(i[17q], 11, \text{ del}(15), 17, \text{ del}(19), \text{ and } -21\) (the IPSS intermediate cytogenetic risk group); and \(-7, \text{ del}(7q), \text{ and derivative } (1;7)(q10;p10) [\text{der}(1;7)(q10;p10)]\) (the IPSS poor cytogenetic risk group).

The most frequent single cytogenetic abnormalities in our series were del(5q) (61 patients; 5.9%), +8 (38 patients; 3.7%), del(20q) (28 patients; 2.7%), and \(-7/\text{del}(7q)/\text{der}(1;7)\) (23 patients; 2.2%) (Table 2). A complex karyotype was observed in 181 patients with an incidence of 17.6% among all patients with MDS and of 39.5% among the patients with abnormal karyotypes. One hundred forty-five patients (80.1%) with complex karyotypes had abnormalities involving chromosome 5 (loss 5q or \(25\)) or chromosome 7 (loss 7q or \(27\)) or both.

### Table 2

**Clinical Characteristics of Cytogenetic Abnormalities, Median Survival, and Acute Myelogenous Leukemia Progression Rate**

<table>
<thead>
<tr>
<th>IPSS Cytogenetic Category</th>
<th>Mean Age [Range], y</th>
<th>Men:Women</th>
<th>Median OS, mo</th>
<th>AML</th>
<th>RA</th>
<th>RABS</th>
<th>RCMD/RCMD-RS</th>
<th>RAEB</th>
<th>MDS-U</th>
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<td>Good, n = 682</td>
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<td>Del(5q), n = 3</td>
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<td>(-Y, n = 22)</td>
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<td>Intermediate, n = 143</td>
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<td>Inv(3); t(3;3), n = 3</td>
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<td>+8, n = 38</td>
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<td>?Del(9q), n = 2</td>
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<td>2 Anomalies n = 55</td>
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<td>Poor, n = 204</td>
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<td>Del(7q), n = 9</td>
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<td>–7, n = 8</td>
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<td>Der(1;7)(q10;p10), n = 6</td>
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<td>Complex, n = 181</td>
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</table>

IPSS indicates International Prognostic Scoring System; OS, overall survival; AML, acute myelogenous leukemia; WHO, World Health Organization; RA, refractory anemia; RABS, refractory anemia with ringed sideroblasts; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, refractory cytopenia with multilineage dysplasia and ringed sideroblasts; RAEB, refractory anemia with excess blasts; MDS-U, myelodysplastic syndrome, unclassifiable; Del, deletion; \(-\), loss; Inv, inversion; \(+\), gain; I, isochromosome; Der, derivative.

* Median OS included all patients in the respective cytogenetic group.

#### Overall Survival Comparison

**Median overall survival by IPSS-defined cytogenetic risk groups**

A comparison of the median overall survival (OS) between the IPSS good, intermediate, and poor cytogenetic risk groups is shown in Figure 1. With a median follow-up of 52 months, the median OS was 64 months for the good cytogenetic risk group, 31 months for the intermediate cytogenetic risk group, and 12 months for the poor cytogenetic risk group.
The difference in OS for the 3 groups was statistically significant (Kaplan-Meier estimate; log-rank \( P < .001 \)).

**Median overall survival in the good cytogenetic risk group**

The good cytogenetic risk category consisted of 682 patients (66.3%). Of those, 571 patients (83.7%) had a normal karyotype, 61 patients (8.9%) had del(5q), 28 patients (4.1%) had del(20q), and 22 patients (3.2%) had \( 2 \mathcal{Y} \) (Table 2). There was no statistically significant difference in the median OS between the different anomalies within the good cytogenetic risk group (\( P = .43 \)) (Fig. 2).

**Median overall survival in the intermediate cytogenetic risk group**

The intermediate cytogenetic risk group consisted of 143 patients (13.9%). Within this group, 88 patients (61.5%) had single cytogenetic abnormalities, whereas 55 patients (38.5%) had 2 cytogenetic abnormalities. Patients who had 1 cytogenetic abnormality did not have a survival benefit compared with patients who had 2 abnormalities (25 months and 32 months, respectively; Kaplan-Meier estimate; log-rank \( P = .95 \)) (Fig. 3A). Of the patients who had 1 cytogenetic abnormality, 38 patients (43.2%) had +8, and 50 patients (35%) had other various single chromosomal abnormalities. Patients who had +8 as the sole abnormality had a median OS comparable to that of patients who had other single chromosomal abnormalities (19 months and 33 months, respectively; Kaplan-Meier estimate; log-rank \( P = .28 \)) (Fig. 3B). It is noteworthy that the patients who had +8 as a sole abnormality had no difference in median OS compared with patients who had +8 present along with another chromosomal abnormality (19 months and 28 months, respectively; Kaplan-Meier estimate; log-rank \( P = .36 \)) (Fig. 3C).

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**TABLE 3**

<table>
<thead>
<tr>
<th>Patient Parameters</th>
<th>No. of Patients (%)</th>
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<tbody>
<tr>
<td>Abnormal karyotype</td>
<td>458 (44.5)</td>
</tr>
<tr>
<td>WHO category*</td>
<td></td>
</tr>
<tr>
<td>Del(5q) syndrome, n = 47</td>
<td>47 (100)</td>
</tr>
<tr>
<td>RA, n = 185</td>
<td>40 (21.6)</td>
</tr>
<tr>
<td>RARS, n = 86</td>
<td>14 (16.3)</td>
</tr>
<tr>
<td>RCMD/RCMD-RS n = 331</td>
<td>138 (41.7)</td>
</tr>
<tr>
<td>RAEB-1, n = 203</td>
<td>119 (58.6)</td>
</tr>
<tr>
<td>RAEB-2, n = 131</td>
<td>89 (67.9)</td>
</tr>
<tr>
<td>MDS-U, n = 46</td>
<td>11 (23.9)</td>
</tr>
<tr>
<td>IPSS score†</td>
<td></td>
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<tr>
<td>Low, n = 295</td>
<td>51 (17.3)</td>
</tr>
<tr>
<td>Intermediate-1, n = 468</td>
<td>182 (38.8)</td>
</tr>
<tr>
<td>Intermediate-2, n = 194</td>
<td>155 (79.9)</td>
</tr>
<tr>
<td>High, n = 72</td>
<td>70 (97.2)</td>
</tr>
<tr>
<td>Cytogenetic risk category</td>
<td></td>
</tr>
<tr>
<td>Good, n = 111‡</td>
<td>111 (10.8)</td>
</tr>
<tr>
<td>Intermediate, n = 143</td>
<td>143 (13.9)</td>
</tr>
<tr>
<td>Poor, n = 204</td>
<td>204 (29.8)</td>
</tr>
</tbody>
</table>

WHO indicates World Health Organization; Del, deletion; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, refractory cytopenia with multilineage dysplasia and ringed sideroblasts; RAEB, refractory anemia with excess blasts; MDS-U, myelodysplastic syndrome, unclassifiable; IPSS, International Prognostic Scoring System.

* The percentage with an abnormal karyotype was calculated within the WHO diagnosis.
† The percentage with an abnormal karyotype was calculated for each IPSS group.
‡ The “good” cytogenetic group also included 571 patients with normal karyotype.

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**FIGURE 1.** Overall survival (OS) comparison among patients with good, intermediate, and poor cytogenetics according to the International Prognostic Scoring System (IPSS). The OS for patients with good, intermediate, and poor cytogenetics was 64 months, 31 months, and 12 months, respectively. The difference in OS for the 3 groups was statistically significant (\( P < .001 \); log-rank test, Kaplan-Meier estimate).

**FIGURE 2.** Comparison of overall survival (OS) among patients with good cytogenetics according to the International Prognostic Scoring System. There were no significant differences in OS noted among patients who had a normal karyotype, \(-\mathcal{Y}\), del(5q), or del(20q) (\( P = .43 \); log-rank test, Kaplan-Meier estimate).
The median OS for the single cytogenetic abnormalities that were identified in more than 1 patient are listed in Table 2. Although the OS varied from 10 months for i(17q) to 69 months for del(12p), no statistically significant difference was achieved because of the small number of patients.

**Median overall survival in the poor cytogenetic risk group**

The poor cytogenetic risk group consisted of 204 patients (19.8%), including 181 patients (88.7%) with complex karyotypes and 23 patients with numeric or structural abnormalities of chromosome 7 (9 patients with 27, 8 patients with del[7q], and 6 patients with der(1;7)] (Fig. 4). It is noteworthy that patients who had del(7q) and der(1;7) demonstrated better OS (26 months and 45.5 months, respectively) compared with patients who had complex karyotypes (Kaplan-Meier estimate; log-rank test, log-rank test, Kaplan-Meier estimate), whereas the OS for patients who had a loss of chromosome 7 (−7) abnormality was comparable to that for patients who had a complex karyotype (P = .70; log-rank test, Kaplan-Meier estimate).

**DISCUSSION**

In the current series, which consisted of 1029 patients with de novo MDS, clonal cytogenetic abnormalities were identified in 458 patients (44.5%), similar the rate reported by others.11-14,18 Of the patients who had with abnormal cytogenetics, single abnormalities comprised 48.5% (222 of 458 patients), including 50 patients who had poorly defined, infrequent, single chromosomal aberrations that currently are categorized in the intermediate cytogenetic risk group according to IPSS criteria. The large number of patients with primary MDS who were included in
this study allowed us to define each of the cytogenetic categories more carefully.

The good cytogenetic risk group comprised the majority of the cases (682 patients; 66.3%) and had a median OS of 64 months. Although the median OS within this group ranged from 39 months (−Y) to 68 months (normal karyotype), the difference was not statistically significant. Our results corroborate previously published data on the cytogenetic abnormalities that are included in this category [del(5q), del(20q) and −Y]. Loss of chromosome Y can represent a normal age-related process or an MDS clone.19 However, even when it represents an abnormal clone, it is uncertain whether the loss of chromosome Y is related to disease pathogenesis.

The intermediate cytogenetic risk group was comprised of 143 patients (13.9%) and represented a cytogenetically diverse population that encompassed patients with 1 or 2 structural and/or numeric abnormalities. The median OS for this group was 31 months (range, 10–69 months) (Table 2). Although our findings overall endorse the 1997 IPSS categorization in defining the intermediate cytogenetic risk group, the median OS for patients with various cytogenetic abnormalities within this group ranged from 10 months to 69 months, with a significant deviation from the mean of 31 months, suggesting different prognoses and potential reclassification of some of the patients into a different risk group.

Del(12p), which is a recurrent cytogenetic abnormality that may carry a good prognosis,11-14 occurred at a very low frequency in our series, with only 3 cases identified, including 1 patient with RAEB-1 and 2 patients with RCMD. All 3 patients with del(12p) had an indolent clinical course; 2 patients were alive at the time of the last follow-up (105 months and 16 months), and 1 patient died at 33 months after the initial diagnosis. It has been reported in some studies that deletion of 11q23 in MDS harbors a good prognosis,11-13,16 but it is classified as intermediate risk by others.14,20 In our series, in total, 5 patients who had del(11)(q23) as a single cytogenetic abnormality were identified, including 1 patient with RCMD, 3 patients with RCMD-RS, and 1 patient with RAEB-1. Although these patients presented with severe anemia and leucopenia with or without thrombocytopenia, all 5 patients had an indolent clinical course, and the median OS for this group was 53 months, which was similar to the reports by Bernasconi et al. and Sole et al.11,18 Although our numbers were small, they suggest that del(12p) and del(11)(q23) may belong to the good cytogenetic group. Rearrangement involving 3q reportedly indicates an intermediate prognosis in some studies13,14,18 but a poor prognosis in others.11,12 In our series, 3 patients (2 with RCMD and 1 with RAEB-1) had 3q rearrangement [t(3;3)(q21;q26) or inv(3)(q21q26)] as a sole abnormality, and all 3 died at 8 months, 25 months, and 30 months after their initial diagnosis, suggesting an aggressive behavior. Trisomy 19, which is a characteristic abnormality in de novo myeloid malignancies,21 was observed in 4 patients who had a median OS of 24 months, similar to what was reported by Haase et al.14 On the basis of these data, both patients with 3q rearrangement and patients with +19 are likely to have been assigned appropriately to the intermediate cytogenetic risk group. Some other cytogenetic aberrations within the intermediate group for which a different prognostic indication has been suggested were extremely rare in our series. Isochromosome 17q reportedly harbors a poor prognosis,13,22,23 and it has been suggested that it should be placed in the poor prognostic category. We encountered only 2 patients who had +17q as a single chromosomal abnormality. Both patients presented with RAEB-1, had a very aggressive clinical course, and died at 9 months and 11 months after the initial diagnosis. Other infrequent single chromosomal abnormalities that may be potential candidates for reclassification, such as +21 (2 patients), del(13q) (1 patient), and +15q (2 patients), were extremely rare in our series. It is noteworthy that we identified 4 patients who had +11 as the sole cytogenetic anomaly, the significance of which has not been reported previously. These patients presented with RA (1 patient), RAEB-1 (1 patient), and RAEB-2 (2 patients). All of these patients died within 21 months after the diagnosis, indicating an aggressive clinical course.

Another intriguing finding within the intermediate cytogenetic group was a heterogeneous clinical course and outcome in patients who had +8 as the sole abnormality, which is considered to confer an intermediate risk. The median OS for this entire group was 19 months (range, 1-123 months). Within the intermediate cytogenetic risk category, the patients who died within 10 months and the patients who survived for >5 years mainly were those who had +8 (data not shown). Furthermore, no significant difference in OS was observed between patients who had +8 as a single abnormality and patients who had +8 associated with another cytogenetic abnormality. Despite the finding that +8 is 1 of the most common myeloid abnormalities and is present in 11% of patients with MDS, its clinical impact on the course of the disease largely remains unknown. For example, the reported transformation rate to AML in patients who have MDS with +8 varies
greatly from 8% to 62%. Recently, Paulsson et al. detected cryptic cytogenetic abnormalities, such as del(7)(p14p14) and del(12)(p13), in 4 of 10 patients with AML and MDS who had +8 as their sole cytogenetic abnormality by using high-resolution, genome-wide, array-based comparative genomic hybridization. In addition, somatic point mutations of leukemia-associated genes, eg, the CCAAT/enhancer binding protein α gene CEBPA, the fms-related tyrosine kinase 3 gene FLT3, the Kirsten rat sarcoma viral oncogene homolog KRAS, the neuroblastoma ras viral oncogene homolog NRAS, and the runt-related transcription factor 1 gene RUNX1, have been detected in patients who have MDS with +8.

Within the poor cytogenetic risk category defined by the current IPSS criteria, our 8 patients who had del(7q) as a sole cytogenetic abnormality had a superior median OS compared to patients who had −7 or a complex karyotype (26 months, 8.5 months, and 11 months, respectively; P < .05). This observation is in agreement with the reports by Bernasconi et al. and Haase et al. and supports the proposal to reclassify interstitial deletion of 7q to the intermediate cytogenetic risk categories. Der(1;7)(q10;p10), a derivative chromosome which is the result of an unbalanced translocation leading to +1q and −7q when it is present with 2 normal chromosomes 1 and 1 normal chromosome 7, was reported first in 3 patients who had myelofibrosis and myeloid metaplasia. Later case reports indicated that der(1;7)(q10;p10) often is associated with therapy-related MDS/AML but is rare in de novo cases and appears to carry an adverse prognosis. In a recent, multi-institutional study that included 64 patients who had primary and therapy-related MDS with der(1;7)(q10;p10), this abnormality appeared to carry a better clinical outcome with a median OS of 23 months. However, that study included both patients with der(1;7)(q10;p10) as an isolated abnormality and patients in whom it was present along with other cytogenetic abnormalities. In our series, we identified 6 patients with primary MDS who had der(1;7)(q10;p10), including 1 patient with RAEB-1, 2 patients with RCM, and 3 patients with MDS-U. These patients had a median OS of 45.5 months, which was significantly superior to the OS of patients who had poor-risk cytogenetics (P = .01) (Table 1). One possible explanation for this finding is the beneficial effect of +1q, which, as a single abnormality, reportedly carries a good prognosis in patients with MDS. It is noteworthy that 5 of 6 patients (83%) who had der(1;7)(q10;p10) had hypocellular bone marrow (mean cellularity, 20%; range, 10%–40%). We have demonstrated that hypocellularity in MDS confers a favorable outcome that is independent of other risk factors, including cytogenetics. Although the underlying pathogenesis is not known, der(1;7)(q10;p10) appears to carry at least an intermediate prognosis if not a good prognosis. This observation is in keeping with our other finding that patients who had der(7q) as a single abnormality had a better OS than patients who had the poor category who had either −7 or a complex karyotype (26 months, 8.5 months, and 11 months, respectively; P < .05) (Table 2). It is noteworthy that some of the new therapeutic/experimental drugs may alter the natural course of these patients and may have a positive impact on OS. It is likely that the adverse biology of MDS can be affected for the better by certain drugs, and it is possible that cytogenetic IPSS risk categorization may be affected by it.

In summary, cytogenetics in MDS plays an important role in disease prognosis and risk stratification for treatment. Our study, which was conducted on a large cohort of patients with primary MDS from the US, confirmed that most of the cytogenetic groups could be defined according to the current IPSS. However, our data also indicated that some single cytogenetic abnormalities, such as del(7q) and der(1;7)(p10;q10), may be excluded from the original IPSS poor-risk category and reclassified into a different category, such as intermediate-risk group. Del(12p) and del(11)(q23), which currently are included in the intermediate-risk group, may belong to the good-risk group; patients who have rearrangement of 3q and +19 are likely to have been assigned appropriately to the intermediate-risk group; and i(17q) and +11 may carry a worse prognosis than their current assignment as intermediate risk. Overall, in patients with primary MDS, the single chromosomal abnormalities other than +8 in the intermediate category are very infrequent, and a multi-institutional study is needed to better define their prognostic significance.

REFERENCES
Cytogenetic study of Brazilian patients with Myelodysplastic Syndrome (MDS)

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Abstract

Bone marrow cytogenetic studies were performed on 93 patients with primary myelodysplastic syndrome (MDS) diagnosed at the Clinical Hospital of the Federal University of Paraná, Brazil. Chromosomal alterations were observed in 69% of the patients. Monosomy of chromosome 7, deletions of 7q, 5q, 12p and 20q, rearrangements of 11q23 and trisomies of chromosomes 8 and 21 were the most frequent abnormalities observed. Among adult patients the most frequent aberrations were rearrangements of 11q23 and 12p deletions. In the pediatric group, 5q deletions and monosomy of chromosome 7 were the most common alterations.

Key words: hematological disorders, myelodysplasias, cancer cytogenetics.

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Introduction

Myelodysplastic syndrome (MDS) represents a heterogeneous group of clonal disorders of hematopoietic stem cells characterized by quantitative and qualitative hematopoietic abnormalities, with cytopenia of one or more peripheral blood cell lineages but usually normal or hypercellular bone marrow (Sanz et al., 1989). Hematopoiesis is ineffective in one or more cell lineage, producing dyserythropoiesis, dysgranulopoiesis and dysmegakaryocytopenia (Goasguen and Bennett, 1992). These disorders are associated with a high risk of progression to acute myeloid leukemia (AML) and overall short survival, the death of MDS patients usually being due to cytopenia or progression to AML (Ganser and Hoelzer, 1992).

Conventionally, primary and secondary MDS are defined taking into account the previous history of the patients. According to the World Health Organization (WHO), primary MDS occurs without a known history of toxic exposure while secondary MDS is therapy-related and observed in patients with a known history of exposure to chemotherapeutic agents and/or radiation therapy (Jaffe et al., 2001). Although MDS occurs predominantly in older adults (median age 70 years) with a general incidence of 3 per 100,000 and reaches 20 per 100,000 over age 70 (Jaffe et al., 2001), MDS in children is usually more aggressive than in adults (Chan et al., 1997).

In order to improve the risk assessment of MDS an International Prognostic Score System (IPSS) was proposed by Greenberg et al. (1997), based on matched cytogenetic, morphologic and clinical data from 816 patients with primary MDS. In this system, variables were reevaluated prioritizing a more refined classification of bone marrow cytogenetic data, and three cytogenetic IPSS subgroups were recognized: good (presence of normal karyotypes, or monosomy of chromosome Y, or long arm deletions of chromosome 5 or 20); poor (presence of complex karyotypes, including three or more abnormalities, or aberrations of chromosome 7), and intermediate (presence of other chromosomal abnormalities).

Clonal chromosomal abnormalities have been reported in more than 3,000 MDS patients (Mitelman Database of Chromosome Aberrations in Cancer 2004), mainly...
adults, supporting the view that this syndrome is neoplastic in nature. The nonrandom distribution of the abnormalities through the different stages of MDS has helped to identify primary (pathogenetically essential for the establishment of the disease) and secondary chromosomal changes (acquired during the evolution of the disease) (Heim and Mitelman, 1995; Mitelman, 2000). Even so, further studies are still necessary for a better characterization of the frequency and nature of the chromosome aberrations in pediatric MDS (Martinez-Climent, 1997). As in the other hematological diseases, the identification of the chromosomal alterations involved in MDS is not only a powerful and essential tool for the clinical management and treatment of patients but is also central to basic research on this syndrome.

In the study described in this paper we determined the spectrum of chromosomal alterations in 93 Brazilian patients with myelodysplastic syndrome and investigated the correlation between clinical and cytogenetic findings.

Material and Methods

This study included 93 patients with primary MDS referred to the Cytogenetic Laboratory of the Clinical Hospital, Federal University of Paraná, Brazil from January 1988 to September 2002. Cytogenetic analysis of bone marrow cells was performed at the time of diagnosis. In our sample, 51 patients were male and 42 were female with a median age of 29 (range 1 to 78 years). These cases were grouped according to the French-American-British (FAB) Co-operative Group classification of Bennett et al. (1982) as: refractory anemia (RA), 39 patients; refractory anemia with ringed sideroblasts (RARS), 6 patients; refractory anemia with excess blasts (RAEB), 17 patients; refractory anemia with excess blast in transformation (RAEBT), 17 patients; and chronic myelomonocytic leukemia (CMML), 14 patients. The sample was subdivided into 66 adult (mean age 40.9 years range 19 to 78 years) and 27 pediatric (mean age 7.9 years, range 1 to 18) patients (Lopes et al., 2002).

Bone marrow cells were cultured for 24 h (Williams et al., 1984) and chromosome analyses performed using a modification of the Giemsa banding technique described by Scheres (1972), the chromosomes being classified according to the International System for Human Cytogenetic Nomenclature (ISCN, 1995).

The differences among the mean values obtained from age and bone marrow blast percentage and the cytogenetic IPSS subgroups (good, intermediate and poor) were analyzed using Fisher’s test and homogeneity among the variances was tested by Bartlett’s test. The number of patients with different cytopenias (number of lineages involved: erythroid, granulocytic and/or megakaryocytic) in the three subgroups was analyzed by the Chi-square test. The survival curves in the cytogenetic IPSS subgroups were estimated and compared using the Kaplan-Meier method and the log rank test, respectively (Bewich et al., 2004).

Results

Clonal chromosome abnormalities were detected in 64 patients (69%) while 29 patients (31%) presented normal karyotypes. The frequencies of abnormal karyotypes were 74% among adults (Table 1) and 56% in children (Table 2).

Table 1 - Clinical data and karyotype of 66 adult MDS patients.

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Cytopenia</th>
<th>Evolution to AML</th>
<th>BM blasts (%)</th>
<th>BMT</th>
<th>Survival (months)</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR</td>
<td>F</td>
<td>78</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>-</td>
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<td></td>
</tr>
<tr>
<td>AR</td>
<td>M</td>
<td>41</td>
<td>2</td>
<td>-</td>
<td>0.6</td>
<td>N</td>
<td>-</td>
<td>46,XY[20]</td>
</tr>
<tr>
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<td>F</td>
<td>21</td>
<td>3</td>
<td>N</td>
<td>1</td>
<td>N</td>
<td>47,XX,+mar[3]/46,XX,del(5)(q?)[2]/46,XX[15]</td>
<td></td>
</tr>
<tr>
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<td>M</td>
<td>75</td>
<td>1</td>
<td>N</td>
<td>1</td>
<td>N</td>
<td>86 d</td>
<td>45,X,-Y[4]/46,XY[12]</td>
</tr>
<tr>
<td>AR</td>
<td>M</td>
<td>36</td>
<td>3</td>
<td>-</td>
<td>N</td>
<td>10 a</td>
<td>47,XY,+8[18]</td>
<td></td>
</tr>
<tr>
<td>AR</td>
<td>M</td>
<td>65</td>
<td>1</td>
<td>N</td>
<td>0,1</td>
<td>N</td>
<td>54 a</td>
<td>46,XY,del(5)(q13q33)[10]/47,XY,idem,+21[3]/46,XY[7]</td>
</tr>
<tr>
<td>AR</td>
<td>F</td>
<td>24</td>
<td>-</td>
<td>-</td>
<td>N</td>
<td>-</td>
<td>46,XX,del(12)(p12)[4]/46,XX[16]</td>
<td></td>
</tr>
<tr>
<td>AR</td>
<td>F</td>
<td>31</td>
<td>2</td>
<td>N</td>
<td>-</td>
<td>Y</td>
<td>10 d</td>
<td>47,XX,del[1][8]/46,XX[5]</td>
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<tr>
<td>AR</td>
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<td>42</td>
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<td>N</td>
<td>0</td>
<td>Y</td>
<td>58 a</td>
<td>47,XY,+8[4]/46,XY[6]</td>
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<td>1</td>
<td>N</td>
<td>1</td>
<td>Y</td>
<td>59 a</td>
<td>46,XY[20]</td>
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<tr>
<td>AR</td>
<td>M</td>
<td>26</td>
<td>3</td>
<td>N</td>
<td>2</td>
<td>Y</td>
<td>21 d</td>
<td>46,XY[30]</td>
</tr>
<tr>
<td>AR</td>
<td>M</td>
<td>39</td>
<td>3</td>
<td>N</td>
<td>-</td>
<td>Y</td>
<td>34 d</td>
<td>46,XY,der(1)(p1;1)(q41;q43),der(1)(p1;1)(q41;q43) dup<a href="q21q25">1</a>[2],dup<a href="q12q32">14</a> [cp3]/46,XY[15]</td>
</tr>
</tbody>
</table>
Classification of MDSs in 1982 was one of the first classification systems that categorized MDS patients for diagnostic and prognostic purposes. This system depended on the morphology of the peripheral blood smear and bone marrow (Table 1), and it became obsolete as the heterogeneity of MDS became more widely appreciated. In response, the World Health Organization (WHO) reported proposals for reclassifying MDS (Table 1). The WHO system now represents the world standard for classification of individual cases. Of note is that, according to the new scheme, chronic myelomonocytic leukemia (CMML) and some cases sharing characteristics of both MDS and myeloproliferative disorders (MPD) were included in a new entity called MDS/MPD.

International Prognostic Scoring System

Many systems have been developed to help predict the prognosis of MDS and the potential for transformation to acute myeloid leukemia (AML). The International Prognostic Scoring System (IPSS) incorporated the peripheral blood and bone marrow blast percentage, number of cytopenias, and cytogenetics to determine survival and risk for transformation to AML. The IPSS applies to de novo MDS, separates patients into 4 risk categories for death or transformation to AML, and was obtained through analysis of patients who received mostly supportive care. These 4 risk categories are: low, intermediate-1, intermediate-2, and high. The IPSS divided chromosomal abnormalities into 3 groups: good risk (normal, isolated del[5q], isolated -Y, and del[20q]) representing 70% of the patients with a median survival of 3.8 years; intermediate risk (14%) with a median survival of 2.4 years; and poor risk, any chromosome 7 abnormality or complex cytogenetic defects of ≥3 abnormalities (16%) with a median survival of 0.8 years. The IPSS applied to CMML cases only if their white blood cell (WBC) count was <14,000/µL.

Cytogenetic Abnormalities

Outcome analysis leading to the establishment of the IPSS recognized the significance of metaphase cytogenetics in predicting the prognosis of MDS. Approximately 40% to 60% of MDS patients will have clonal cytogenetic abnormalities by routine metaphase analysis, and this percentage increases when using more sophisticated methods like single nucleotide polymorphism (SNP) array. Analysis of a very large database of MDS patients described recently by Haase and colleagues, demonstrated the diversity of chromosomal defects in MDS. The most common chromosomal abnormalities are del(5q), del(6q), -7, del(7q), trisomy 8, -18/del(18q), del(20q), -17/del(17p), trisomy 21, -13/del(13q), -12, trisomy 1, t(1q), del(12p), del(12q), t(7q), and inversion 3. They were divided into 3 risk groups according to survival. The good prognosis group included 22 different abnormalities, intermediate prognosis had 13 different abnormalities, and 3 were in the poor prognosis group. This study, and others, show that cytogenetics has a role in predicting survival and the risk of AML transformation.

Good Risk

5q deletion (del[5q])

Van den Berghe and colleagues described a syndrome characterized by an isolated 5q31 deletion (Figure 1) associated with...
refractory macrocytic anemia, less than 5% myeloblasts, lack of ring sideroblasts in the bone marrow, normal to elevated platelet count, and a mostly older female predominance.6,10 Deletions of the long arm of chromosome 5 are one of the most common findings in MDS. The frequency of del(5q) in patients with MDS is 16% to 28%.2,5 Del(5q) can be found as an isolated cytogenetic abnormality, which carries a relatively good prognosis, or as part of a complex karyotype in MDS patients, which carries a poor prognosis. The most common band region involved in the interstitial deletion of del(5q) extends between 5q31 and 5q32, but the size of this deletion varies.8 It is possible that 2 distinct common deleted regions (CDRs) exist, one specific for 5q- syndrome and the other present in other primary and secondary forms of MDS.9 This former group of patients has a good prognosis, better survival (median of 77 months), and low AML transformation rate. 5q- syndrome is classified as a separate group of MDS patients by the WHO classification.3,21

Normal Cytogenetics
Normal cytogenetics in de novo MDS patients is found in about 50% of cases and predicts a low likelihood of transforming into AML, with a median survival of 53.4 months.6 Treatment decisions are based on IPSS score and age. Younger patients with higher scores are considered for allogeneic stem cell transplant, while older patients with lower scores are eligible for less intense therapies such as lenalidomide or hypomethylating agents (eg, 5-azacytidine and decitabine).

Del(20q)
Deletion 20q was categorized among the good risk cytogenetic abnormalities according to the IPSS and has a low AML transformation rate. Del(20q) frequency in MDS patients is around 2% to 5% whether isolated or associated with other cytogenetics, and the median survival is about 71 months.6,11

-Y
The frequency of -Y as an isolated abnormality or with 1 other defect is 3.5% and 2.7%, respectively, and is highly dependent on the age of the patient.6 It is also considered a good risk defect by IPSS. While the pathogenetic role of loss of Y is not clear, this defect points toward clonality of hematopoiesis but may be either an age-dependent non-neoplastic process or associated with a neoplastic process.

Poor Risk

Chromosome 7 Abnormalities
The poor risk group includes monosomy 7 (-7) or del(7q) with or without other chromosome defects. The presence of -7 or del(7q) in MDS or AML patients is a very poor prognostic indicator and correlates with short survival and high risk for AML transformation.1 Treatment with bone marrow transplant is required for long-term survival, but results are poor.20 Recent studies using SNP array-based karyotyping suggest that uniparental disomy 7q may have similarly poor prognosis.4,5 Newer studies suggest that patients with LOH of chromosome 7 show good responsiveness to hypomethylating agents.3

Complex Cytogenetics
Complex cytogenetics is defined as the presence of 3 or more cytogenetic abnormalities within 1 clone. Survival is significantly affected by the number of defects. The median survival is 17 months for MDS patients with 3 abnormalities and 9 months for patients with 4 to 6 abnormalities. They are also at high risk for AML transformation.6

Chronic Myelomonocytic Leukemia With t(5;12)
Chronic myelomonocytic leukemia (CMML) is a subset group of patients recognized by the FAB classification of MDS. It is characterized by splenomegaly, hypercellular bone marrow, dysplastic monocytosis, and bone marrow fibrosis. A chromosome translocation t(5;12)(q33;p13) has been identified in a small group of CMML patients. The presence of t(5;12) leads to the expression of the Tel/PDGF beta fusion protein, inducing myeloproliferative disease (MPD).12,13 This subset of CMML patients may respond to treatment with imatinib mesylate, a PDGFR beta tyrosine kinase inhibitor.14,22

Treatment Options

Growth Factors
Until recently, growth factors and blood product transfusions were the only treatment options available for low-risk MDS. The use of hematopoietic growth factors, including granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), and erythropoietin in low-risk MDS patients, may result in significant improvement in quality of life and survival.15,16 The response to erythropoietin varies and correlates with many factors such as erythropoietin serum level and the number of monthly transfusions. The best response is seen in MDS patients with serum erythropoietin less than 500 MIU/mL and a red cell transfusion need of less than 2 units per month.17

Lenalidomide
This immunomodulating agent has activity in transfusion-dependent, low-risk MDS patients, particularly those characterized by del(5q). The identification of 5q- syndrome has gained greater importance now that specific and effective therapy has been found. Approximately two-thirds of low-risk MDS patients with isolated del(5q) who are transfusion-dependent responded and become transfusion-independent by taking oral lenalidomide. Furthermore, MDS patients with del(5q) in the presence of other cytogenetic abnormalities will have an approximately 25% response rate to lenalidomide.8 Complete cytogenetic remission occurred in 45% of the responding patients with a del(5q) clone treated with lenalidomide. The abnormal bone marrow morphology changed to normal in 36% of the patients.8 The median survivals of untreated MDS patients with isolated del(5q) or del(5q) in the presence of other cytogenetics are 80 and 47 months, respectively.6

The exact mechanism of action of lenalidomide is unknown. The starting dose is 10 mg daily, and the common side effects of neutropenia and thrombocytopenia occur in approximately 80% of patients.

Lenalidomide has the best effect on MDS patients with isolated deletion 5q syndrome, but other transfusion-dependent, low-risk MDS patients with cytogenetic abnormalities other than del(5q) have a 26% response rate to lenalidomide.6

Hypomethylating Agents
Gene hypermethylation may have a role in MDS pathogenesis. Two hypomethylating agents (azacitidine and decitabine) have been approved recently for the treatment of MDS.
Cytogenetic abnormalities in the myelodysplastic syndromes and occupational or environmental exposure

R. R. West, D. A. Stafford, A. D. White, D. T. Bowen, and R. A. Padua

Patients with myelodysplastic syndromes (MDS) have high frequencies of cytogenetic abnormalities and evidence is accumulating of associations between exposure history and primary MDS. The objective of this article is to examine the relationship between histories of occupational or environmental exposure and presence of cytogenetic abnormalities. A case control study of MDS patients estimated lifetime exposure to more than 90 potential hazards in 400 age, sex, and area of residence matched patient and control pairs. A parallel cytogenetics study undertaken at time of diagnosis, independently of any knowledge of exposure history, identified 75 cytogenetically abnormal and 139 normal (186 not studied). Odds ratios of MDS patients and their matched controls were compared for 3 groups: cytogenetically abnormal, normal, and not known. The odds ratios for all exposures combined were possibly higher among cytogenetically abnormal 2.0 (95% confidence interval 0.8-5.9) than among normal 1.0 (0.6-1.8). This pattern was observed for exposure to semimetal, abnormal 4.0 (0.4-195.1) and normal 0.5 (0.1-1.0) and inorganic dusts, 1.6 (0.6-3.8) and 0.4 (0.1-1.4) respectively. The pattern was principally in abnormalities in chromosomes 5 and 7. For organic chemicals and radiation, the odds ratios for both cytogenetically abnormal and normal were marginally raised: organic 1.8 (0.6-6.0) and 1.3 (0.6-2.9), respectively, and radiation 1.7 (0.5-5.6) and 1.3 (0.4-4.7) respectively. For radiation, abnormalities were mostly in chromosome 8. This study of association between exposures and cytogenetics in primary MDS complements those previously reported in secondary MDS and may provide some insight into pathogenetic mechanisms that lead to development of MDS.

Introduction

Cytogenetic abnormalities are identified at diagnosis in 30% to 70% patients with de novo myelodysplastic syndrome (MDS); the frequency increasing with higher risk disease.1,2 Chromosome translocations in MDS are rare and the most common karyotypic lesions involve chromosomes 8 (gain), 5 (loss/deletion), and 7 (loss/deletion).3 The accumulation of karyotypic abnormalities with disease progression provides some support for the multistep process of malignant transformation from MDS to acute myeloid leukemia (AML). Survival of patients with abnormalities involving chromosomes 5, 7, and 8 has been shown to be significantly reduced compared with patients with normal karyotypes.2,4

The etiologic insults leading to the development of MDS and the latency period between the initial genomic insult and disease manifestation are largely unknown. The best-defined xenobiotic insult in the development of MDS is that which follows cytotoxic chemotherapy for cancer with alkylating agents. Therapy related MDS (t-MDS) is associated with a higher frequency of karyotypic abnormalities than de novo MDS.5,6 The majority of these abnormalities involve chromosomes 5 and/or 7 suggesting that these chromosomes are particularly susceptible to genomic damage and that this leads to proliferative advantage. Furthermore, it has been shown that chemotherapy treated patients in clinical remission also harbour RAS and/or FMS oncogene mutations in peripheral blood DNA in the absence of hematologic disease and this may be a manifestation of genomic instability or damage.7-10 Postchemotherapy patients do not, however, show increased chromosome aberration frequencies compared with normal subjects, although they do show qualitative differences in the type of aberrations. A higher frequency of exchanges is seen amongst patients, particularly in those who received multiple compared with single courses of therapy and the frequency of gaps is lower.11

There have been many reports of associations between histories of exposures to certain organic chemicals, notably benzene solvents, pesticides, and radiation and MDS.12-14 However, only benzene has been strongly implicated in the etiology, with an elevated relative risk identified in a large cohort study of benzene-exposed workers compared with nonexposed controls.15 Benzene exposed workers developing hematologic abnormalities also showed polymorphism in metabolic pathways, which would predispose to the accumulation of the highly genotoxic quinone benzene metabolic intermediates.13 In vitro benzene metabolites induce peripheral blood lymphocyte chromosome 5 and 7 loss and long arm deletion.14 It has also been suggested that exposure to pesticides and organic solvents are associated with aberrations in chromosomes 5 and 7 in both AML and MDS. These were consecutive patients referred to the centers for specialist treatment of their conditions, not by reason of suspected past exposures. Exclusions were only for early death (less than 1 month of diagnosis) or severe illness (too ill to be interviewed).18-22 To elucidate the role of environmental mutagens in the pathogenesis of MDS, this study...
investigates the relationship between a history of exposure to chemicals/hazards and cytogenetic changes in primary MDS.

**Materials and methods**

**Case-control study of lifetime exposure**

Four hundred primary myelodysplastic syndrome (MDS) patients, diagnosed in 3 specialist regional centers (Bournemouth, Cardiff, and Leeds), were referred to the study. Controls were selected from hospital outpatients and inpatients, with a broad range of diagnoses but excluding malignancy, and were matched by age, sex, and area of residence. Lifetime occupational or environmental exposures of MDS patients and matched controls were estimated by questionnaire and semistructured interview at home after discharge by trained interviewers “blind” as to the case/control status of the patients. Lifetime exposure histories were estimated for more than 90 chemicals or putative hazards, including radiation. Exposures at 3 subjective intensities: (1) light (for example, being in the same room as open chemicals), (2) moderate (for example, working directly with chemicals), and (3) heavy (for example, working with volatile substances in confined space with poor ventilation and/or poor protection) were estimated as hours per day, days per year, and years to give lifetime hours. In analysis, patients were classified as “exposed” at 4 thresholds: (1) 10 hours per day at low intensity (a practical minimum detectable memorable level), (2) 2500 hours per day at low intensity, (3) 50 hours per day at moderate intensity, and (4) 2500 hours per day at moderate intensity to each of 90 chemicals or hazards (for example, arsenic), 13 groups of chemicals or hazards (for example, semimetals), 3 major groupings (organics, inorganics, and radiation), and any potential hazard.

**Cytogenetic analysis**

Bone marrow karyotype analysis was carried out according to conventional cytogenetic procedures. Approximately 20 metaphases were karyotyped by G banding. Clonal abnormalities were defined as 2 or more cells with the same additional whole chromosome or chromosome rearrangement, or 3 or more cells with the same chromosome missing. In line with other studies, the most frequent clonal abnormalities were trisomy 8, loss or deletion of chromosome 5, and loss or deletion of chromosome 7. Karyotypes were defined according to the Cytogenetic Nomenclature. Karyotype analysis was opportunistic and depended on availability of bone marrow aspirate. There were no recognizable biases in selecting patients for karyotyping but, in case there were unrecognized biases, the study design compared the odds ratios of cytogenetics known with cytogenetics not known.

**Statistical analysis**

Odds ratios (ORs) for each putative exposure were calculated as the ratio of discordant pairs and 95% confidence intervals were based on the binomial distribution. The analysis compares the ORs of matched pairs (averaged 1.2) and for several hazards significantly exceed 1.0, an association between exposure and cytogenetic abnormality is indicated not by an absolute OR but by comparison of ORs among cytogenetically normal with ORs among cytogenetically normal. The comparison thus seeks OR (abnormal) > OR (normal). Furthermore, because the cytogenetics known group includes normals and normals, the OR of this group would be expected to lie between the above 2. The analysis starts with all potential hazards combined and focuses progressively through 3 major groupings, 13 groups to 90 potential hazards combined and focuses progressively through 3 major groupings, 13 groups to 90.

**Results**

**Patient characteristics**

Cytogenetic analysis was completed in 214 MDS patients. There was no significant difference in patient characteristics, age, sex, and clinical diagnosis between these and patients for whom cytogenetic status was not known. Seventy-five (35%) had abnormal cytogenetics; the more common chromosomal abnormalities were in chromosome 8 (21,18 trisomy 8), chromosome 5 (14,7 monosomy 5) and chromosome 7 (9,6 monosomy 7). Lifetime exposure histories were obtained for a further 186 MDS patients, for whom cytogenetics were not known. Patients with cytogenetic abnormalities were possibly older than those who were cytogenetically normal ($\chi^2 = 7.94$, df = 4, $P < .15$), and also possibly more likely to be diagnosed with the poor prognostic FAB subtypes RAEB and RAEB t ($\chi^2 = 6.6$, df = 3, $P < .10$), but none of these differences were statistically significant.

**Exposure and cytogenetic abnormality**

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Case Report:

CD19-positive acute myeloblastic leukemia with trisomy 21 as a sole acquired karyotypic abnormality

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Abstract: We report that a 63-year-old Chinese female had acute myeloblastic leukemia (AML) in which trisomy 21 (+21) was found as the sole acquired karyotypic abnormality. The blasts were positive for myeloperoxidase, and the immunophenotype was positive for cluster of differentiation 19 (CD19), CD33, CD34, and human leukocyte antigens (HLA)-DR. The chromosomal analysis of bone marrow showed 47,XX,+21[2]/46,XX[18]. Fluorescent in situ hybridization (FISH) showed that three copies of AML1 were situated in separate chromosomes, and that t(8;21) was negative. The patient did not have any features of Down syndrome. A diagnosis of CD19-positive AML-M5 was established with trisomy 21 as a sole acquired karyotypic abnormality. The patient did not respond well to chemotherapy and died three months after the diagnosis. This is the first reported case of CD19-positive AML with trisomy 21 as the sole cytogenetic abnormality. The possible prognostic significance of the finding in AML with +21 as the sole acquired karyotypic abnormality was discussed.

Key words: Trisomy 21, Acute myeloid leukemia, Cluster of differentiation 19 (CD19)
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INTRODUCTION

Trisomy 21 (+21) is one of the five most frequent numerical abnormalities occurring in human neoplasms (Heim and Mitelman, 1986); however, in most cases, the extra chromosome 21 is present together with other numerical and/or structural changes. Acquired trisomy 21 is the single karyotypic abnormality in only 0.4% of human neoplasms, and the frequency was slightly lower in lymphoid than in myeloid malignancies (0.2% vs. 1.0%) (Mitelman et al., 1990). The incidence of trisomy 21 as a sole abnormality was between 0.3% and 0.6% in all patients with acute myeloblastic leukemia (AML). Morphologically, AML with trisomy 21 as a sole abnormality preferentially shows M2 or M4 phenotype according to the French-American-British (FAB) classification (Gallego et al., 1997; Wan et al., 1999). Patients with this aberration appear to have a poor prognosis (Wan et al., 1999; Cortes et al., 1995; Wei et al., 1996), but the clinical and prognostic implications of this karyotypic abnormality in AML remain unclear. Recently, a small number of cases (Wei et al., 1996; Kondo et al., 2001; Yamamoto et al., 2002; Udayakumar et al., 2007) reported the relationship between AML with trisomy 21 as a sole acquired abnormality and expression of cluster of differentiation 7 (CD7), and reported that co-expression of CD7 is probably indicative of the very early stage at which the cell became malignant (Udayakumar et al., 2007). Here, we presented a case of CD19-positive AML with trisomy 21 as a sole acquired karyotypic abnormality and discussed its possible prognostic significance.

1 Corresponding author
We considered trisomy 21 to be acquired when the patient showed no phenotypic characteristics of Down syndrome, such as developmental retardation, brachycephaly, epicantthal folds, and speckled iris. The patient was diagnosed with AML-M5 with +21 as the sole acquired karyotypic abnormality, and treated with cytosine arabinoside (100 mg/m\(^2\), continuous infusion, Days 1–7) and homoharringtonine (6 mg, Days 1–3). However, a repeated bone marrow showed a refractory state. The patient was then treated with cytosine arabinoside (100 mg/m\(^2\), continuous infusion, Days 1–7) and etoposide (100 mg, Days 1–7). Unfortunately, she died of refractory leukemia after the second treatment, with survival duration of three months.

**DISCUSSION**

Trisomy 21 has been demonstrated to be a recurring cytogenetic abnormality in AML and myelodysplastic syndrome (MDS). Trisomy may contribute to leukemogenesis by a gene dosage effect, whereby the presence of an increased copy number of certain genes gives a cell survival advantage and hence neoplastic potential (Sacchi, 1992). Cells trisomic for chromosome 21 could be over-proliferating due to the enhanced expression of a tumourigenic gene (Shen et al., 1995). The incidence of trisomy 21 as a sole cytogenetic anomaly in the de novo AML varies from 0.3% to 0.6% and from 2% to 6.7%, when it is associated with other anomalies whose presence rather than trisomy 21 determines the clinical outcome (Mitelman, 1994; Mitelman et al., 1990; Cortes et al., 1995; Wei et al., 1996; Berger et al., 1987). Dewald et al. (1990) reported that trisomy 21 was observed as a sole acquired abnormality in 13 patients who had hematologic malignancies, of whom 12 had myeloid, including 5 myelodysplastic syndromes, 3 AML-M4, 3 AML-M2, and 1 AML-M7, and the remaining one had lymphoid leukemia. Many AML cases with trisomy 21 as a sole anomaly had been reported. Most of them were associated with AML-M2 or AML-M4, and the remaining cases included AML-M1, AML-M3, AML-M5, AML-M6, and AML-M7 (Table 1) (Dewald et al., 1990; Mitelman, 1994; Cortes et al., 1995; Gallego et al., 1997; Wei et al., 1996; Wan et al., 1999; Kondo et al., 2001; Yamamoto et al., 2002; Udayakumar et al., 2007). Trisomy 21 also had been documented in 16 patients with myelodysplastic syndromes, 6 chronic myelomonocytic leukemia, 2 acute lymphocytic leukemia (ALL), 2 bilineal leukemia, and 2 undifferentiated leukemia (Mitelman, 1994). By comparison, the most common hematological malignancies in patients with constitutional trisomy 21 (Down syndrome) are ALL and AML-M7 (Wan et al., 1999; Hasle et al., 2000). Here, we reported a new case of AML-M5 with trisomy 21 as the sole acquired karyotypic abnormality, which is in accordance with the observation reported in most cases that trisomy 21 was present along with normal clones (Gallego et al., 1997; Wan et al., 1999; Wei et al., 1996; Yamamoto et al., 2002; Dewald et al., 1990).

**Table 1** Reports of trisomy 21 as a sole acquired abnormality in AML

<table>
<thead>
<tr>
<th>References</th>
<th>Year</th>
<th>Country</th>
<th>Sex/age</th>
<th>FAB subtype</th>
<th>CD7</th>
<th>CD19</th>
<th>Outcome</th>
<th>Os (month)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wei et al. (1996)</td>
<td>1996</td>
<td>China</td>
<td>M/35</td>
<td>AML-M4</td>
<td>NA</td>
<td>NA</td>
<td>Died of refractory leukemia</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Taiwan, China</td>
<td>M/30</td>
<td>AML</td>
<td>NA</td>
<td>NA</td>
<td>Died of internal bleeding 3 d after chemotherapy</td>
<td>0.1</td>
</tr>
<tr>
<td>Gallego et al. (1997)</td>
<td>1997</td>
<td>Spain</td>
<td>M/86</td>
<td>AML-M5b</td>
<td>NA</td>
<td>NA</td>
<td>No treatment</td>
<td>NA</td>
</tr>
<tr>
<td>Wan et al. (1999)</td>
<td>1999</td>
<td>China</td>
<td>M/28</td>
<td>AML-M2</td>
<td>NA</td>
<td>NA</td>
<td>CR, received an ABMT</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F/78</td>
<td>AML-M4</td>
<td>NA</td>
<td>NA</td>
<td>No treatment</td>
<td>NA</td>
</tr>
<tr>
<td>Kondo et al. (2001)</td>
<td>2001</td>
<td>Japan</td>
<td>M/21</td>
<td>AML-M2</td>
<td>+</td>
<td>–</td>
<td>CR well at 4 months</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M/49</td>
<td>AML-M2</td>
<td>+</td>
<td>–</td>
<td>CR, MDS 2 years later, leukemia 3 years later, died at 4 years</td>
<td>48</td>
</tr>
<tr>
<td>Present case</td>
<td>2008</td>
<td>China</td>
<td>F/61</td>
<td>AML-M5b</td>
<td>–</td>
<td>+</td>
<td>Did not receive CR</td>
<td>NA</td>
</tr>
</tbody>
</table>

*When the patients were first diagnosed. M: male; F: female; NA: not available; +: positive; –: negative; CR: complete remission; FAB: French-American-British; Os: overall survival; ABMT: autologous bone marrow transplant; MDS: myelodysplastic syndrome
Comparison of interphase FISH and metaphase cytogenetics to study myelodysplastic syndrome: an Eastern Cooperative Oncology Group (ECOG) study

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Abstract

Cytogenetic analysis can be important in determining the prognosis and diagnosis of a number of hematological disorders, including myelodysplastic syndromes (MDS). Here, we compared metaphase chromosomal analyses on bone marrow aspirates from MDS patients with interphase fluorescence in situ hybridization (FISH) using probes specific for chromosomes nos. 5, 7, 8, 11, 13 and 20. Forty-three patients enrolled in ECOG protocol E1996 for low risk MDS and five patients enrolled in ECOG protocol E3996 for high risk MDS were studied by both metaphase chromosomal analysis and interphase FISH. Excluding those with a clonal loss of the Y chromosome, an abnormal clone was detected by cytogenetic analysis in 18 of 48 samples (37.5%). In comparison, our FISH panel detected an abnormal clone in 17 of 48 samples (35.4%). Twenty-nine of 30 samples with apparently normal karyotypes, including those with a missing Y chromosome, were also normal by our FISH panel. One patient had an occult deletion of chromosome 11 that was detected by FISH.

These results indicate that around 60% of patients with MDS do not have abnormalities that are detectable by either chromosomal or FISH studies. In addition, it appears that interphase FISH studies are nearly as sensitive as cytogenetic analyses and can be a useful tool in studying bone marrow aspirates where cytogenetic analysis is not possible.

Keywords: FISH, Myelodysplastic syndrome, Cytogenetics, Chromosome, Hematology

1. Introduction

Myelodysplastic syndromes (MDS) are primarily diseases of the elderly. These disorders are usually associated

Abbreviations: MDS, myelodysplastic syndrome; FISH, fluorescence in situ hybridization; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RAEB, refractory anemia with excess blasts; RAEB-T, refractory anemia with excess blasts in transformation; CMML, chronic myelomonocytic leukemia; MLL, myeloid/lymphoid or mixed lineage leukemia gene; G-CSF, granulocyte colony-stimulating factor; EPO, erythropoietin

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with morphological dysplasia, impaired differentiation, defective cellular function, ineffective hematopoiesis and genetic instability. They also have significant morbidity and mortality [1]. Patients either have bone marrow failure or are transformed to acute myeloid leukemia (AML) in 20–40% of patients [1]. Clonal chromosomal abnormalities are typically detected in 40–60% of patients with primary (or de novo) MDS [2]. Chromosomal abnormalities commonly described in MDS include (in order of their frequency): −5/5q−, −7/7q−, +8, 20q−, +21, abn(3), 11q−/t(11;var)/+11, 12p−, 13q− and 17p− [3].

The importance of clonal chromosomal abnormalities in predicting the outcome in MDS patients has been recognized.
internationally. Karyotype, along with the percentage of bone marrow myeloblasts and the number of cytopenias, has been identified by the International Prognostic Scoring System (IPSS) as an important variable for the determination of prognosis in MDS [4]. The IPSS classified patients into three cytogenetic categories: Low risk MDS was defined as normal karyotype, loss of Y chromosome, del(5q) or del(20q) as sole anomalies. Intermediate risk MDS was defined as having structural abnormalities or loss of chromosome no. 7 and/or a complex karyotype with ≥5 abnormalities. High risk MDS was defined as having any other anomalies. At diagnosis, ≤35% of MDS patients present with normal karyotypes when assessed by conventional cytogenetic analysis [4–11]. However, patients with MDS and a normal karyotype are a heterogeneous group from a biologic standpoint and their outcome is often unpredictable [8,11].

Acquired monosomy 7 is among the most common abnormalities. This aberration is associated with a high rate of progression to AML [12]. Since cytogenetics can be used as a prognostic indicator for patients with MDS, a number of investigators have performed fluorescence in situ hybridization (FISH) to determine if some clonal anomalies are missed by conventional cytogenetic analysis [13–22]. Anomalies that are found by FISH but not by conventional cytogenetic analysis are typically termed “masked” or “occult”. In our study, we compared chromosomal analyses with interphase FISH analyses on patients with MDS to determine if there is a difference in the sensitivity between methods.

2. Materials and methods

Forty-eight bone marrow samples were collected from 43 patients on E1996 for low risk MDS and 5 patients on E3996 for high risk MDS. E3996 was a phase II study of combination human recombinant G-CSF with intermediate dose cytarabine and mitoxantrone chemotherapy in patients with high risk MDS [23]. E1996 was a phase III evaluation of EPO with or without G-CSF versus supportive therapy alone in treatment of myelodysplastic syndromes. As defined by the ECOG working committee, patients eligible for E1996 had a diagnosis of RA, RARS or RAEB and patients eligible for E3996 had RAEB, RAEB-T or CMML with at least 11% myeloblasts in their bone marrow.

There were 43 patients studied from E1996 and 5 patients studied from E3996 (see Table 1). The bone marrow specimens for E3996 were obtained between February 1997 and January 1998, while the bone marrow specimens for E1996 were obtained between December 1997 and March 2001. Harvested bone marrow cell pellets were received from participating ECOG cytogenetic laboratories, without knowledge of their cytogenetic diagnosis. Interphase FISH was done on 39 fixed cell pellets from cytogenetic studies and nine bone marrow aspirate smears from morphology studies. Normal control values were previously established using 16 normal bone marrow samples from bone marrow transplant donors [21] (see Table 2).

The bone marrow samples were analyzed independently by two different individuals in the order in which the specimens were received. A total of 200 nuclei were scored per probe set per patient. Two regions were analyzed by a dual color FISH probe strategy: −5/5q− and −7/7q−. Three regions were analyzed using a single color FISH probe strategy: +8, 13q− and 20q−. The remaining region, MLL, was analyzed using a dual color break-apart probe strategy (Table 2).

Slides were prepared, treated, hybridized and analyzed per manufacturer’s protocol (VysisTM, Downers Grove, IL) and as previously described [21].

3. Results

Bone marrow samples from 48 patients with MDS were analyzed by both metaphase chromosome analysis and FISH studies. Forty-three of these patients were on the low risk E1996 ECOG protocol. If the four samples with loss of the Y chromosome as their sole clonal abnormality (L10, L17, L29, L33) and one sample with non-clonal anomalies (L28) are included as chromosomally normal samples, an abnormal clone was identified in 15 of 43 samples (34.9%). In comparison, our FISH panel showed that 14 of 43 samples (32.6%) were abnormal. There were five samples in our study from five patients with high risk MDS who were on the E3996 ECOG protocol. All five cases had both cytogenetic and FISH analysis. Cytogenetics detected three patients with abnormal clones (60%). Our FISH panel also detected abnormal clones in the same three patients (60%). When the data of both the low and high risk patients were combined, 18 of 48 samples (37.5%) had an abnormal clone identified by FISH. A normal karyotype was described in 30 of the 48 samples (62.5%) with chromosomal analysis. Twenty-nine of these 30 samples were also normal by FISH.

Table 1

<table>
<thead>
<tr>
<th>MDS classification</th>
<th>Number of patients</th>
<th>MDS classification</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>5</td>
<td>RAEB</td>
<td>2</td>
</tr>
<tr>
<td>RARS</td>
<td>16</td>
<td>RAEB-T</td>
<td>2</td>
</tr>
<tr>
<td>RA vs. RARS</td>
<td>1</td>
<td>CMML</td>
<td>1</td>
</tr>
<tr>
<td>RAEB</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAEB-I</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAEB-II</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDS/RARS with del(5q)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDS, NOS</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOS: not otherwise specified.
References


Clinical and cytogenetic features of 508 Chinese patients with myelodysplastic syndrome and comparison with those in Western countries

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Myelodysplastic syndrome (MDS) is a clonal hematopoietic stem cell disorder characterized by ineffective hematopoiesis and leukemia progression. Racial differences may exist on clinical pictures and the molecular events leading to MDS, which are heterogeneous. To better define the clinical and cytogenetic features in Chinese patients, a retrospective multicentric study was performed in 508 MDS cases. Compared with Western countries, Chinese patients showed younger age (median: 49 vs 65–73 years), lower percentages of RARS (2.8 vs 6.6–15.3%), and CMML (5.2 vs 11.7–30.6%). Cytogenetically, among 367 cases with evaluable data, abnormal karyotypes were found in 136 cases, including 56 numerical and 80 structural changes. Incidences of single chromosome 5 and 7 abnormalities were lower than those in Western countries (2.2 vs 17.8–42.5%). However, complex cytogenetic aberrations and chromosome translocations were frequently observed and related to poor prognosis. Both multiple chromosome deletions and translocations were detected in advanced subtypes (RAEB and RAEB-T). Analysis of 200 cases revealed a higher incidence of hepatitis-B-virus infection than that in non-MDS population (21.00 vs 9.75%). This study further confirmed: (1) different genetic/environmental backgrounds between Asian and Western MDS populations; (2) a strong predictive value of cytogenetic abnormalities on disease outcome and involvement of genomic instability in leukemia clone development.

Materials and methods

Patients

From January 1990 to March 2003, 508 consecutive patients with primary MDS were included in this retrospective study. Thorough morphological review and immunohistochemistry studies were performed and the diagnosis was established according to the FAB criteria. Patients with a history of prior chemoradiotherapy and those with secondary MDS were excluded from this analysis. All patients provided informed consent and the University and Institutional Review Boards approved all research studies.

Cytogenetics

Cytogenetic analyses from bone marrow cells were available from 367 patients. Chromosomes were R- and G-banded on unstimulated bone marrow cells after 24-h culture. Karyotypes were classified according to International System for Human Cytogenetic Nomenclature. Inclusion in the study required the analysis of ≥20 metaphase cells per patient. For multiplex fluorescence in situ hybridization (M-FISH) analysis, the SpectraVysion Assay (Vysis, USA) was performed according to the manufacturers’ recommendations. The whole chromosome paint (WCP DNA probe) panel provided by SpectraVysion™ is directly labeled with the five different fluorochromes in a combinatorial labeling format to provide 24 distinct colors to identify the 24 chromosomes when analyzed with the CytoVision System (Applied Imaging, UK).

For clinical/cytogenetic categorization, patients were classified according to scoring systems related to karyotype, including international prognostic scoring system (IPSS), Lille system (calculated by platelet counts, bone marrow blasts and karyotype) and Tokyo system (calculated by neutrophil counts, hemoglobin level, platelet counts, bone marrow blasts and karyotype).

Statistics

The percentages of cytogenetic abnormalities according to FAB subtypes were compared using χ2 analyses and the Fisher exact test. Overall survival (OS) was measured from the date of
aberration, the number and the percentage of these abnormalities were only 16 (11.8%), 11 (8.1%), 2 (1.5%), 1 (0.7%), and 1 (0.7%), respectively.

According to the MDS subtypes, cytogenetic abnormalities were present in 80 chromosome segments in RA, nine in RARS, 144 in RAEB, and 142 in RAEB-T. Duplications were mostly seen in RA (37/80, 46.3%), while translocations were predominantly found in RAEB (40/144, 27.8%) and RAEB-T (47/142, 33.1%). As shown in Figure 2, in RA, the most frequent aberrations were dup(1)(q21q41), +8, and −20/20q−[del(20)(q11)]. In RAEB, −5/5q−, +8, and −20/20q−[del(20)(q11)] were major abnormalities observed. Cytogenetic abnormalities, especially chromosome deletions, were revealed on almost every chromosome. Simple and complex translocations, marker chromosome and other kind of structural abnormalities were also detected. In RAEB-T, dup(3)(q21q29), −5/5q−, −7/7q−[del(7)(q22q35)], +8, −18, marker chromosome, especially the simple and complex translocations constituted the special pattern of abnormalities.

Chromosome translocations in MDS

The chromosome translocations and the genes involved were listed in Table 3. Simple translocation involving chromosome 8, 22, 21, and 7 were found in young patients. t(8;21) was found in three cases, t(7;11), t(3;5), and t(1;7) were found in two cases. +8 was found in five cases as additional abnormalities. Complex translocations tended to be observed in older patients, mainly involving chromosome 3 (case 2, 3, 4, 6, 7, 8), 7 (case 1–4, 6, 8), 10 (case 2, 4, 5, 8), 12 (case 3, 4, 6, 8), 20 (case 1, 2, 4, 5, 7, 8), and 21 (case 3, 5, 8, 9). −5/5q− were found in six cases as additional abnormalities. In case 8, the most complex translocations involved 14 chromosomes with seven different translocations. Figure 3 showed the complex translocations confirmed by M-FISH analysis.

Impact of cytogenetics on leukemia progression and survival time

Among 329 patients with both available karyotype and follow-up data, survival analysis showed that IPSS had more significant impact on survival. The survival curves with respect to FAB classification, IPSS and cytogenetic findings were presented in Figure 4. In numerical changes, patients with hypodiploid presented with significantly early leukemia progression and short survival time ($P=0.0066$ and 0.0012, respectively). Although +8 and −20/20q− failed to indicate any statistically prognostic significance, survival curves of +8 and −20/20q− cases showed a tendency of decreased survival time (Figure 4c). −5/5q−, −21, and −7/7q− were not listed because of the limited number of patients with those cytogenetic abnormalities as a single aberration. In structural changes, double or complex aberrations, either with or without translocation, were related to poor disease outcome ($P=0.0008$ and 0.0008, respectively). In these 25 patients, all of them with follow-up data transformed to AL and survived within 19 months (Table 3).

Discussion

Despite of heterogeneous morphologic, biologic, and clinical features, MDS is generally considered as a preleukemic disorder in which the established neoplastic clone may progress to AL.8 The disease usually occurs in the elder group in Western population.2,4,9,10 However, the median age of our patients was a decade younger, which, interestingly, is in agreement with the previous reports from Asian countries, such as Korea,5,11
Case report

Trisomy 21 as the sole acquired karyotypic abnormality in acute myeloid leukemia and myelodysplastic syndrome

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Abstract

We report five cases of myeloid disorders in which trisomy 21 (+21) was found as the sole acquired karyotypic abnormality, comprising two cases of acute myeloid leukemia (AML) and three cases of myelodysplastic syndrome (MDS). In this series, MDS patients with +21 presented as high grade disease, which included two cases of refractory anemia with excess of blasts (RAEB) and one case of refractory anemia with excess of blasts in transformation (RAEBt), and showed rapid disease progression. Significant thrombocytopenia was observed in all three patients, and bone marrow examination showed a marked reduction in megakaryocytes. AML patients with +21 included one case each of AML-M2 and M4. Despite the poor prognosis reported in AML patients with +21 as the sole abnormality, the patient in our series who was able to complete intensive treatment was cured of disease. The role of +21 in leukemogenesis is reviewed. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Acute myeloid leukemia; Myelodysplastic syndrome; Trisomy 21; Pathogenesis; Cytogenetics

1. Introduction

Trisomy 21 (+21) is the second commonest trisomy in AML and MDS after trisomy 8. Nevertheless, the clinical and prognostic implications of this karyotypic abnormality in myeloid disorders remain incompletely characterized. In the largest series on +21 in myeloid disorders to date from a single institution [1], 27 out of 1187 patients (3.3%) with untreated AML or MDS seen over a 13-year period had an acquired +21, and in four patients (0.3%) it occurred as the sole cytogenetic abnormality. Secondly, among 13 patients who had hematologic malignancies in which +21 was found as the sole acquired abnormality [2], 12 had myeloid disease (AML or MDS), with the remaining case being acute lymphoblastic leukemia (ALL). There appeared to be no morphologic specificity. Furthermore, while a higher complete remission (CR) had previously been reported in AML with +21 [3], a more recent study [1] showed that +21 in AML typically presented in conjunction with other cytogenetic changes, whose presence rather than +21 determined the clinical outcome. We present clinicopathologic features and outcome in five cases of AML and MDS in which +21 was found as the sole karyotypic abnormality and review the possible role of +21 in leukemogenesis.

2. Material and methods

Cytogenetic records from 1990 to 1997 in our center were reviewed and patients with +21 as the sole karyotypic abnormality in myeloid disorders were retrieved. We considered +21 to be acquired when the patient showed no phenotypic characteristics of Down syndrome (DS). A total of five patients were identified,
comprising two patients with AML and three patients with MDS. Among AML and MDS cases successfully karyotyped at diagnosis in our laboratory, +21 as the sole acquired cytogenetic abnormality was found in 0.8% of AML (2 out of 242) and 1.7% of MDS (3 out of 171) respectively. Patient files were reviewed for clinical presentation, treatment received and clinical outcome.

Cytogenetic analysis was performed on synchronized and non-synchronized short term culture of bone marrow cells supplemented by direct harvest, in accordance with previously published protocols [4].

3. Results

The clinical presentation, hematologic findings and diagnosis of the five AML/MDS patients with +21 as the sole acquired karyotypic abnormality were tabulated in Table 1. Among AML patients, there was one case each of AML-M2 and M4. In this series, MDS patients were diagnosed with high-grade disease, including two cases of RAEB and one case of RAEBt. All three MDS patients presented with significant thrombocytopenia and bleeding. Bone marrow aspiration of these patients showed hypercellular marrow particles with markedly reduced megakaryocytes in addition to granulocytic hyperplasia, increased blasts, dysplastic myeloid maturation and depressed erythropoiesis (Fig. 1).

Table 2 showed the karyotype, treatment and clinical outcome of the five patients in this study. Normal metaphases were encountered together with the abnormal clone harboring +21 in all cases. Patient 1 had two extra copies of chromosome 21, but was included in the present study since aneuploidy involving chromosome 21 was the only abnormality identified.

Only Patient 1 among the two AML patients was able to complete induction chemotherapy and was subsequently consolidated with autologous bone marrow transplantation (BMT). Cytogenetic study was repeated twice after BMT, which showed no clonal abnormality. He was considered cured after enjoying a disease-free survival of eight years. All MDS patients with +21 showed rapid disease progression and succumbed within six months of initial diagnosis.

4. Discussion

Clinical observations, including that there are a 10 to 20 times increased risk of leukemia in DS [5], that trisomic 21 cells are the ones involved in leukemic transformation in DS mosaics [6], and that +21 is a common cytogenetic change in AML and ALL, lead Rowley [7] to suggest an etiologic role for +21 in leukemogenesis, although this has been disputed by others [8,9]. While hypothetical models were subsequently developed to explain the contribution of an extra chromosome 21 to leukemogenesis [summarized in [10]], the basis of the association between trisomy 21 and leukemia remains to be defined.

Trisomy may contribute to leukemogenesis by a gene dosage effect whereby the presence of an increased copy number of certain genes gives a cell survival advantage and hence neoplastic potential [10]. Gains and losses of whole chromosomes in neoplastic diseases could be explained in terms of amplification of an active primary aberration [11]. The active primary aberration may be a submicroscopic chromosomal change. Trisomies or monosomies may be due to subsequent nondisjunction events. If the trisomy includes two deranged chromosomes (disomic homozygosity) and only one normal chromosome, amplification of the primary change may take place and imparts the cell a neoplastic potential.

Cells trisomic for chromosome 21 could be over-proliferating due to enhanced expression of a tumourigenic protein coded by a chromosome 21 gene [5]. Chromosome 21 represents 0.8% of the human genome and is estimated to encode for approximate 800 genes. The
identification of transcribed sequences in chromosome 21 will facilitate the understanding of the candidate gene for leukemogenesis. Recently, increased levels of a chromosome 21-encoded tumor invasion and metastasis factor (TIAM1) mRNA have been detected in bone marrow of DS children during the acute phase of AML-M7 [12]. The human homologue of TIAM1 has been mapped to chromosome 21q22.1 [13]. Furthermore, increased disomic homozygosity in the pericentromeric region of 21q has been found amongst DS subjects with transient abnormal myelopoiesis and/or AML-M7 leukemia compared to DS individuals with normal myelopoiesis by use of both cytogenetic [14] and molecular techniques [5,15,16]. It has been proposed that 21q11 region may house a gene encoding a product with tumor suppressor or growth control functions [15,17,18].

Although +21 is one of the commonest acquired chromosomal abnormalities in hematologic malignancies [19], +21 as the sole anomaly is a very rare event [20]. In this study, we report five patients with +21 as the sole karyotypic abnormality in myeloid disorders. AML/MDS patients in which +21 is found as the sole abnormality appear to have a poor prognosis. Dewald et al. [2] reported a median survival of 4.2 months from chromosome analysis, although the treatment given was not specified. In two AML patients who were intensively treated [21], the overall survival was 3 days and 9 months respectively. In our series, one AML patient (case 1) received intensive chemotherapy. Owing to young age and good premorbid status, he was able to withstand intensive treatment and could be considered cured. The clone with +21 was no longer detectable after autologous BMT, and this cytogenetic change served as a useful marker for disease monitoring. For MDS patients on expectant management, disease progression is rapid and all succumbed within 6 months after initial presentation. Hence more patients who are intensively treated will have to be analyzed before a firm conclusion on prognostic implication can be drawn.

The three MDS patients in our series had significant thrombocytopenia. Bone marrow of these patients showed a marked reduction in megakaryocytes. Throm-
bocytopena associated with decreased or absent megakaryocytes is seen in a number of disorders, including aplastic anemia, acute leukemia, MDS and post-chemotherapy and/or radiation therapy [22]. Moreover, acquired megakaryocytic thrombocytopenia may progress into MDS [23]. It will be interesting to look for +21 in MDS with decreased or absent megakaryocytes to determine if it is a consistent abnormality in this situation.

Finally, it has been proposed that primary and secondary genetic aberrations in neoplasia are functionally distinct. Primary abnormalities lead to specific gene rearrangements, while secondary changes result in genomic imbalance [24]. It follows that unbalanced genetic changes, for example chromosomal trisomies like +21, may always be secondary events and there are no unbalanced primary aberrations. The importance of studying unbalanced genetic changes would be to identify any associated submicroscopic disease-specific gene rearrangements that are relevant to diagnosis or prognosis. This is exemplified by the recent observation that +21 is a recurrent secondary aberration in childhood ALL with TEL/AML1 gene fusion [25]. Since t(12;21)(p13;q22) giving rise to TEL/AML1 fusion is in most instances cryptic in nature, the presence of +21 in childhood ALL may serve as a cytogenetic pointer for t(12;21) [26]. Whether +21 is preferentially found with specific underlying abnormalities in myeloid disorders to which it may point will await pooling and analysis of as much patient information as possible in this relatively rare aberration.

Acknowledgements

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References


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Cytogenetic analysis of 52 Indian patients with de novo myelodysplastic syndromes—a comparative analysis of results with reports from Asia

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Abstract Most published data on myelodysplastic syndromes (MDS) are derived from Western countries, which report MDS as a disease of the elderly. However, it was observed that Asian MDS patients were younger than subjects in Western reports. With this in mind, the study was conducted prospectively on 52 Indian patients to define chromosomal abnormalities and to understand ethnogeographical differences, if any, underlying the pathogenesis of MDS among this Asian population. Cytogenetic analysis was performed using GTG banding and karyotyped according to the International System for Human Cytogenetic Nomenclature (ISCN). The incidence of MDS was predominant in the age group of 41–60 years (44.23%), with a median age at diagnosis of 55 years. The disease was more frequent in males (33 patients, 63.46%) than females (19 patients, 36.53%). Of 48 patients successfully karyotyped, 17 had normal karyotype (35.4%) and 31 patients (64.5%) had a chromosomal abnormality. The most frequent chromosome abnormalities were del 5q/−5i in 3 patients (42%), −7/7q− in 10 patients (32.2%), +8 and del 20q− in 6 cases each (19.3%) and i(17)(q10) in 1 patient (3.2%). In addition to these non-random chromosomal abnormalities, some rare abnormalities were also encountered. A higher rate of transformation to acute myeloid leukaemia (AML) was observed in the Chinese population compared to other Asian countries. The incidence of chromosomal abnormalities varied considerably across the different Asian populations. The overall frequency of chromosomal abnormalities in our study was comparable to most Western reports. Further prospective studies are warranted to elucidate precisely the ethnic differences in the pathogenesis of MDS in the Indian population.

Keywords Myelodysplastic syndromes · Cytogenetics · Indian population

Introduction

Myelodysplastic syndromes (MDS) comprise a heterogeneous group of acquired clonal neoplastic disorders of the haematopoietic cells. MDS is characterised by morphological abnormalities with evidence of trilineage dysplasia, ineffective haemopoiesis and a propensity of transformation to acute myeloid leukaemia (AML) [10]. In MDS, haematopoietic precursor cell differentiation is progressively impaired and evidence suggests that the disorder occurs at the level of the pluripotential stem cell. The distinctive hallmark of MDS is the paradox of variable peripheral cytopenia despite hypercellular or normocellular bone marrow and is attributed to excessive apoptosis in the initial stages of disease. In 1982, the French–American–British (FAB) group classified MDS based on morphological characteristics into five subsets and included refractory anaemia (RA), refractory anaemia with ringed sideroblasts (RARS), chronic myelomonocytic leukaemia (CMML), refractory anaemia with excess blasts (RAEB) and refractory anaemia with excess blasts in transformation (RAEB-t) [3].

In most series reported, a chromosomally abnormal clone has been demonstrated in the bone marrow of 40–60% of patients with primary MDS [4, 11]. An increasing number of non-random chromosomal abnormalities are recognised in MDS. The five single chromosomal abnormalities that appear most common in MDS are del(5q)/−5, −7/del(7q), +8, del(20q) and iso17q. The other less common isolated
Cytogenetic analysis of 52 Indian patients with de novo myelodysplastic syndromes—a comparative analysis of results with reports from Asia

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Abstract Most published data on myelodysplastic syndromes (MDS) are derived from Western countries, which report MDS as a disease of the elderly. However, it was observed that Asian MDS patients were younger than subjects in Western reports. With this in mind, the study was conducted prospectively on 52 Indian patients to define chromosomal abnormalities and to understand ethno-geographical differences, if any, underlying the pathogenesis of MDS among this Asian population. Cytogenetic analysis was performed using GTG banding and karyotyped according to the International System for Human Cytogenetic Nomenclature (ISCN). The incidence of MDS was predominant in the age group of 41–60 years (44.23%), with a median age at diagnosis of 55 years. The disease was more frequent in males (33 patients, 63.46%) than females (19 patients, 36.53%). Of 48 patients successfully karyotyped, 17 had normal karyotype (35.4%) and 31 patients (64.5%) had a chromosomal abnormality. The most frequent chromosome abnormalities were del 5q/−5i in 3 patients (42%), −7/7q− in 10 patients (32.2%), +8 and del 20q− in 6 cases each (19.3%) and i(17)(q10) in 1 patient (3.2%). In addition to these non-random chromosomal abnormalities, some rare abnormalities were also encountered. A higher rate of transformation to acute myeloid leukaemia (AML) was observed in the Chinese population compared to other Asian countries. The incidence of chromosomal abnormalities varied considerably across the different Asian populations. The overall frequency of chromosomal abnormalities in our study was comparable to most Western reports. Further prospective studies are warranted to elucidate precisely the ethnic differences in the pathogenesis of MDS in the Indian population.

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Introduction

Myelodysplastic syndromes (MDS) comprise a heterogeneous group of acquired clonal neoplastic disorders of the haematopoietic cells. MDS is characterised by morphological abnormalities with evidence of trilineage dysplasia, ineffective haemopoiesis and a propensity of transformation to acute myeloid leukaemia (AML) [10]. In MDS, haematopoietic precursor cell differentiation is progressively impaired and evidence suggests that the disorder occurs at the level of the pluripotential stem cell. The distinctive hallmark of MDS is the paradox of variable peripheral cytopenia despite hypercellular or normocellular bone marrow and is attributed to excessive apoptosis in the initial stages of disease. In 1982, the French–American–British (FAB) group classified MDS based on morphological characteristics into five subsets and included refractory anaemia (RA), refractory anaemia with ringed sideroblasts (RARS), chronic myelomonocytic leukaemia (CMML), refractory anaemia with excess blasts (RAEB), and refractory anaemia with excess blasts in transformation (RAEB-t) [3]. In most series reported, a chromosomally abnormal clone has been demonstrated in the bone marrow of 40–60% of patients with primary MDS [4, 11]. An increasing number of non-random chromosomal abnormalities are recognised in MDS. The five single chromosomal abnormalities that appear most common in MDS are del(5q)/−5, −7/del(7q), +8, del(20q) and iso17q. The other less common isolated...
abnormalities include 12p−, deletions and translocations involving band 11q23 and loss of Y chromosome. Chromosomal findings are independent prognostic variables that contribute to a better definition of prognosis in MDS patients [13, 22, 30, 36, 37]. In general, clones with complex karyotypes are more frequent in the advanced FAB groups of MDS and often associated with shortened survival and an increased frequency of transformation to AML [5, 34].

Most information on MDS is derived from Western countries on Caucasian patients, which report MDS as a disease of the elderly. Studies on MDS in Asian countries are scarce except for reports from Japan. It was observed that non-Caucasian MDS patients from Asian countries are at least 8–10 years younger than those reported from Western countries. The variability in the natural history of MDS makes it difficult for diagnosis and classification, which results in lack of substantial epidemiological data for comparison across the globe. We report herein cytogenetic studies on 52 Indian patients with primary MDS and discuss chromosomal abnormalities encountered and correlation with prognosis of the disease. In addition, we also compare our demographic and cytogenetic results with those reported from other Asian countries to throw light on the ethnogeographical variation of the disease amongst the Asian population.

**Materials and methods**

**Patients**

Morphological classification (FAB) and cytogenetic analysis were performed on 52 consecutive patients diagnosed with de novo MDS attending the Department of Haematology/Oncology, Government General Hospital, Chennai, from 1998 to 2002. This government-run hospital is one of the major referral centres in the region it serves. It is worth mentioning that Chennai with a population of over 4.2 million is one of the four major metropolitan cities in India. The location and reputation of the hospital attracts patients from all parts of the state of Tamil Nadu, which ensures an unbiased sample collection. Informed consent was obtained from all patients for the collection of bone marrow (BM) and peripheral blood (PB) samples. Patients who had previously received chemotherapy and those with MDS secondary to a previous malignancy were not included in the study.

**Methods**

Bone marrow cells were cultured in RPMI 1640 supplemented with 20% fetal calf serum, 2 mM l-glutamine, penicillin and streptomycin (100 U/ml) for 24 h. After 24-h incubation, colcemid was added at a final concentration of 0.1 μg/ml for 30 min. Then, the cells were treated with hypotonic KCl (0.075 M) for 12–15 min and fixed with methanol/acetic acid (3:1). Metaphase chromosomes were banded using the conventional GTG banding technique and karyotyped according to the International System for Human Cytogenetic Nomenclature (ISCN) [20]. Twenty metaphases were analysed whenever possible to demonstrate the clonal nature of the aberrations. A karyotype was considered complex if there was an involvement of three or more chromosomes.

We also searched the database from the National Library of Medicine (PubMed) for literature on MDS from other non-Caucasian ethnic groups to compare and contrast demographic and cytogenetic profiles reported from other Asian countries. We searched the database with key words “MDS and Asia”, which resulted in 97 articles. From this group, the articles which reported cytogenetic, clinical and demographic data as occurring in their MDS population were scrutinised. These articles were obtained and cytogenetic results as reported from different Asian countries are compared in our analysis. The requirements were that these articles had been published in English and studies involving large number of patients were given priority for analysis and comparison of results. Also, it was of crucial importance that these reports employed the FAB classification system of MDS, which permits us a clearer and better understanding and appreciation of the comparative analysis of results from Asian countries.

**Results**

**FAB, age and sex distribution**

The incidence of MDS was predominant in the age group of 41–60 years (44.23%). The incidences in the other age groups of 0–20, 21–40 and >60 years were 5.76, 11.53 and 38.46%, respectively. The disease was more preponderant in males (33 patients, 63.46%) than females (19 patients, 36.53%). The FAB, age and sex distribution of the 52 patients studied and distribution in the various subgroups of MDS are presented in Table 1.

**Table 1** Age and sex distribution of the 52 MDS patients studied across the various FAB subtypes

<table>
<thead>
<tr>
<th>FAB subtype</th>
<th>No. of patients (%)</th>
<th>Age in years (%)a</th>
<th>Sex (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0–20</td>
<td>21–40</td>
</tr>
<tr>
<td>RA</td>
<td>24 (46.1)</td>
<td>3 (12.5)</td>
<td>5 (20.8)</td>
</tr>
<tr>
<td>RARS</td>
<td>8 (15.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>CMML</td>
<td>4 (7.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>RAEB</td>
<td>10 (19.2)</td>
<td>0 (0)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>RAEB-t</td>
<td>6 (11.5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>52 (100)</td>
<td>3 (5.7)</td>
<td>6 (11.5)</td>
</tr>
</tbody>
</table>

*Percentage calculated on the number of patients in the various FAB subtypes (indicated in bold in the respective rows)
Cytogenetics

Of the 52 patients, 48 patients (92.3%) were successfully karyotyped. Two patients with RA and one each in the RARS and RAEB subgroups, respectively, could not be karyotyped for reasons of inadequate metaphases and culture failure. Of the 48 patients karyotyped, 17 patients (35.4%) had normal karyotype and 31 patients (64.5%) had a chromosomal abnormality. In the RA group of 12 patients with a chromosomal abnormality, 9 patients had a single chromosomal abnormality, 2 patients had more than one chromosome involvement and 1 patient had a complex karyotype. In the RARS group all four patients had single chromosome involvement. In the CMML category of three patients with a chromosomal abnormality all had single chromosomal involvement. In the RAEB subgroup, in which seven patients had a chromosomal abnormality, three of the seven patients had single chromosomal abnormality, two patients had more than a single chromosome involvement and two patients had complex karyotype.

Non-random chromosomal abnormalities in the FAB subgroups

In our study, of the 48 patients successfully karyotyped, 13 patients (42%) had del 5q/−5. Abnormality of chromosome 7 was found in ten patients (32.2%). Trisomy 8 was found in six cases (19.3%). Non-random chromosomal abnormalities of 20q− was found in six patients (19.3%) and i(17q) was found in one patient (3.2%). In our study, we also observed some less common chromosomal abnormalities such as trisomy 14 (3.2%), trisomy 4 (3.2%) and a translocation involving chromosome band 11q23 (3.2%). The incidences of chromosomal abnormalities encountered in the patients along with their respective FAB distributions are presented in Table 2.

Transformation to AML

Of the 52 patients studied, 10 patients (19.2%) progressed to AML. Of the ten patients who transformed to AML, four patients (40%) were in the RAEB-t subgroup, three patients (30%) were in the RAEB subgroup, two patients (20%) were in the RA category and one patient (10%) had CMML. It is interesting that transformation to AML was frequently associated with abnormality of chromosome 7 as presented in Table 3.

Comparison of data from other Asian countries

The demographic, FAB distribution and transformation characteristics of patients reported from other Asian countries are presented in Table 4. It is interesting that the median

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No. of patients (%)</th>
<th>FAB distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karyotypes</td>
<td></td>
<td>RA</td>
</tr>
<tr>
<td>Normal</td>
<td>17 (35.4)</td>
<td>10</td>
</tr>
<tr>
<td>Abnormal</td>
<td>31 (64.5)</td>
<td>12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. and type of chromosomal abnormalities of the 31 abnormal karyotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
</tr>
<tr>
<td>Double</td>
</tr>
<tr>
<td>Complex</td>
</tr>
<tr>
<td>Del 5q−5</td>
</tr>
<tr>
<td>Chromosome 7</td>
</tr>
<tr>
<td>Trisomy 8</td>
</tr>
<tr>
<td>Del 20q</td>
</tr>
<tr>
<td>i(17q)</td>
</tr>
<tr>
<td>−Y</td>
</tr>
<tr>
<td>Trisomy 4</td>
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<tr>
<td>Trisomy 14</td>
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<table>
<thead>
<tr>
<th>Table 2 Chromosomal abnormalities in the 48 MDS patients successfully karyotyped and distribution across the FAB subtypes</th>
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<tr>
<td>Parameter</td>
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<td>Karyotypes</td>
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<td>No. and type of chromosomal abnormalities of the 31 abnormal karyotypes</td>
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<td>Trisomy 8</td>
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<td>Del 20q</td>
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<tr>
<td>i(17q)</td>
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<tr>
<td>−Y</td>
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<tr>
<td>Trisomy 4</td>
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<td>Trisomy 14</td>
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<table>
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<th>Table 3 FAB subtypes and chromosomal abnormalities of ten patients transformed to AML</th>
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<tbody>
<tr>
<td>FAB type</td>
</tr>
<tr>
<td>RA</td>
</tr>
<tr>
<td>RA</td>
</tr>
<tr>
<td>CMML</td>
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<tr>
<td>RAEB</td>
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<td>RAEB</td>
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<tr>
<td>RAEB</td>
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<td>RAEB-t</td>
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</table>
and 74 years [2, 7, 9, 15]. In our study, the median age of patients at diagnosis was 55 years and is in concordance with reports of investigators from Southeast Asia, Turkey and Central Africa. They have reported that MDS patients in these areas are generally younger than those from Western countries [25], and the reason for this difference is unclear. However, it is speculated that the younger age of Asian patients may be due to differential action of various environmental factors, including an increase in exposure to aetiologically relevant risk factors such as organic solvents, pesticides, radiation and environmental pollution. In our study the sex ratio was 1.7:1, in favour of men. This unbalanced sex ratio suggests the importance of hormonal and/or exposure to occupational factors in the development of MDS.

The incidence of clonal abnormalities in MDS bone marrow cells analysed by conventional banding techniques varies between 23 and 78%. Cytogenetic abnormalities in MDS are associated with a strong prognostic value in the pathogenesis of the disease [24, 26, 28, 33]. In our study, of the ten patients who transformed to AML, seven patients (70%) had only abnormal karyotypes and three patients (30%) had a mix of normal and abnormal karyotypes as shown in Table 3. We also observed that leukaemic transformation occurred more frequently in patients with double or complex defects than in those patients with a single defect or a normal karyotype. In the current analysis, we observed non-random chromosomal abnormalities of del (5q), −7, +8, del 20q, i(17q) in the same decreasing order of preponderance as reported by most Western investigators on Caucasian patients.

Many reports have suggested that the distribution of specific chromosomal abnormalities characterised in the subtypes of leukaemia vary between Western and Asian counties [14]. Few reports have also indicated geographical and ethnic differences in the frequency of specific chromosomal aberrations [19]. The incidence of all chromosomal abnormalities was between 37 and 88% in the Indian population and varied between 37 and 50% in China, Thailand, Hong Kong and Japan. Complex abnormalities were observed more frequently in patients from Hong Kong, China and Japan compared to other Asian countries as presented in Table 5. It also needs to be emphasised that the Chinese report had the youngest cohort associated with the highest rate of transformation to AML among other Asian countries. On the other hand, the Koreans had the lowest percentage of transformation as shown in Table 4. In the present study, we found del 5q/−5 and trisomy 8 in a large number of patients compared to other reports from India and also a higher incidence of del 20q than any other Asian country as shown in Table 5.

While the pathogenesis of MDS is still poorly understood, environmental, biological and occupational factors could induce mechanisms that are associated with diverse karyotypes and variable frequency of chromosomal abnormalities [8, 21, 29]. Thus, genetic load and degree of exposure to aetiological agents in various countries owing to socio-economic standards may explain the differences in the incidence of non-random chromosomal abnormalities among MDS patients in different geographical locations. Taken together, these factors may have a significant contribution in the pathogenesis and variability in the demographic and cytogenetic profile of MDS patients in various Asian countries. In conclusion, although at present cytogenetic investigations in India are performed in only a few hospitals, prospective studies on a large number of patients are warranted to elucidate more precisely the demographic and ethnic differences in the pathogenesis of MDS amongst the Asian population.

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References

Identification of novel cytogenetic markers with prognostic significance in a series of 968 patients with primary myelodysplastic syndromes

8. Sole2005 & Haase2007 (condensed)

Background and Objectives. The main prognostic factors in myelodysplastic syndromes (MDS) are chromosomal abnormalities, the proportion of blasts in bone marrow and number and degree of cytopenias. A consensus-defined International Prognostic Scoring System (IPSS) for predicting outcome and planning therapy in MDS has been developed, but its prognostic value in a large and independent series remains unproven. Furthermore, the intermediate-risk cytogenetic subgroup defined by the IPSS includes a miscellaneous number of different single abnormalities of uncertain prognostic significance at present. The main aim of the present study was to identify chromosomal abnormalities with a previously unrecognized good or poor prognosis in order to find new cytogenetic markers with predictive value.

Design and Methods. We report the cytogenetic findings in a series of 968 patients with primary MDS from the Spanish Cytogenetics Working Group, Grupo Cooperativo Español de Citogenética Hematológica (GCEGH).

Results. In this series of 968 MDS patients, we found various cytogenetic aberrations with a new prognostic impact. Complex karyotype, -7/7q- and i(17q) had a poor prognosis; normal karyotype, deletion 11q, deletion 12p and deletion 20q as single alterations had a good prognosis. Intermediate prognosis aberrations were rearrangements of 3q21q26, trisomy 8, trisomy 9, translocations of 11q and del(17p). Finally, a new group of single or double cytogenetic abnormalities, most of which are considered rare cytogenetic events and are usually included in the intermediate category of the IPSS, showed a trend to poor prognosis.

Interpretations and Conclusions. This study suggests that some specific chromosomal abnormalities could be segregated from the IPSS intermediate-risk cytogenetic prognostic subgroup and included in the low risk or in the poor risk groups.

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Key words: myelodysplastic syndromes, karyotype, cytogenetics, prognosis, IPSS.
Statistical analysis

$\chi^2$ tests were used to compare proportions. The Kaplan-Meier product limit method was used to estimate the actuarial probability of survival and the cumulative risk of leukemic transformation. Survival was measured from diagnosis to death or last follow-up. All deaths, related or not to MDS, were considered as the endpoint of the follow-up interval. The time to transformation into acute leukemia was measured from diagnosis to development of acute leukemia. Data from patients dying from any cause before developing acute leukemia were censored at the date of death for the calculation of risk of leukemic transformation. Statistical comparisons between different actuarial curves were based on log rank tests or, if applicable, the test for trend, as recommended by Peto et al.

According to the prognostic significance of the cytogenetic abnormalities found as single anomalies, we proposed four categories of karyotypes, called GCECGH categories. These categories are as follows: good prognosis: normal karyotype, loss of $Y$ chromosome, del(5q), del(20q), del(11q) and del(12p) as a single anomaly; intermediate prognosis: trisomy 8, rearrangements of 3q21q26, translocations of 11q del(17p), trisomy 18 and trisomy 19; poor prognosis: complex karyotypes, monosomy 7, deletion 7q and i(17q); unknown prognosis: all remaining cases with single or double abnormalities. Further multivariate analysis by the Cox proportional hazards regression method was used to identify the most significant independent prognostic factors related to survival and acute leukemic transformation. In a first phase the prognostic variables, with
New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: evidence from a core dataset of 2124 patients

Detlef Haase,1 Ulrich Germing,2 Julie Schanz,1 Michael Pfeilstöcker,3 Thomas Nösslinger,3 Barbara Hildebrandt,4 Andrea Kundgen,2 Michael Lübbert,5 Regina Kunzmann,5 Aristoteles A. N. Giagounidis,6 Carlo Aul,6 Lorenz Trümper,1 Otto Krieger,7 Reinhard Stauder,8 Thomas H. Müller,9 Friedrich Wimazal,10 Peter Valent,10 Christa Fonatsch,11 and Christian Steidl1

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We have generated a large, unique database that includes morphologic, clinical, cytogenetic, and follow-up data from 2124 patients with myelodysplastic syndromes (MDSs) at 4 institutions in Austria and 4 in Germany. Cytogenetic analyses were successfully performed in 2072 (97.6%) patients, revealing clonal abnormalities in 1084 (52.3%) patients. Numeric and structural chromosomal abnormalities were documented for each patient and subdivided further according to the number of additional abnormalities. Thus, 684 different cytogenetic categories were identified. The impact of the karyotype on the natural course of the disease was studied in 1286 patients treated with supportive care only. Median survival was 53.4 months for patients with normal karyotypes (n = 612) and 8.7 months for those with complex abnormalities (n = 166). A total of 13 rare abnormalities were identified with good (+1/1q, t(1q), t(7q), del(9q), del(12p)), chromosome 15 anomalies, t(17q), monosomy 21, trisomy 21, and −X), intermediate (del(11q), chromosome 19 anomalies), or poor (t(5q)) prognostic impact, respectively. The prognostic relevance of additional abnormalities varied considerably depending on the chromosomes affected. For all World Health Organization (WHO) and French-American-British (FAB) classification system subtypes, the karyotype provided additional prognostic information. Our analyses offer new insights into the prognostic significance of rare chromosomal abnormalities and specific karyotypic combinations in MDS. (Blood. 2007;110:4385-4395)

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Introduction

The myelodysplastic syndromes (MDSs) are a heterogeneous group of clonal hematopoietic stem cell disorders that cause one or more peripheral cytopenias due to ineffective hematopoiesis.4 The sequelae of MDS result from the underlying cytopenias and include hemorrhage (thrombocytopenia), infections (neutropenia), and, most commonly, anemia. Complications from cytopenias lead to higher morbidity and mortality among patients with MDS compared with an age-matched population.9 The variable risk of progression to acute myeloid leukemia (AML) is of major prognostic significance in early MDS without blast excess.2

A succession of MDS classification systems have been developed to facilitate prediction of the risk of progression to AML and overall survival.10-15 The first of these was the French-American-British (FAB) system,10 which used cytomorphologic abnormalities and blast percentage as criteria for classification. The World Health Organization (WHO) classification system subsequently improved homogeneity and discrimination between lower-risk MDS categories11,12,16,17 and was prospectively validated recently on a large number of patients.18 The International Prognostic Scoring System (IPSS), which applies only to de novo MDS, assigns 4 categories of risk for death or transformation to AML (Low, Int-1, Int-2, and High) based on a numeric score that reflects the percentage of bone marrow blasts, number of cytopenias, and presence or absence and type of chromosomal abnormalities.14 Cytogenetic risk groups are defined by the IPSS as good (normal, isolated −Y, del(5q), and del(20q)), poor (complex [≥ 3 abnormalities] and/or any chromosome 7 anomalies), and intermediate (all other abnormalities).14 Due to the growing awareness of shortcomings of established classification and prognostic scoring systems, and the increasing number of emerging therapeutic targets, a consensus statement was published recently revising standards for diagnostic criteria and prognostication.19,20

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successful cytogenetic analyses, 988 (48%) had no karyotype anomalies and 1084 (52%) had clonal cytogenetic abnormalities, with 605 (29%) having 1, 180 (9%) having 2, and 299 (14%) having complex (≥ 3) abnormalities. In the latter group, 65 patients (3%) had 3 abnormalities, 39 (2%) had 4 abnormalities, 31 (1%) had 5 abnormalities, 38 (2%) had 6 abnormalities, 27 (1%) had 7 abnormalities, 26 (1%) had 8 abnormalities, and 47 (2%) had more than 8 abnormalities. In 26 patients with complex karyotype, no ISCN code was available.

A systematic documentation of cytogenetic abnormalities (monosomies, trisomies, deletions of short or long arms, trisomies of short or long arms, additional material on short or long arms, translocations involving short or long arms, inversions, isochromosomes, dicentrics, or derivative chromosomes or chromosome arms) was performed for every patient. Furthermore, occurrences as isolated abnormalities, accompanied by 1 additional change, as noncomplex abnormalities (sole and +1 abnormality), and as part of complex abnormalities were recorded (Table S1, available on the Blood website; see the Supplemental Tables link at the top of the online article).

Karyotype abnormalities involving deletions of 5q were the most frequent, occurring in 30% of the 1080 patients with clonal cytogenetic abnormalities (15% of the 2072 patients with successful cytogenetic analyses; Figure 1). Other frequent anomalies were −7/del(7q) (21% of 1080 patients with chromosomal abnormalities), +8 (16%), −18/18q− (7%), 20q− (7%), −5 (6%), −Y (5%), −17/17p− (including isochromosome (17q)) (5%), +Mar (5%), +21 (4%), inv/t(3q) (4%), −13/13q− (4%), +1/1q (3%), −21 (3%), +11 (3%), 12p− (2%), t(5q) (2%), 11q− (2%), and t(7q) (2%). Isolated del(5q) was seen in 14% of patients with clonal abnormalities, del(5q) with 1 additional anomaly occurred in 5% of patients, and complex anomalies including del(5q) were seen in 11% of patients. The −7/del(7q) anomaly was seen in 8% of patients in isolation, in 3% of patients with 1 additional anomaly, and in 10% of patients as a complex karyotype. Trisomy 8 was seen in 8% of patients in isolation, in 3% of patients with an additional anomaly, and in 5% of patients as a complex anomaly. Loss of the Y chromosome as a sole anomaly was present in 4%, with 1 additional change in less than 1%, and as a complex abnormality in 2%. Isolated 20q− was seen in 3% of patients, 20q− with 1 additional anomaly was seen in less than 1% of patients, and complex anomalies involving 20q− were seen in 4% of patients. Partial or total monosomy 18 occurred nearly exclusively as part of

### Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, no.</td>
<td>2124</td>
</tr>
<tr>
<td>Median age, y (range)</td>
<td>65.7 (0.1-96.1)</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>1197/927</td>
</tr>
<tr>
<td>Type of MDS, no. (%)</td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>1981 (93.3)</td>
</tr>
<tr>
<td>Secondary</td>
<td>143 (6.7)</td>
</tr>
<tr>
<td>FAB classification (n = 2124), n (%)</td>
<td></td>
</tr>
<tr>
<td>RA</td>
<td>590 (27.8)</td>
</tr>
<tr>
<td>RARS</td>
<td>256 (12.1)</td>
</tr>
<tr>
<td>RAEB</td>
<td>425 (20.0)</td>
</tr>
<tr>
<td>RAEB-I</td>
<td>311 (14.6)</td>
</tr>
<tr>
<td>CMML</td>
<td>287 (13.5)</td>
</tr>
<tr>
<td>MDS-AL</td>
<td>132 (6.2)</td>
</tr>
<tr>
<td>Unknown</td>
<td>123 (5.8)</td>
</tr>
<tr>
<td>WHO classification (n = 598), no. (%)</td>
<td></td>
</tr>
<tr>
<td>5q− syndrome</td>
<td>61 (10.2)</td>
</tr>
<tr>
<td>RA</td>
<td>56 (9.4)</td>
</tr>
<tr>
<td>RARS</td>
<td>26 (4.3)</td>
</tr>
<tr>
<td>RCMD</td>
<td>165 (27.6)</td>
</tr>
<tr>
<td>RSCMD</td>
<td>77 (12.9)</td>
</tr>
<tr>
<td>RAEB-I</td>
<td>90 (15.1)</td>
</tr>
<tr>
<td>RAEB-II</td>
<td>123 (20.6)</td>
</tr>
<tr>
<td>Follow-up Patients with follow-up data, no. (%)</td>
<td>1841 (86.7)</td>
</tr>
<tr>
<td>Mean observation time, mo</td>
<td>29.2</td>
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<tr>
<td>Therapies, no. (%)</td>
<td></td>
</tr>
<tr>
<td>Supportive care only</td>
<td>1286 (60.5)</td>
</tr>
<tr>
<td>Chemotherapy (intensive and low-dose)</td>
<td>462 (21.8)</td>
</tr>
<tr>
<td>Amifostine</td>
<td>22 (1.0)</td>
</tr>
<tr>
<td>No therapeutic data</td>
<td>354 (16.7)</td>
</tr>
<tr>
<td>Cytogenetic overview</td>
<td></td>
</tr>
<tr>
<td>Mean no. of metaphases analyzed</td>
<td>21.9</td>
</tr>
<tr>
<td>Successful cytogenetic analyses, no. (%)</td>
<td>2072 (97.6)</td>
</tr>
<tr>
<td>Clonal cytogenetic abnormalities, no. (%)</td>
<td>1084 (52.3)</td>
</tr>
<tr>
<td>Mean number of aberrations per case</td>
<td>1.52</td>
</tr>
<tr>
<td>Clonal abnormalities in primary MDS, no. (%)</td>
<td>986 (49.8)</td>
</tr>
<tr>
<td>Clonal abnormalities in secondary MDS, no. (%)</td>
<td>96 (47.1)</td>
</tr>
<tr>
<td>IPSS cytogenetic risk group, no. (%)</td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>1217 (58.7)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>401 (19.4)</td>
</tr>
<tr>
<td>Poor</td>
<td>454 (21.9)</td>
</tr>
</tbody>
</table>

![Figure 1](image-url)

**Figure 1.** Frequencies of most common cytogenetic anomalies subdivided into isolated, with 1 additional anomaly, and complex anomalies.
Adequate cytogenetic examination in myelodysplastic syndromes: analysis of 529 patients

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Abstract

In a retrospective study, we examined karyotypes of bone marrow specimens of an oligocentric cohort comprising 529 patients with MDS to address the question how many metaphases need to be analyzed to detect even small cell clones with an appropriate expenditure. We found a statistically significant difference of the frequency of normal karyotypes in the patient group with 19 or less analyzed metaphases compared to the group with 20 or more metaphases analyzed (56% versus 47%, \( p = 0.041 \)). Furthermore, we demonstrate that the analysis of 25 or more metaphases can further improve the sensitivity of karyotype analysis and leads to the identification of additional clinically relevant abnormal clones or subclones in a substantial proportion of patients. In summary, our data suggest the examination of at least 20 metaphases in MDS.

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Keywords: MDS; Cytogenetics; Sensitivity; Metaphase; Diagnostic accuracy; Prognosis

1. Introduction

Myelodysplastic syndromes (MDS) represent a heterogeneous group of clonal diseases affecting the stem cell compartment in bone marrow [1,2]. The stem cell defect leads to an ineffective and dysplastic hematopoiesis resulting in anemia, leukocytopenia and/or thrombocytopenia. Current pathogenetic models consider initiation and progression of MDS to be a multi-step process associated with accumulation of genetic alterations [3,4]. Normal hematopoietic cells can be transformed into leukemia by the acquisition of genetic alterations leading to abnormal clones [5]. One-third of patients with MDS transform into secondary acute leukemia characterized by a poor prognosis with rapid expansion of the aberrant clone [6,7]. Expanding but not yet fully infiltrating neoplastic clones result in mosaic karyotypes and subclones, which are frequently found in bone marrow specimens of patients with MDS or early after transformation into AML, reflecting the juxtaposition of cytogenetically distinct cells [8].

The diagnosis of myelodysplastic syndromes is mainly based on cytomorphology or histomorphology and cytogenetic analysis. For risk-adapted treatment ranging from best supportive care to AML-like intensive chemotherapy
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order to demonstrate the shape of the curve for small clone sizes, in Fig. 2 the assumed relative clone size was 20%, which was smaller than the average in our cohort.

In our opinion, the additional expenses for analysis of more metaphases to reach a standard of at least 20–25 MPs is appropriate concerning the clinical impact. To increase the number of MPs, in many cases culturing and metaphase preparation again is not necessary, since in most laboratories reserve slides or cell suspension in fixative are available for supplementary metaphase capturing and examination. Provided that the laboratories have an automated metaphase finding and capturing system at their disposal, the extra expenses are restricted to the pure time of analyzing the additional MPs. In contrast, for supplementary FISH examinations, probe costs have to be considered besides the extra personal resources. As proposed above, FISH should be performed only in selected cases.

In summary, our study provides a quantitative basis for recommendations on how many metaphases need to be analyzed in MDS, confirming the current guidelines of most international committees to completely analyze at least 20 metaphases. The present data suggest that examination of 25 or more metaphases could further increase the sensitivity of cytogenetic analyses with clinical impact in individual cases by identifying additional abnormal clones or subclones. If less than 20 MPs are examined, we suggest to indicate the restricted sensitivity of the investigation in these cases. Based on our data, an adjustment of the IPSS recommendations on how many metaphases need to be analyzed in MDS, confirming the current guidelines of most international committees to completely analyze at least 20 metaphases. The present data suggest that examination of 25 or more metaphases could further increase the sensitivity of cytogenetic analyses with clinical impact in individual cases by identifying additional abnormal clones or subclones. If less than 20 MPs are examined, we suggest to indicate the restricted sensitivity of the investigation in these cases. Based on our data, an adjustment of the IPSS recommendations concerning the minimal number of completely analyzed metaphases (to more than 20) should be considered, at least in cases with normal karyotypes.

Acknowledgement

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References

Abstract
Cytogenetic abnormalities are seen in approximately 50% of cases of myelodysplastic syndrome (MDS) and 80% of cases of secondary MDS (following chemotherapy or radiotherapy). These abnormalities generally consist of partial or complete chromosome deletion or addition (del5q, –7, +8, –Y, del20q), whereas balanced or unbalanced translocations are rarely found in MDS. Fluorescence hybridization techniques (fluorescence in situ hybridization [FISH], multiplex FISH, and spectral karyotyping) are useful in detecting chromosomal anomalies in cases in which few mitoses are obtained or rearrangements are complex. Ras mutations are the molecular abnormalities most frequently found in MDS, followed by p15 gene hypermethylation, FLT3 duplications, and p53 mutations, but none of these abnormalities are specific for MDS. The rare cases of balanced translocations in MDS have allowed the identification of genes whose rearrangements appear to play a role in the pathogenesis of some cases of MDS. These genes include MDS1-EVI1 in t(3;3) or t(3;21) translocations, TEL in t(5;12), HIP1 in t(5;7), MLF1 in t(3;5), and MEL1 in t(1;3). Genes more frequently implicated in the pathogenesis of MDS cases, such as those involving del5q, remain unknown, although some candidate genes are currently being studied. Cytogenetic and known molecular abnormalities generally carry a poor prognosis in MDS and can be incorporated into prognostic scoring systems such as the International Prognostic Scoring System. Int J Hematol. 2001;73:429-437.

Key words: Myelodysplastic syndromes; Chromosomes; Gene rearrangements

1. Introduction
Myelodysplastic syndromes (MDS) are clonal stem cell disorders characterized by ineffective hematopoiesis and a high risk of progression to acute myeloid leukemia (AML) [1]. The etiology of MDS is generally unknown, but some cases of MDS (secondary MDS) can occur after the use of antineoplastic agents (mainly alkylating agents) or after exposure to benzene derivatives [2-4].

Cytogenetic abnormalities can be found in approximately one half of the cases of MDS by using conventional banding techniques, and these abnormalities carry strong prognostic value [5-13]. Other cytogenetic techniques, including fluorescence in situ hybridization (FISH), multiplex FISH (M-FISH), and spectral karyotyping (SKY) can give further information on chromosomal rearrangements [14-17].

Due largely to the rare occurrence of balanced translocations in MDS (as opposed to AML), specific gene abnormalities playing a role in the myelodysplastic process have been difficult to identify in myelodysplastic disorders. Some gene rearrangements that can be observed in other myeloid malignancies and are therefore not specific to MDS, are, however, observed in a substantial percentage of MDS cases.

2. Cytogenetic Abnormalities
2.1. Chromosomal Abnormalities Observed in MDS by Using Conventional Cytogenetic Analysis

The primary cytogenetic abnormalities observed in MDS are shown in Table 1 [5-13]. The incidence of abnormal karyotypes is higher (80%) in secondary MDS. The most frequent rearrangements are partial and complete chromosome loss (especially del5q, –7, –Y, and del20q) or chromosome gain (most frequently +8), whereas balanced translocations are rare. Unbalanced chromosomal translocations are somewhat more frequent, leading to partial monosomy or trisomy [ie, 17p monosomy in t(5;17) or 7q monosomy in t(1;7)]. None of
1. Introduction

Myelodysplastic syndromes (MDS) are clonal stem cell disorders characterized by ineffective hematopoiesis and a high risk of progression to acute myeloid leukemia (AML) [1]. The etiology of MDS is generally unknown, but some cases of MDS (secondary MDS) can occur after the use of antineoplastic agents (mainly alkylating agents) or after exposure to benzene derivatives [2-4].

Cytogenetic abnormalities can be found in approximately 50% of cases of myelodysplastic syndrome (MDS) and 80% of cases of secondary MDS (following chemotherapy or radiotherapy). These abnormalities generally consist of partial or complete chromosome deletion or addition (del5q, –7, +8, –Y, del20q), whereas balanced or unbalanced translocations are rarely found in MDS. Fluorescence hybridization techniques (fluorescence in situ hybridization [FISH], multiplex FISH, and spectral karyotyping) are useful in detecting chromosomal anomalies in cases in which few mitoses are obtained or rearrangements are complex. Ras mutations are the molecular abnormalities most frequently found in MDS, followed by p15 gene hypermethylation, FLT3 duplications, and p53 mutations, but none of these abnormalities are specific for MDS. The rare cases of balanced translocations in MDS have allowed the identification of genes whose rearrangements appear to play a role in the pathogenesis of some cases of MDS. These genes include MDS1-EVI1 in t(3;3) or t(3;21) translocations, TEL in t(5;12), HIP1 in t(5;7), MLF1 in t(3;5), and MEL1 in t(1;3). Genes more frequently implicated in the pathogenesis of MDS cases, such as those involving del5q, remain unknown, although some candidate genes are currently being studied. Cytogenetic and known molecular abnormalities generally carry a poor prognosis in MDS and can be incorporated into prognostic scoring systems such as the International Prognostic Scoring System. Int J Hematol. 2001;73:429-437.

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Key words: Myelodysplastic syndromes; Chromosomes; Gene rearrangements
Tetraploidy and 5q deletion in myelodysplastic syndrome: A case report

Iya Znoyko, Robert K. Stuart, Tara Ellingham, Jennifer Winters, Daynna J. Wolff, Denise I. Quigley

Abstract

Tetraploidy is a very rare cytogenetic abnormality in myelocytic malignancies, and its significance is unclear to date. We report here on a 68-year-old male diagnosed with myelodysplastic syndrome/refractory anemia with excess blasts (MDS/RAEB). Cytogenetic analysis of his bone marrow biopsy at initial clinical presentation and in subsequent studies revealed the presence of two abnormal clones, 92,XXYY and 92,XXYY,del(5)(q13q33). Interphase fluorescence in situ hybridization analysis of abnormal cells confirmed interstitial deletion in 5q, demonstrated predominance of the tetraploid clone and persistent presence of the tetraploid clone with 5q deletion. The patient was not responsive to Revlimid (lenalidomide) treatment, which is routinely used in patients with 5q syndrome. However, a subsequent course of therapy with the methyl-transferase inhibitor decitabine resulted in clinical and cytogenetic remission. Our data suggest that the unique complex abnormality of tetraploidy and 5q deletion described here for the first time in MDS is characterized by distinct disease etiology, the mechanism of which could involve epigenetic inactivation of gene expression via methylation.

1. Introduction

Myelodysplastic syndromes (MDS) are a group of hematopoietic stem cell disorders characterized by ineffective hematopoiesis and peripheral blood cytopenias [1]. They are categorized into high-risk (> 10% marrow blasts) and low-risk (< 10%) groups [1]. High-risk MDS bears considerable resemblance to acute myeloid leukemia (AML) and is more likely to evolve into AML [1]. Approximately 50–60% of patients with de novo MDS and more than 85% of individuals with secondary MDS (after cytotoxic therapy) show nonrandom chromosomal aberrations that may involve isolated or multiple abnormalities [2]. Therefore, analysis of recurrent cytogenetic abnormalities in MDS is widely used for diagnosis and for determining prognosis and management.

Deletion of the long-arm of chromosome 5 [del(5q)] is the most common chromosomal abnormality in MDS, occurring at a frequency of 10–15% [3–4]. Del(5q) also occurs in AML [4] and several other cancers [5–7]. While the deleted region of chromosome 5 can vary greatly in size between individual patients, two regions have been identified that correspond to subtypes of MDS. A 4-megabase interval within 5q31 has been defined for patients with more aggressive MDS, including secondary MDS, while a more telomeric region is deleted in the patients with del(5q) syndrome [8]. The commonly deleted region (CDR) or critical region has been defined as 5q31–q33 [8,9] and contains multiple genes involved in cellular growth, hematopoiesis, cell cycle control, cell adhesion, and tumor suppression [2,10,11].

Massive hyperdiploidy (>50 chromosomes) and tetraploidy (4n) are rare cytogenetic abnormalities in myelocytic malignancies. A handful of MDS cases with massive hyperdiploidy have been published to date [12–18], whereas there is only one report, to the best of our knowledge, of two MDS patients with tetraploidy [15]. The combination of tetraploidy and del(5q) has not been reported previously in MDS. Hyperdiploidy has been associated with poor prognosis in MDS [12,14–16], but in light of the small numbers of reported cases, its significance is unclear.

In this report, we describe the clinical, cytogenetic, and molecular cytogenetic findings in a case of high-risk MDS with the unique finding of tetraploidy and 5q deletion.

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Short communication

Tetraploidy and 5q deletion in myelodysplastic syndrome:
A case report

Iya Znoyko\textsuperscript{a}, Robert K. Stuart\textsuperscript{b}, Tara Ellingham\textsuperscript{a}, Jennifer Winters\textsuperscript{c}, Daynna J. Wolff\textsuperscript{a}, Denise I. Quigley\textsuperscript{a,}\textsuperscript{*}

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In this report, we describe the clinical, cytogenetic, and molecular cytogenetic findings in a case of high-risk MDS with the unique finding of tetraploidy and 5q deletion.
2. Materials and methods

2.1. Case report

The patient was a 68-year-old generally healthy man referred by his local primary care physician due to decreased hemoglobin and white cell count. Analysis of his blood and bone marrow aspirate demonstrated macrocytic anemia, severe neutropenia, megaloblastic changes in the red cell line, and occasional uninuclear morphology of megakaryocytes. Excess of blasts evaluated by differential cell count and flow cytometry with myeloid markers was 13 and 19%, respectively. Though the patient demonstrated clinical features of the 5q syndrome (macrocytic anemia, normal to elevated platelet count, and neutropenia), he did not respond to treatment with Revlimid (lenalidomide), which was administered daily for 12 weeks. Moreover, during this period, his platelet count decreased from 350,000 to 118,000, and the patient developed AML, with a repeat bone marrow examination showing 35% myeloblasts marking for myelomonocytic lineage. However, subsequent treatment with two cycles of decitabine therapy resulted in marked improvement in the patient’s condition, with resolution of severe neutropenia and decrease in blast count to 2 and 3% (estimated by differential cell count and flow cytometry, respectively). Finally, the patient achieved clinical and cytogenetic remission.

2.2. Cytogenetics and fluorescence in situ hybridization (FISH) analysis

Bone marrow was cultured in RPMI 1640 media (Invitrogen, Carlsbad, CA) and Chang BMC media (Irvine Scientific, Santa Ana, CA), harvested, and slides were prepared according to standard laboratory methods. Metaphase cells were imaged and karyotypes were generated using the Cytovision System version 3.6 (Applied Imaging, Santa Clara, CA). Cytogenetic abnormalities were described according to the International System of Human Cytogenetic Nomenclature (ISCN) 2005[19].

FISH analysis for del(5q) was performed using probes EGR1 (5q31), CSF1R (5q33~q34), and control probes D5S23 and D5S721 (5p15.2) on slides prepared from fixed cell pellets according to the manufacturer’s recommended procedure (Abbott Molecular/Vysis, Des Plains, IL).

3. Results

Cytogenetic analysis of the bone marrow aspirate revealed the presence of cells with normal karyotypes as well as two abnormal cell lines: a tetraploid clone and a clone with tetraploidy and del(5q) (Fig. 1; Table 1). It is noteworthy that the abnormal mitotic cell population at presentation and subsequent cytogenetic tests at 1 and 3 months were dominated by the tetraploid cell line with 5q deletion. However, at the 5-month follow-up (when AML was diagnosed), while the tetraploid/del (5q) clone was persistent, the majority of abnormal dividing cells were represented by the tetraploid clone without 5q deletion (Table 1). Later, the patient was started on decitabine therapy, and by the next cytogenetic evaluation at 10 months, no abnormal cells were observed by routine cytogenetic analysis; the patient had reached cytogenetic remission (Table 1).

To detect the presence of residual disease, we performed interphase FISH. We used two probes specific for the 5q CDR, namely CSF1R and EGR1 (see Materials and methods). Both probes have shown similar results (data not shown) and proved to be equally suitable for the identification of the 5q deletion in our case. According to the FISH results, tumor burden at 1 month was 12.4%, and it increased twofold by the fifth month (Table 2), when the patient evolved to AML (see Materials and methods). However, after decitabine treatment, at the 10-month follow-up, the number of abnormal cells had fallen to 3.5%. This value correlates well with the number of blasts measured by differential cell count and flow cytometry (2 and 3%, respectively, see Materials and methods). Interestingly, during the whole observation period, despite of the overall number of abnormal cells, the ratio between the two clones (tetraploid and tetraploid with 5q deletion) remained the same, 2:1 (Table 2).

4. Discussion

Chromosomal abnormalities in neoplastic marrow cells often correlate closely with specific clinical and biologic characteristics of the disease and serve as a tool to predict the clinical outcome and develop effective therapeutic approaches. In this paper, we describe the successful treatment of a myelodysplastic syndrome-refractory anemia with excess blasts patient with the unique finding of tetraploidy and 5q deletion.

Tetraploidy is a very rare abnormality in hematologic malignancies, especially in MDS. In fact, we are aware

![Figure 1: Representative chromosomes 5 showing tetraploidy and deletion of one 5q (arrow).](image-url)
clinical remission (CR) rate of 9% [29]. Fortunately, our patient has shown remarkable response to treatment with decitabine and eventually achieved clinical and cytogenetic remission.

The effect of decitabine, an S-phase—specific inhibitor of DNA methyltransferase, relies on its ability to decrease levels of methylation, which is responsible for abnormal silencing of many genes, including tumor suppressor genes in neoplasia. Therefore, a possible mechanism of disease underlying the observed cytogenetic abnormalities, tetrariploidy and 5q deletion, may involve epigenetic inactivation of gene expression via methylation. This finding is of particular interest in light of the recently published data involving the model of alpha-catenin therapy [11]. The study demonstrated that silencing of key tumor-suppressor genes within the 5q CDR results from the loss of one of the alleles via deletion and epigenetic inactivation of the remaining allele. In vitro experiments indicate that inactivation of the remaining allele is caused by promoter methylation and histone deacetylation and can be reversed by using inhibitors of methyltransferase and histone deacetylase [11]. These data suggest that combinational treatment with methyltransferase inhibitors (such as decitabine or azacitidine) and histone deacetylase inhibitors (such as valproic or suberoylanilide hydroxamic acid), which can be reversed by using inhibitors of methyltransferase and histone deacetylase [11].

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References
Abstract Myelodysplastic syndrome (MDS) involves myeloid cells of the bone marrow, which is important in progressive bone marrow insufficiency. Of all MDS patients, 40%–50% have at least one chromosomal rearrangement. Loss of specific chromosomal regions like 5q- and 7q- are usually the secondary cytogenetic abnormalities associated with MDS. In order to detect chromosome abnormalities associated with MDS, bone marrow samples from 26 patients diagnosed as MDS were obtained prior to chemotherapy. Both conventional cytogenetic analyses and fluorescence in situ hybridisation (FISH) methods were performed and locus-specific probes for 5q and 7q were used. Results obtained were compared. Twenty-one patients had normal karyotypes and four patients had abnormal karyotypes, while in one patient we could not obtain metaphases from cultures. Three patients with normal karyotypes revealed del (5q), two patients had del (7q) and one patient had monosomy (7). A total of 10 of 26 patients had chromosome changes visualised by either conventional or molecular cytogenetics (~38.5%). Our results show that both methods are important in diagnosis and follow up of MDS patients. When used together, conventional cytogenetics and FISH detect clinically significant chromosome abnormalities in MDS patients.

Key words Myelodysplastic syndrome • Cytogenetics • FISH • 5q, 7q deletion

Introduction

Myelodysplastic syndromes (MDS) are clonal haematopoietic stem cell disorders, which are characterised by ineffective haematopoesis, peripheral cytopenias and substantial risk of acute myeloid leukaemia (AML) development.

MDS usually occurs in the 6th and 7th decades of life and disease progression begins as benign anaemia that changes form to AML. Cytogenetic changes have been reported to play an important role in MDS pathogenesis and progression to AML.

Deletions in specific chromosomal regions such as 5q-, 7q-, 12p-, +8 and point mutations or hypermethylation of proto-oncogenes and tumour suppressor genes are responsible for disease progression [1].

Cytogenetic results have a critical role in correct diagnosis and identification of prognostic subgroups of MDS. In patients with bone marrow hypoplasia, cytogenetics is important in differential diagnosis between aplastic anaemia and MDS [1].

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MDS patients, comparison of conventional cytogenetic analysis and fluorescence in situ hybridisation (FISH) has been investigated. FISH has been reported as an effective and rapid diagnostic test in MDS, although interpretation of results may be complex. Masked or occult abnormalities have been found to be detected by FISH, but not by conventional cytogenetic analysis [2, 3].

In this study, we compared conventional cytogenetic and FISH analysis results of MDS patients in order to detect the difference between these two methods.

Materials and methods

Twenty-six patients, newly diagnosed as MDS, were included in the study. Bone marrow specimens were obtained and direct, overnight, 48- and 72-h cultures were set up in RPMI 1640 culture medium as described [4]. Conventional cytogenetic analysis was performed on Trypsin-Giemsa banded chromosomes under light microscopy. The resolution was at the 200–400 band level. Two separate investigators analysed a total of at least 20 cells in order to detect chromosome abnormalities. Cases with two or more metaphases involving the same chromosome trisomy or structural abnormality and cases with three or more metaphases involving the same chromosome monosomy were considered to have clonal abnormalities [5].

FISH analyses were performed on fixed cells obtained from cultures with Vysis probes 5q31/CEP5, 7q31/CEP 7. Slides were prepared, treated, hybridised and analysed according to the manufacturer’s instructions. At least 200 nuclei or metaphase spreads were analysed for each probe by two separate investigators. 5q and 7q regions were analysed by dual colour FISH probe strategy (Table 1). Nuclei that were overlapping or in which the number of signals was ambiguous were not scored. Cut-off values for each probe were calculated in peripheral blood samples of 10 healthy volunteers by adding three times the standard deviation to the mean percentage of abnormal cells analysed. For the 5q probe, the cut-off value was calculated as 2.46% for the 7q probe 2.96%.

Results

Bone marrow samples from 26 patients were analysed by both chromosome analysis and FISH studies. In one patient we observed 46, XY, del (20)(q11–qter) karyotype (Patient 2, Table 2). In another patient (Patient 3, Table 2) we observed a mosaic karyotype with a marker chromosome. Conventional cytogenetic analysis from another patient revealed a complex karyotype (Patient 20, Table 2). We observed hypodiploidy as the sole abnormality in metaphases of one patient and could not obtain metaphases suitable for analysis from cultures in one patient. Twenty-one patients had normal karyotypes.

FISH results revealed deletion of 5q31 in three patients who had normal karyotypes. Two patients had 7q31 deletion detected by FISH although they had normal karyotypes and another patient with normal karyotype had monosomy 7. FISH findings occurred in various ratios of cells, which are also shown in Table 2.

According to the cytogenetic risk status, 23 patients have been classified in the good, 1 in the intermediate and 2 in the poor risk group. After interphase FISH, three patients in the good risk group in the cytogenetic risk status, patients 5, 14 and 23 in Table 2, were determined to be in the poor risk status in the FISH group. On the contrary, patient 3 had a marker chromosome detected in his karyotype and was classified in the intermediate group by the cytogenetic risk status. According to FISH, the same patient did not have a 5q or 7q deletion and was classified in the good risk group. The disease seems to be stable during the follow-up period. Patient 20 had a complex karyotype and a poor risk cytogenetically. The karyotype did not reveal chromosome 5 and 7 abnormalities; nor did the FISH results. This patient was classified in the good risk group by FISH analysis. Patient 14 had a normal karyotype by cytogenetic analysis and a 7q deletion by FISH analysis. The patient was classified in the good risk group by cytogenetic analysis and poor risk group by FISH analysis.

Discussion

Karyotype, percentage of bone marrow myeloblasts and number of cytopenias have been identified as important prognostic variables in MDS by the International Prognostic Scoring System (IPSS). According to this classification, there are three cytogenetic categories. Normal karyotype; loss of Y, del (5q) or del (20q) as sole abnormality

Table 1 Probes used during the study and hybridisation results of the patients

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High risk MDS has been defined as having structural abnormalities or >1 abnormal clone [7]. Loss of chromosome 7 has been reported in a variety of haematological disorders both as the sole chromosomal abnormality and as a secondary finding associated with other abnormalities. Loss or deletion of chromosome 7 is particularly noteworthy in MDS where, if detected, the prognosis for development of acute leukaemia has been reported to be especially high [13]. Eight of our patients belong to this group. Intermediate risk MDS was defined as having any other abnormalities such as trisomy 8 [6].

At primary diagnosis, 40%–60% of MDS patients present with normal karyotypes. In our study, this ratio was 80.5%, which seemed higher than the previous reports. Patients with MDS and a normal karyotype are a heterogeneous group and their prognosis has been reported to be unpredictable [7].

In the current study, we aimed to compare conventional cytogenetic analysis results with FISH. The combination of cytogenetic and FISH analysis has been reported to bring together the screening potential of cytogenetics with the accuracy of a molecular genetic technique [8].

Similar studies have been reported before in which different percentages have been reported for patients with occult deletions, monosomies or trisomies [2, 9]. In our study we detected deletion of 5q31 region in 3, deletion of 7q31 region in 2 and monosomy 7 in one patient, all with normal karyotypes. FISH abnormalities were more frequently observed among patients with an increased percentage of bone marrow blasts in a study reported by Rigolin et al. [10]. They suggested that cryptic deletions beyond the resolution power of conventional band analysis might not be detected and result in a normal karyotype, however FISH detects the minor clone with the submicroscopic deletion. This may explain our percentage of deletions. In patients 5 and 14 (Table 2) we observed 7q deletions in 5% and 8% of cells. In patient 23 we observed monosomy 7 in 20% of cells. Also, 5q deletions were observed in patients 8, 12 and 15 in 5%, 90% and 5% of cells respectively. All patients had normal karyotypes. Interphase FISH may be an additional tool in MDS to categorise patients. As reported previously, interphase FISH is a very useful method to display the chromosome abnormalities that are associated with unfavourable prognosis at an early stage of MDS [9–12].

As a molecular cytogenetic technique, FISH allows identification of specific regions of DNA in a metaphase or in an interphase cell [13]. There are several advantages of using FISH as a diagnostic tool in haematological malignancies such as detection of cryptic deletions, follow up of patients and detection of minimal residual disease. However, factors like cell type, level of detection, type and stage of disease, chromosome heteromorphism, structural rearrangement and hybridisation efficiency can make interpretation of FISH results complex [3]. When all these factors are regarded together, FISH has both advantages and complexities in detection of chromosomal rearrangements.

Conventional cytogenetics has its own importance in bone marrow diseases [14]. Solé et al. [15] reported that IPSS cytogenetic prognostic groups, bone marrow blast ratios and haemoglobin levels are the main prognostic factors for survival, and cytogenetic findings, blast ratios and platelet counts are important for leukaemic transformation risk. In this regard, FISH is the method to confirm and reveal details of cytogenetic results. Our results indicate that, when used together, conventional cytogenetics and FISH detect clinically significant chromosome abnormalities in MDS patients.

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References

Clinical Relevance of Cytogenetics in Myelodysplastic Syndromes

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ABSTRACT: Myelodysplastic syndromes (MDS) are a group of heterogeneous stem cell disorders with different clinical behaviors and outcomes. Conventional cytogenetics (CC) studies have demonstrated that the majority of MDS patients harbor clonal chromosome defects. The probability of discovering a chromosomal abnormality has been increased by fluorescence in situ hybridization (FISH), which has revealed that about 15% of patients with a normal chromosome pattern on CC may instead present cryptic defects. Cytogenetic abnormalities, except for the interstitial long-arm deletion of chromosome 5 (5q−), are not specific for any French-American-British (FAB)/World Health Organization (WHO) MDS subtypes, demonstrate the clonality of the disease, and identify peculiar morphological entities, thus confirming clinical diagnosis. In addition, chromosome abnormalities are independent prognostic factors predicting overall survival and the likelihood of progression in acute myeloid leukemia.

KEYWORDS: myelodysplastic syndromes; FAB subtype; chromosomal abnormalities; fluorescence in situ hybridization

INTRODUCTION

Myelodysplastic syndromes (MDS) are clonal stem cell disorders most frequently affecting the elderly. They are characterized by a hypercellular marrow that exhibits a defective maturation of all marrow cell lineages, determining ineffective hemopoiesis and peripheral blood cytopenia. Apart from constitutional conditions (such as Fanconi’s anemia and other congenital dyserythropoietic anemias), these oncohematological disorders are distinguished in de novo MDS, which arises without exposure to any well-known carcinogenic
hypolobulations, small granules, and vacuoles in the cytoplasm. From a clinical point of view the deletion causes a poor response to chemotherapy and a short survival.

**Loss of the Y Chromosome**

The clinical significance of this cytogenetic defect is still undefined and the defect seems to be determined by the ageing process. In fact, –Y is not only discovered in 10% of MDS patients, but also in 7% of the elderly people without any haematological disorder. So, the IPSS has established that an MDS diagnosis cannot be based on the presence of –Y alone; instead, when biological and clinical parameters point to an MDS diagnosis, the loss of the Y chromosome identifies patients with a favorable clinical outcome. Despite these data, elderly people with a high percentage of –Y marrow cells are at risk of developing a hematological disorder and in MDS patients the abnormality is surely clonal because it is present at the onset of the disease and disappears upon achievement of complete remission.

**Trisomy 8**

This chromosomal abnormality is not specific to MDS because it can be discovered in other oncohematological disorders. The incidence of trisomy 8 varies between 5% and 20%; it occurs in 19% of chromosomally abnormal patients and in 10% of all MDS patients. It can be hypothesized that in half of the patients such a trisomy would be a secondary event, appearing during the clinical course. However, it is worth noting that the percentage of +8 cells varies during the follow-up independently of response to chemotherapy. This condition has been clarified by FISH, which has documented that some patients may present a constitutional mosaicism. In other patients +8 is surely an acquired clonal abnormality and FISH has demonstrated its presence in CD33+, CD34+ hemopoietic precursors.

The incidence of trisomy 8 varies among the different MDS subtypes. It is more often discovered in RARS and in RAEB. Some patients who harbor two cell populations, one marked by trisomy 8 and the other by tetrasomy 8, disease frequently evolves to AML and they develop extramedullary disease, often involving the skin. From a prognostic point of view +8 is associated with an intermediate outcome.

**20q Deletion**

20q deletion is a recurrent chromosomal abnormality observed in myeloproliferative disorders. It is discovered in 5% of patients with de novo MDS and
Cytogenetic aspects of adult primary myelodysplastic syndromes: Clinical implications

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Abstract

Myelodysplastic syndrome (MDS) is a heterogeneous disease from the clinical, biological and morphological point of view. The pathogenesis of MDS is not well established and it appears to occur complex changes in the stem cell biology. Clonal chromosomal aberrations are found in 30–50% of primary MDS and no specific cytogenetic abnormality has as yet been defined. The chromosomal abnormalities are predominantly characterized by partial/total chromosomal losses or chromosomal gains. These chromosomal abnormalities include mainly -5/del(5q), -7/del(7q), del(11q), del(12p), del(20q), -Y, and +8. The role of cytogenetic analysis in the diagnosis, prognosis, taking treatment decisions and follow up of patients with MDS has been clearly defined. Despite its difficulties in obtaining for analysis high quality metaphases conventional cytogenetics continues to be the basic technique for cytogenetic evaluation of a MDS patient. Other molecular cytogenetic methods have been shown to be complementary, without replacing the information obtained with this technique. Further investigations with both conventional and molecular cytogenetics in relation to clinical features as well as other molecular methods will undoubtedly contribute to improve understanding of the underlying genetic events responsible for the development and evolution of MDS leading to more accurate classification and management of MDS patients.

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Keywords: Myelodysplastic syndromes; Chromosomal abnormalities; Clinical implications; Conventional cytogenetics; Molecular cytogenetics

1. Introduction

Myelodysplastic syndrome (MDS) is a clonal disorder of the pluripotent hematopoietic stem cells characterized by ineffective and dysplastic hematopoiesis. Patients with MDS have peripheral blood cytopenia, a hypercellular bone marrow and an increased risk (30%) to evolve to acute leukemia. They have generally a short overall survival, death being usually due to the progression to acute leukemia or to consequences of cytopenias. MDS appear to be the most common hematological malignancy to affect the elderly with a median age of 60–75 years at diagnosis. MDS constitutes a heterogeneous group with a wide variability in prognosis survival ranging
The 5q-syndrome is well established among patients with MDS and it includes patients with primary MDS RA-FAB subtype, who have del(5q) as the sole karyotypic abnormality. Patients with the 5q-anomaly have been presented as a separate entity in the new WHO classification of hematological malignancies. 5q-syndrome has a striking female predominance occurring in the elderly with median age of 60 years old. Patients with this syndrome have a macrocytic refractory anemia with monolobulated small megakaryocytes, less than 5% marrow myeloblasts and a very good prognosis [44,45,74–76].

Deletion of 5q is interstitial in the majority of cases. Three types of interstitial deletions have been identified: del(5)(q12–13q31–33), del(5)(q12q23) and del(5)(q23q32). Although the interstitial deletion limits vary among patients the del(5q13q33) constitutes a major subset [4,5,9]. The long arm of chromosome 5 has a particular interest because it encodes many hematopoietic growth factors and growth factors receptors. Among the most important genes mapped on chromosome 5q13–q33 are interleukin-4 (IL-4), IL-5, interferon regulator factor 1 (IRF1), granulocyte-macrophage colony stimulating factor, IL-9, IL-3, early growth response 1 (EGR-1). However, the exact role of any of those genes in the pathogenesis of MDS has not been well established. A critical region of consistent loss at 5q31 (in > 90% of the cases) has led the investigators to look for the presence of a key negative regulator of leukemogenesis. However, molecular studies have not definitively identified critical suppressor genes in 5q-associated MDS [77–83]. Deletions and translocations of 5q13 have also been reported and it was suggested that this region may be of particular interest as it may encode a critical gene for leukemogenesis [84]. Patients with del(5q) and additional aberrations have a poor prognosis with early progression to leukemia, resistance to treatment and a short survival. Notably, many patients with de novo MDS and −5/del(5q) have a significant occupational exposure to potentially carcinogens, suggesting that abnormalities of chromosome 5 may be a marker of mutagen-induced MDS. In the MDS with 5q- within complex karyotypes chromosomal changes include mainly deletions involving 3p, 7, 12p and 17p. Mutation of the p53 gene are also frequent in this cytogenetic subgroup [2,5,9,46,83]. Crescenzi et al. [83] studied 30 patients with MDS or AML with the karyotypic anomaly 5q-. They applied FISH technique using a panel of 11 probes for known suppressor genes or loci deleted in MDS/AML and they investigated whether putative suppressor sequences other than those at 5q are lost in myeloid malignancies associated with the 5q-anomaly. FISH data strongly supported a mutator phenotype underlying complex karyotype with a 5q-deletion. The authors concluded that their results add strong evidence that MDS/AML with only 5q- and those with 5q- in complex karyotypes are the products of different genomic backgrounds and possibly of different micro-environmental influences.

Chromosome 7 abnormalities. Monosomy 7 is a frequent finding in MDS patients. Patients with monosomy 7 have a very aggressive disease with short survival time. Del(7q) is also a common finding in MDS and it is most frequently seen in association with other chromosomal changes such as 5q-. Most of the patients with del(7q) show two major breakpoints, at 7q11–q22 and at 7q31–q36. Several genes have been mapped on chromosome 7q, such as T-cell receptor β (TCR β), erythropoietin (EPO), asparagines synthase gene (ASNS). The deleted segment of 7q may encode a tumor suppressor gene implicated in the pathogenesis of MDS. Patients with −7/7q-associated with additional changes have a high risk of evolution to leukemia. As with −5/del(5q) occupational or environmental exposure to mutagens has been associated with −7/del(7q). Notably, −7/del(7q) is the most frequent karyotypic abnormality detected in patients with constitutional predisposition to myeloid malignancies, including Fanconi anemia and neurofibromatosis type 1. An entity designated ‘monosomy 7 syndrome’ has been described in young children. This syndrome is characterized by refractory anemia, leucocytosis, thrombocytopenia and common evolution to AML [5,6,46,85–87].

Del(20q) can occur as the sole anomaly or in association with other changes and it is seen in about 5% of MDS. Del(20q) is usually interstitial with the most common deleted region being between 20q11 and 20q13. The International MDS Risk analysis Workshop found that patients with a del(20q) observed in association with a complex karyotype had a poorer prognosis, whereas the prognosis for patients with an isolated del(20q) was favorable.
However, a few studies reported that this anomaly is associated with a poor prognosis [5, 46, 56, 88, 89].

Deletion or unbalanced translocations involving 12p13 region, in which the TEL gene is located, have been found in about 10% of patients with CMMoL and in about 5% of patients with RAEB and RAEB-\textsuperscript{t}. TEL gene is a negative regulator of transcription and is likely a candidate tumor suppressor gene. On the 12p13 the KIP1 gene is also located, which encodes the p27 protein, a cyclin-dependent kinase inhibitor, playing a role in inhibiting cell proliferation. Loss of p27 function has been shown in cancer progression [90–92]. Patients with del(12p) as the sole anomaly according to the IPSS are in the intermediate-risk cytogenetic subgroup [46]. However, Sole et al. [49] reported that patients with del(12p) seem to have a better prognosis than patients in the IPSS intermediate-risk group.

Loss of Y occurs in MDS in about 10% of cases, while it can also be seen as the sole cytogenetic abnormality in the bone marrow of healthy old men in about 7–8% [93, 94]. The clinical and biological significance of loss of Y in MDS is not well known. Wictor et al. [94] reported that patients with a hematological disease have a significantly higher percentage of cells with $\neg$Y anomaly. They also noted a neutral or favorable prognosis for an isolated $\neg$Y. Patients with loss of Y as the sole anomaly according to the IPSS are of good prognosis [46].

Loss of 17p. Abnormalities resulting in loss of 17p material have been reported in up to 4% of MDS patients. These include simple deletions, i(17q) or unbalanced translocations. Del(17p) is frequently associated with additional changes involving mainly chromosomes 5 or 7. The 17p-syndrome is morphologically characterized by a typical form of dysgranulopoiesis with pseudo-Pelger-Huet hypolobulation and the presence of small granules in granulocytes. Clinically the disease is aggressive with resistance to treatment and short survival. TP53 located on 17p13 is typically lost in these cases, while an inactivating mutation in the second allele on the homologous chromosome 17 occurs in ~70% of cases [95–97].

Trisomy 8. The incidence of a gain of chromosome 8 in MDS is about 10% and it is observed in all FAB subgroups. Trisomy 8 as the sole anomaly appears to have a male predominance accounting for two-thirds of cases [98]. The significance of trisomy 8 as a risk factor in MDS is not well defined. The International MDS Risk analysis Workshop ranked this abnormality in the intermediate risk group. However, Sole et al. [49] found that patients with trisomy 8 as the sole chromosomal abnormality have a significant risk of leukemic transformation and worse behavior than expected in the IPSS intermediate risk group. Also Fernandez et al. [56] considered that $+8$ may be a predictor of MDS evolution and that it is correlated with short survival. Pfeilstocker et al. [48] using database of 386 MDS patients reported that cytogenetic findings are a strong independent prognostic factor in MDS with aberrations of chromosome 8 being detected to carry a poor prognosis.

Chromosome 3 abnormalities. Several abnormalities involving chromosome 3 have been described in MDS. These aberrations include del(3p), inv(3)(q21q26), t(3;3)(q21;q26), t(3;12)(q26;p13), t(3;5)(q25;q34).They frequently seen in association with other aberrations and they have poor prognosis with resistance to conventional chemotherapy. MDS patients with 3q21q26 involvement are mostly female and relatively of young age <55 years [99].

Chromosome 11 abnormalities. Abnormalities of 11q are among the most common found in myeloid malignancies and they often harbor a breakpoint at 11q23. Aberrations of 11q23 region on which the MLL (mixed-lineage leukemia) gene is located, are found in about 5% of MDS patients. They frequently accompanied by additional abnormalities including complex karyotypes and have an unfavorable prognosis. Deletion of 11q13 has also been described in MDS and in few of them it was reported as the sole anomaly [100–102].

Rare recurrent translocations. The identification of genes involved in recurrent chromosomal translocations are of major importance. The consequence of a recurring translocation is the deregulation of a gene expression or the generation of a novel fusion gene and the production of a fusion protein. In contrast to leukemias, in MDS rare recurrent translocations have been identified and investigated by molecular methods.

$t(5;12)(q33;p13)$ is a rare cytogenetic anomaly seen mainly in CMMoL. This translocation results in a fusion transcripts between the TEL gene on 12p13 and the platelet derived growth factor receptor $\beta$.


Trisomy 19 as the sole chromosomal abnormality in proliferative chronic myelomonocytic leukemia

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Abstract

Distinct morphologic and clinical features associated with specific chromosomal abnormalities have been described in subgroups of myelodysplastic syndromes (MDS), which often are losses or gains and only rarely translocations. Among 103 consecutive MDS patients diagnosed and karyotyped at the Albert-Ludwigs University of Freiburg (ALU) between 1993 and 1999, two chronic myelomonocytic leukemias (CMMoL) displayed trisomy 19 (+19) as the sole chromosomal abnormality. Three further CMMoL cases with +19 as the single abnormality, two of which previously reported, were collected from other centers. Four of the five patients presented with leukocytosis and splenomegaly, and an increased number of ringed sideroblasts was observed in two cases. Treatment was low-dose Decitabine (cases 1 and 2), oral steroids (case 3), hydroxyurea (case 4), and daunorubicin/Ara-C (case 5). Transformation to acute myeloid leukemias (AML) occurred in three/five patients (cases 1, 2, and 4) 26, 12, and 22 months after diagnosis of CMMoL, respectively. We conclude that +19 as the sole anomaly is a rare but recurrent change in CMMoL, in particular of the proliferative type. It is at present unclear which gene(s) located on chromosome 19 might have a functional role for the development of this phenotype.

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Keywords: Myelodysplastic/myeloproliferative overlap syndrome; Ringed sideroblasts; Karyotype; Decitabine

1. Introduction

Among the unbalanced chromosomal abnormalities found in myelodysplastic syndromes (MDS) only a few, for example monosomy 7 in children and whole or partial losses of chromosomes 5 and 17, have been linked to distinct phenotypes [1–4]. Most numerical chromosomal aberrations are not preferentially found in specific FAB subgroups of MDS or associated with characteristic morphologic features. Trisomy 19 has previously been described as a sole abnormality in 12 cases of MDS and 21 acute myeloid leukemias (AML), the latter including two cases evolving from refractory anemia with excess blasts (RAEB) and two evolving from refractory anemia with ringed sideroblasts (RARS) [5,6]. Based on the cases reported and reviewed by Johansson et al. [5], which included a single case of chronic myelomonocytic leukemia (CMMoL) with leukocytosis, they concluded that trisomy 19, as sole chromosomal abnormality, in AML is associated either with a monocytic/myelomonocytic phenotype or with a very immature phenotype of AML.

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Cytogenetic abnormalities in Tunisian de novo myelodysplastic syndrome: A comparison with other populations

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Abstract  
Cytogenetic analysis was performed on 224 bone marrow (BM) of Tunisian patients with de novo myelodysplastic syndrome (MDS) at our institution from January 1993 to December 2006.  

According to French–American–British (FAB) criteria, there were 36% of patients with refractory anaemia (RA), 26% with refractory anaemia with excess of blasts (RAEB), 10% with refractory anaemia with ringed sideroblasts (RARS), 12% with chronic myelomonocytic leukaemia (CMML), 9% refractory anaemia with excess of blasts in transformation (RAEB-t) and 7% of unclassified MDS. A clonal chromosomal abnormality was observed in 51% of the patients. The most frequent karyotypic change was 5q− in 30 cases (13%), followed by −7/7q− in 17 cases (8%), del(12p) in 8 cases (4%), del(20q) and trisomy 8 in 7 cases each (3%), i(17q) in 2 cases (1%) and −y in only one case (0.4%).  

This is the first large comparative series of MDS from an Arab country, with cytogenetic analysis showing haematological and cytogenetic features similar to those of MDS population of European or mixed European-subsaharian African origin (like Brazil), but different from those seen in Eastern populations.  
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1. Introduction  
Myelodysplastic syndromes (MDS) are clonal disorders involving all myeloid lineages, characterized by dyshematopoiensis, peripheral blood cytopenias [1] and frequent progression to acute myeloid leukaemia (AML) [2]. MDS occurs predominantly in the elderly and are extremely rare in pediatric populations. According to the French–American–British (FAB) classification, MDS can be divided into refractory anaemia (RA), refractory anaemia with ring sideroblasts (RARS), refractory anaemia with excess of blasts (RAEB) and refractory anaemia with excess of blasts in transformation (RAEB-t); considered AML by the WHO classification which is a more refined classification, taking into account in particular the extent of dysplasia on non-erythoblastic lineages [3–6].  

Cytogenetic analyses are presumed to be strongly predictor of clinical outcome in MDS. They allowed the definition of a risk-based classification system for MDS: the International Prognostic Scoring System (IPSS). So, patients were divided into cytogenetic categories. Poor risk MDS was defined as
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normal karyotype, loss of Y chromosome, del(5q) or del(20q) as sole anomalies. High risk MDS was defined as having structural abnormalities or loss of chromosome 7 and/or a complex karyotype with $\geq 3$ abnormalities. Intermediate-risk MDS was defined as having any other anomalies, e.g. trisomy 8 [7].

Cytogenetic abnormalities are found in greater than 50% of MDS, and include complete or partial chromosome loss (especially del(5q), −7, del(7q), del(20q) or gain (+8). The combination of morphological and cytogenetic features has allowed to identify “specific” entities in MDS, including the so-called “5q− syndrome” and MDS with thrombocytosis, dysmegacaryopoiesis and 3q26 abnormalities.

Interestingly, it has recently been reported that types of MDS could differ in different regions of the globe. For example, MDS diagnosed in Japan are characterized by a lower incidence of the 5q-syndrome, and a higher incidence of RA with normal karyotype than MDS diagnosed in Germany, and those differences probably arise from ethnic and/or environmental differences between those two populations.

To our knowledge, we are the first in North African and Arab countries to report haematological and cytogenetic characteristics of a consecutive series of 224 Tunisian MDS and to compare their features with those of MDS series from Western and Eastern countries.

2. Patients and methods

2.1. Patients

Between January 1993 and December 2006, 216 adults and 8 children with primary MDS were diagnosed in the divisions of haematology of the university hospitals of Tunis, Sousse and Sfax (Tunisia), and their bone marrow (BM) samples were sent to the cytogenetic laboratory at Farhat Hached University Hospital, of Sousse (Tunisia).

2.2. Methods

Cytogenetic studies were performed using standard methods for preparations and R-Banding. Chromosome identification and classification of chromosomal abnormalities were made according to the International System for Human Cytogenetic Nomenclature (ISCN) [8].

At least 20 metaphases were analyzed after 24 h and/or 48 h bone marrow cell culture.

Clonal abnormalities were defined as 2 or more cells with the same whole chromosome gain or chromosome rearrangement, or 3 or more cells with the same chromosome loss. A complex karyotype was defined as three or more cytogenetic abnormalities.

Cytogenetic abnormalities were analyzed for their prognostic value on survival by using the log rank test. Overall survival was measured from time of diagnosis and curves were constructed by the Kaplan–Meier method.

3. Results

3.1. Haematological characteristics

Haematological characteristics of the 224 cases (216 adults and 8 children) of de novo MDS are summarized in Table 1.

<table>
<thead>
<tr>
<th>Cytophenic subgroup</th>
<th>Karyotypes</th>
<th>Number of patients (%)</th>
<th>RA</th>
<th>RARS</th>
<th>RAEB</th>
<th>RAEB-t</th>
<th>CMML</th>
<th>No FAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>Normal</td>
<td>110 (49)</td>
<td>36</td>
<td>10</td>
<td>29</td>
<td>6</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>del(5q)</td>
<td>30 (13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>del(20q)</td>
<td>7 (3)</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>−y</td>
<td>1 (0.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>−</td>
</tr>
<tr>
<td>Poor</td>
<td>−7</td>
<td>17 (8)</td>
<td>5</td>
<td>1</td>
<td>10</td>
<td>1</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>Complex karyotype</td>
<td>18 (8)</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td>5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Trisomy 8</td>
<td>7 (3)</td>
<td>3</td>
<td>−</td>
<td>3</td>
<td>1</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>Trisomy 21</td>
<td>1 (0.4)</td>
<td></td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>del(12)(p12p13)</td>
<td>8 (4)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>−</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>i(17q)</td>
<td>2 (1)</td>
<td></td>
<td>1</td>
<td>−</td>
<td>1</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>46,XY,del(5)(q13q33),t(2;11)(p21;q23)</td>
<td>1 (0.4)</td>
<td>1</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>t(3:5)(q21q26)</td>
<td>1 (0.4)</td>
<td></td>
<td>1</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>del(12)(12p13),i(14) (q11)</td>
<td>1 (0.4)</td>
<td></td>
<td>1</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>Trisomy 19</td>
<td>1 (0.4)</td>
<td></td>
<td>1</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>T(6;14)(q23;q32),+13,+21</td>
<td>1 (0.4)</td>
<td></td>
<td>−</td>
<td>1</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>Del(5q), del(13q)</td>
<td>3 (2)</td>
<td>2</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Monosomy 19, +mar</td>
<td>1 (0.4)</td>
<td></td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

Abbreviations: CMML, chronic myelomonocytic leukemia; RA, refractory anemia; RAEB, refractory anemia with excess of blasts; RAEB-t, refractory anemia with excess of blasts in transformation; RARS, refractory anemia with ringed sideroblast; (−): 0.
Table 2
Clinical, haematological and cytogenetic features at diagnosis of pediatric MDS cases

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age (years)/sex</th>
<th>WBC count (L−1)</th>
<th>FAB</th>
<th>Karyotypes</th>
<th>Follow-up (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15/M</td>
<td>6,000</td>
<td>JMML</td>
<td>46,XY[20]</td>
<td>36 (d)</td>
</tr>
<tr>
<td>2</td>
<td>15/M</td>
<td>1,500</td>
<td>RAEB</td>
<td>45,XY,−7[17]</td>
<td>7 (a)</td>
</tr>
<tr>
<td>3</td>
<td>7/F</td>
<td>8,000</td>
<td>CMML</td>
<td>46,XX[23]</td>
<td>18 (d)</td>
</tr>
<tr>
<td>4</td>
<td>3/M</td>
<td>6,000</td>
<td>RAEB</td>
<td>45,XY,−7[12]/46,XY,−7,+mar[8]</td>
<td>32 (a)</td>
</tr>
<tr>
<td>5</td>
<td>16/M</td>
<td>7,000</td>
<td>JMML</td>
<td>46,XY[21]</td>
<td>12 (a)</td>
</tr>
<tr>
<td>6</td>
<td>17/M</td>
<td>8,000</td>
<td>RAEB</td>
<td>45,XY,−7[15]</td>
<td>7 (a)</td>
</tr>
<tr>
<td>7</td>
<td>2/M</td>
<td>&gt;50,000</td>
<td>JMML</td>
<td>46,XY[20]</td>
<td>5 (a)</td>
</tr>
<tr>
<td>8</td>
<td>10/F</td>
<td>&gt;50,000</td>
<td>JMML</td>
<td>46,XX[21]</td>
<td>4 (a)</td>
</tr>
</tbody>
</table>

Abbreviations: WBC, peripheral white blood cell count; JMML, juvenile myelomonocytic leukaemia; (a), alive; (d), died.

According to FAB criteria, 36% of our patients had RA, 26% had RAEB, 12% had CMML, 10% had RARS and 9% had RAEB-t, and the 7% remaining patients were non-classifiable.

Haematological characteristics of the pediatric cases are summarised in Table 2. There were six males and two females, in the age range of 2–17 years (mean 10 years). There were four cases of juvenile myelomonocytic leukaemia (JMML), defined as an entity similar to the adult form of CMML and four cases of RAEB.

3.2. Cytogenetic results (Table 1)

Cytogenetic analysis showed an abnormal karyotype in 114 cases. Good-, intermediate- and poor-risk cytogenetic results were found in 67%, 17% and 16% of the patients, respectively.

3.2.1. Good-risk patients

- **Normal karyotypes:** 110 (49%) of our patients had normal metaphases. Of whom five were children.

They had predominately RA and RAEB with 36 (33%) and 29 (27%) cases, respectively (Table 1).

- **5q deletion:** Del(5q) was identified as a single anomaly in 30 patients (13%). This abnormality is associated with RA in 20 cases (67%). All del(5q) were interstitial with variable proximal and distal breakpoints. The most common breakpoints occurred at band 5q12–14 and 5q31–33, respectively.

- **20q deletion:** Deletion of the long arm of chromosome 20 was detected as a single anomaly in seven cases (3%). Five of them were classified as RA, one as RAEB and one as RARS.

Breakpoints occurred between bands 20q11.2 and 20q13.2.

- **Loss of chromosome Y:** only one (0.4%), 30-year-old man, of our patients had a −Y clone (45, X−,−Y[4]/44,XY,−5,−7[3]/46,XY[15]).

3.2.2. Poor-risk patients

- **Monosomy 7/7q−:** Isolated chromosome 7 abnormalities were found in 17 cases (8%) (monosomy 7 in 17 cases

Table 3
Complex karyotypes found at the time of diagnosis in 18 patients classified according to the FAB criteria

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age (years)</th>
<th>FAB</th>
<th>Karyotype</th>
<th>Follow-up (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>51</td>
<td>RAEB</td>
<td>46,XY,−7,+8[8]/47,idem,+11[10]</td>
<td>3 (a)</td>
</tr>
<tr>
<td>2</td>
<td>79</td>
<td>RAEB-t</td>
<td>44,XX,del(5)(q13q33),−7,+8,+19,+mar[5]/44,XX[16]</td>
<td>1 (l)</td>
</tr>
<tr>
<td>3</td>
<td>61</td>
<td>RAEB</td>
<td>44,XY,del(5)(q22q34),del(7)(q21q31),−12,+16,+19,+mar[12]</td>
<td>8 (a)</td>
</tr>
<tr>
<td>4</td>
<td>68</td>
<td>RAEB-t</td>
<td>46,XY,del(5)(q13q34),add(13)(p13),del(17)(t:12;17)[15]</td>
<td>9 (a)</td>
</tr>
<tr>
<td>5</td>
<td>65</td>
<td>RA</td>
<td>45,XY,−X,−5,del(7)(q21q31),+mar[4]/44,idem,−19[6]/44,XX,−5,del(7)(q21q31),del(12),−19[3]/46,XX[2]</td>
<td>3 (d)</td>
</tr>
<tr>
<td>6</td>
<td>70</td>
<td>RAEB</td>
<td>46,XY,del(1q)(q21),del(8)(q21),del(11)(q22),inv(12)(q12q14)[17]</td>
<td>5 (d)</td>
</tr>
<tr>
<td>7</td>
<td>73</td>
<td>CMML</td>
<td>43,XY,add(3)(q13),del(4)(t:12),del(5)(q14),−7,add(12)(q24),−14,add(14)(q32),−20[5]/46,XY[18]</td>
<td>1 (d)</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>RAEB</td>
<td>44,XY,−5,add(7)(q22),−15,−17,+mar[3]/46,XY,−5,add(20)(q12)[15]</td>
<td>5 (d)</td>
</tr>
<tr>
<td>9</td>
<td>82</td>
<td>RA</td>
<td>47,XY,del(5)(q13q34),−7,+mar[5]/46,XY[15]</td>
<td>7 (a)</td>
</tr>
<tr>
<td>10</td>
<td>67</td>
<td>RARS</td>
<td>51,XXY,+3,der(4),del(5)(q12q31)[18]</td>
<td>12 (d)</td>
</tr>
<tr>
<td>11</td>
<td>52</td>
<td>RAEB-t</td>
<td>46,XY,del(5)(q14q34),−7,+21[15]/47,XY,del(5)(q14q34),+21[2]/46,XY[2]</td>
<td>6 (d)</td>
</tr>
<tr>
<td>12</td>
<td>49</td>
<td>RAEB-t</td>
<td>47,XY,del(5)(q12q32),ins(13)(q21q21q31),+21[14]</td>
<td>9 (a)</td>
</tr>
<tr>
<td>13</td>
<td>62</td>
<td>RAEB</td>
<td>44,XY,del(5)(q13q33),−7,−8,+19,+mar[5]/46,XX[16]</td>
<td>5 (d)</td>
</tr>
<tr>
<td>14</td>
<td>73</td>
<td>RAEB-t</td>
<td>46,XY,del(1)(p35p11),t(3;3)(q21q26),del(19)(q11)[17]/46,XY,idem,t(9;12),q13p13[3]</td>
<td>3 (l)</td>
</tr>
<tr>
<td>15</td>
<td>89</td>
<td>CMML</td>
<td>43,XY,del(13)(q25p13),del(5)(q13q33),−8,−12,+20[10]/46,XY[7]</td>
<td>8 (d)</td>
</tr>
<tr>
<td>16</td>
<td>55</td>
<td>RAEB-t</td>
<td>47,XY,−8[6]/44,XY,del(4)(q12),−8,del(9)(q12),+9,+14,+15,add(17)(q25),−22,+mar[15]</td>
<td>5 (a)</td>
</tr>
<tr>
<td>17</td>
<td>46</td>
<td>RARS</td>
<td>47,XY,−12,t(14;16)(q11q14),+mar[5]/46,XY[12]</td>
<td>6 (d)</td>
</tr>
</tbody>
</table>

Abbreviations: CMML, chronic myelomonocytic leukaemia; RA, refractory anemia; RAEB, refractory anemia with excess of blasts; RAEB-t, refractory anemia with excess of blasts in transformation; RARS, refractory anemia with ringed sideroblast; (a) alive; (d) died; (l) lost to follow-up.
including three children and del(7q) in one). Ten of whom had RAEB, five had RA, one had RARS and the last one had RAEB-t.

- Complex karyotypes: Complex abnormalities were observed in 18 cases (8%) with primary MDS. Six of whom had a diagnosis of RAEB, five had RAEB-t, three CMML, two had RARS, one had RA and one was unclassified according to the FAB criteria. In this subgroup, chromosomes 5/7/12 were predominantly implicated (Table 3).

3.2.3. Intermediate-risk patients

Thirty-eight patients in our study had intermediate-risk cytogenetics including trisomy 8 in seven cases, del(12)(p13) in eight cases, iso(17)(q10) in two, trisomy 21 in one, 11q23 rearrangement in one, 3q26 abnormalities in one (t(3;3)(q21;q26)) and some rare anomalies in 18 cases (Table 1).

3.3. Outcome and prognostic factors

Median survival was 30, 33 and 10 months for patients in the good, intermediate and poor cytogenetic subgroups, respectively \((p=0.03)\) (Fig. 1).

Although median survival did not differ significantly between patients aged less and more than 50 years, survival curves according to FAB subgroups were significantly different \((p=0.0007)\) (Fig. 2).

Median survival in patients with RARS (36 months) and RA (24 months) were significantly longer than those in patients with CMML (5 months). Median survival with RAEB was 7 months and with RAEB-t was 6 months.

Pediatric MDS cases were followed up from 4 to 36 months (mean 15 months). All three cases of RAEB are alive and under regular follow-up. Of the four cases of CMML, two died and the other two cases are alive with progressively increasing blast counts (Table 2).

4. Discussion

We compared characteristics of our patients to those published in different populations (Table 4). Median age of this Tunisian MDS population analyzed was 60 years as found in Japanese MDS patients [9] compared to 72 years in German [3] but only 49 years in Korean [10] and 53 years in Chinese [11]. This age difference at diagnosis between world regions [12,13] was previously suggested Komorkji to uncover possible hereditary, genetic, immunologic, infectious and other environmental factor differences [14].

Our incidence of abnormal karyotype (51%), was comparable to that described in German and Korean MDS patients where 52.1% and 45% had clonal anomalies, respectively [10,15] but higher than that found in a Chinese series where Irons et al. reported only 31% of abnormal karyotypes [11] (Table 4).

According to FAB classification, the highest frequency of chromosome abnormalities was observed in RA and RAEB subtypes, and the lowest in CMML. This was different from that reported by Solé et al. [16] in Spanish MDS cases, who found that RAEB and RAEB-t subtypes presented generally the highest rates of chromosomal anomalies and RARS and CMML, the lowest.

In Korea, Lee et al. [10] reported that abnormal clones were commonest in patients with RARS and RAEB-t followed by those with RAEB, and least common in patients with RA and CMMoL.

The frequency of cytogenetic abnormalities in MDS subtypes in Tunisian patients was therefore somewhat different to that found in both Eastern and Western countries and this may be explained by ethno-geographical differences.

No difference in the distribution of chromosomal abnormalities according to MDS subtypes was noted and no specific cytogenetic abnormality was associated with a particular MDS FAB subtype with the exception of del(5q) that was more frequent in RA while most of cases of monosomy 7 were detected in RAEB and complex karyotype in
Table 4
Comparison of distribution of MDS subtypes and frequencies of chromosome abnormalities

<table>
<thead>
<tr>
<th></th>
<th>Tunisia</th>
<th>Germany</th>
<th>Switzerland</th>
<th>USA</th>
<th>Brazil</th>
<th>Shanghai</th>
<th>Korea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>224</td>
<td>2124</td>
<td>109</td>
<td>247</td>
<td>93</td>
<td>218</td>
<td>205</td>
</tr>
<tr>
<td>RA</td>
<td>36</td>
<td>27.7</td>
<td>25</td>
<td>37.2</td>
<td>42</td>
<td>64.2</td>
<td>36.5</td>
</tr>
<tr>
<td>RARS</td>
<td>10</td>
<td>12.1</td>
<td>24</td>
<td>14.6</td>
<td>6.4</td>
<td>3.6</td>
<td>4.4</td>
</tr>
<tr>
<td>RAEB</td>
<td>26</td>
<td>20</td>
<td>15</td>
<td>31.6</td>
<td>18.2</td>
<td>13.3</td>
<td>31.2</td>
</tr>
<tr>
<td>RAEB-t</td>
<td>9</td>
<td>14.6</td>
<td>9</td>
<td>8.5</td>
<td>18.2</td>
<td>12.8</td>
<td>18.5</td>
</tr>
<tr>
<td>CMMoL</td>
<td>12</td>
<td>13.5</td>
<td>23</td>
<td>8.1</td>
<td>15</td>
<td>5.9</td>
<td>6.8</td>
</tr>
<tr>
<td>No FAB</td>
<td>7</td>
<td>4.2</td>
<td>46</td>
<td>0</td>
<td>0</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Median age (range)</td>
<td>60 (1–90)</td>
<td>65.7 (0.1–96.1)</td>
<td>69 (30–92)</td>
<td>NA</td>
<td>29 (1–78)</td>
<td>53 (19–82)</td>
<td>49 (NA)</td>
</tr>
</tbody>
</table>

Sex

<table>
<thead>
<tr>
<th></th>
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<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tunisia</td>
<td>129</td>
<td>95</td>
</tr>
<tr>
<td>Germany</td>
<td>1197</td>
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<td>USA</td>
<td>151</td>
<td>96</td>
</tr>
<tr>
<td>Brazil</td>
<td>51</td>
<td>42</td>
</tr>
<tr>
<td>Shanghai</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Korea</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

% of cases with abnormal karyotype

<table>
<thead>
<tr>
<th></th>
<th>Tunisia</th>
<th>Germany</th>
<th>Switzerland</th>
<th>USA</th>
<th>Brazil</th>
<th>Shanghai</th>
<th>Korea</th>
</tr>
</thead>
<tbody>
<tr>
<td>5q/5q-</td>
<td>13^b</td>
<td>35^c</td>
<td>34.4^c</td>
<td>21.6^c</td>
<td>12.5^c</td>
<td>2.4^b</td>
<td>13^c</td>
</tr>
<tr>
<td>7q/7q-</td>
<td>8</td>
<td>21</td>
<td>14.8</td>
<td>25.0</td>
<td>14.2</td>
<td>4.9</td>
<td>11.9</td>
</tr>
<tr>
<td>+8</td>
<td>3</td>
<td>16</td>
<td>14.8</td>
<td>13.8</td>
<td>9.4</td>
<td>12.2</td>
<td>19.5</td>
</tr>
<tr>
<td>−20q</td>
<td>3</td>
<td>9</td>
<td>13.2</td>
<td>4.3</td>
<td>7.8</td>
<td>4.3</td>
<td>5.4</td>
</tr>
<tr>
<td>−Y</td>
<td>0.4</td>
<td>7</td>
<td>7.8</td>
<td>0</td>
<td>0</td>
<td>9.8</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CMMoL, chronic myelomonocytic leukemia; RA, refractory anemia; RAEB, refractory anemia with excess of blasts; RAEB-t, refractory anemia with excess of blasts in transformation; RARS, refractory anemia with ringed sideroblast; NA: not available.

RAEB and RAEB-t (Table 1) as described in other countries [6,17,18]. In Tunisian patients, chromosomal deletions (by decreasing order del(5q), monosomy 7, del(12p) and del(20q), were the most frequent structural alterations.

Similar findings were reported in Western countries although with slightly higher frequencies of deletions [17,19,20], while Toyama et al. [13], Matsushima et al. [21] and Matsuda et al. [9], reported a much lower incidence of del(5q) in the east (2%, 1.5% and 2.9%, respectively) (Table 4).

Trisomy 8 was detected in only 3% of our patients, whereas Irons et al. [11] found it to be the commonest abnormality observed in a Chinese MDS population.

Only one of our patient (0.4%) had loss of chromosome Y; he is 30 years old. This anomaly is also seen as the sole cytogenetic abnormality in the bone marrow of 7–8% healthy old men [22–24] and its clonal nature is regularly questioned.

The majority of nonrandom chromosomal changes described in MDS were found in our series. We noted however, cytogenetic anomalies rarely described before such as i(14)(q10), t(6;14) (q23,q32), and a loss of chromosome 19 and a ring of chromosome 12.

The proportion of good, intermediate and poor prognosis cytogenetic subgroups was similar to that previously reported in both Western and Eastern MDS cases [7,11,15,25] with 67%, 17% and 16%, respectively. However, the median survival time for the intermediate-risk patients was slightly longer than the good-risk patients (33 months versus 30 months), contrary to the large published series [25,26]. This finding might be explained by the relatively small size of our cohort.

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In conclusion, our series that constitutes to our knowledge the first comparative series of MDS from an Arab country studied cytogenetically, had clinical haematological and cytogenetic characteristics comparable with those reported in Western countries [29–33] including those among in populations of European origin (Europe, North America), or of mixed origin (European and Subsaharian African ascent, like Brazil). Some rearrangements, especially deletions (del(5q), del(7q) and del(20q)) and trisomy 8 however, seemed to occur at slightly lower frequency than in European/North American series. Whether this was due to the fact that few of our patients had complementary interphase FISH, or to real differences, remains to be established.

Differences in frequency of MDS subtypes, age at diagnosis, and incidence of some of cytogenetic abnormalities were however noted in Eastern populations, as reported before, probably reflecting different hereditary, genetic and environmental factors.

Thus, we agree with Komorkji [14] that MDS may behave differently where the sun rises and where the sun sets, but...
Table 4
Comparison of distribution of MDS subtypes and frequencies of chromosome abnormalities

<table>
<thead>
<tr>
<th></th>
<th>Tunisia</th>
<th>Germanya</th>
<th>Switzerlanda</th>
<th>USAa</th>
<th>Brazila</th>
<th>Shanghaia</th>
<th>Koreaa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>224</td>
<td>2124</td>
<td>109</td>
<td>247</td>
<td>93</td>
<td>218</td>
<td>205</td>
</tr>
<tr>
<td>RA</td>
<td>36</td>
<td>27.7</td>
<td>25</td>
<td>37.2</td>
<td>42</td>
<td>64.2</td>
<td>36.5</td>
</tr>
<tr>
<td>RARS</td>
<td>10</td>
<td>12.1</td>
<td>24</td>
<td>14.6</td>
<td>6.4</td>
<td>3.6</td>
<td>4.4</td>
</tr>
<tr>
<td>RAEB</td>
<td>26</td>
<td>20</td>
<td>15</td>
<td>31.6</td>
<td>18.2</td>
<td>13.3</td>
<td>31.2</td>
</tr>
<tr>
<td>RAEB-t</td>
<td>9</td>
<td>14.6</td>
<td>9</td>
<td>8.5</td>
<td>18.2</td>
<td>12.8</td>
<td>18.5</td>
</tr>
<tr>
<td>CMMoL</td>
<td>12</td>
<td>13.5</td>
<td>23</td>
<td>8.1</td>
<td>15</td>
<td>5.9</td>
<td>6.8</td>
</tr>
<tr>
<td>No FAB</td>
<td>7</td>
<td>4.2</td>
<td>46</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.4</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>60 (1–90)</td>
<td>65.7 (0.1–96.1)</td>
<td>69 (30–92)</td>
<td>NA</td>
<td>29 (1–78)</td>
<td>53 (19–82)</td>
<td>49 (NA)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th>Tunisia</th>
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<th>Switzerlanda</th>
<th>USAa</th>
<th>Brazila</th>
<th>Shanghaia</th>
<th>Koreaa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>129</td>
<td>1197</td>
<td>66</td>
<td>151</td>
<td>51</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Female</td>
<td>95</td>
<td>927</td>
<td>43</td>
<td>96</td>
<td>42</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>% of cases with abnormal karyotype</td>
<td>51</td>
<td>52.1</td>
<td>56</td>
<td>47</td>
<td>69</td>
<td>31</td>
<td>44.8</td>
</tr>
</tbody>
</table>

% of nonrandom abnormality

<table>
<thead>
<tr>
<th></th>
<th>Tunisia</th>
<th>Germanya</th>
<th>Switzerlanda</th>
<th>USAa</th>
<th>Brazila</th>
<th>Shanghaia</th>
<th>Koreaa</th>
</tr>
</thead>
<tbody>
<tr>
<td>−5q/5q−</td>
<td>13b</td>
<td>35c</td>
<td>34.4c</td>
<td>21.6c</td>
<td>12.5c</td>
<td>2.4b</td>
<td>13c</td>
</tr>
<tr>
<td>−7/7q−</td>
<td>8</td>
<td>21</td>
<td>14.8</td>
<td>25.0</td>
<td>14.2</td>
<td>4.9</td>
<td>11.9</td>
</tr>
<tr>
<td>+8</td>
<td>3</td>
<td>16</td>
<td>14.8</td>
<td>13.8</td>
<td>9.4</td>
<td>12.2</td>
<td>19.5</td>
</tr>
<tr>
<td>−20q</td>
<td>3</td>
<td>9</td>
<td>13.2</td>
<td>4.3</td>
<td>7.8</td>
<td>4.3</td>
<td>5.4</td>
</tr>
<tr>
<td>−Y</td>
<td>0.4</td>
<td>7</td>
<td>0</td>
<td>7.8</td>
<td>0</td>
<td>0</td>
<td>9.8</td>
</tr>
</tbody>
</table>

Abbreviations: CMMoL, chronic myelomonocytic leukemia; RA, refractory anemia; RAEB, refractory anemia with excess of blasts; RAEB-t, refractory anemia with excess of blasts in transformation; RARS, refractory anemia with ringed sideroblast; NA: not available.

b Among total myelodysplastic syndrome (MDS).
c Among total MDS cases with abnormal karyotype.

RAEB and RAEB-t (Table 1) as described in other countries [6,17,18].

In Tunisian patients, chromosomal deletions (by decreasing order del(5q), monosomy 7, del(12p) and del(20q), were the most frequent structural alterations.

Similar findings were reported in Western countries although with slightly higher frequencies of deletions [17,19,20], while Toyama et al. [13], Matsushima et al. [21] and Matsuda et al. [9], reported a much lower incidence of del(5q) in the east (2%, 1.5% and 2.9%, respectively) (Table 4).

Trisomy 8 was detected in only 3% of our patients, whereas Irons et al. [11] found it to be the commonest abnormality observed in a Chinese MDS population.

Only one of our patient (0.4%) had loss of chromosome Y; he is 30 years old. This anomaly is also seen as the sole cytogenetic abnormality in the bone marrow of 7–8% healthy old men [22–24] and its clonal nature is regularly questioned.

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Conflict of interest statement

None.

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