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Research paper

Prognostic impact of a suboptimal number of analyzed metaphases in normal karyotype lower-risk MDS

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ABSTRACT

Conventional karyotype is one of the most relevant prognostic factors in MDS. However, about 50% of patients with MDS have a normal karyotype. Usually, 20–25 normal metaphases (nMP) are considered to be optimal to exclude small abnormal clones which might be associated with poor prognosis. This study evaluated the impact of examining a suboptimal number of metaphases in patients recruited to the EUMDS Registry with low and intermediate-1 risk according to IPSS. Only 179/1049 (17%) of patients with a normal karyotype (35%) had a suboptimal number of MP assessed, but this did not impact on outcome. For patients with a normal karyotype and suboptimal numbers of analyzable metaphases standard evaluation might be acceptable for...
1. Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal myeloid disorders characterized by peripheral blood cytopenias and increased risk of transformation to acute myelogenous leukemia (AML) [1]. Classical karyotype analyses detect clonal chromosome abnormalities in about 50% of patients with MDS [2]. The karyotype is one of the strongest prognostic parameters in the currently applied prognostic models, including the revised International Prognostic Scoring System (IPSS-R) [3]. In general, the aim is to analyze 20 or more metaphases (MP) before a karyotype is considered to lack specificity.

The primary aim of the present study was to assess whether the number of MP examined in patients with normal karyotype provide any outcome in patients with an abnormal karyotype. To the best of our knowledge, the prognostic relevance of these smaller clones remains to be elucidated in lower-risk MDS [4].

The results of the IPSS-R cytogenetic scores were retrospective assigned by one of the investigators of the EUMDS registry and verified by an independent expert of the international IPSS working group (D. Haase). IPSS and IPSS-R cytogenetic scores were calculated and the IPSS-R cytogenetic risk category of patients with only nMP was assigned as good-risk. In these cases no abnormal MP were reported. Patients with abnormal MP were categorized to the IPSS-R cytogenetic risk score: very good, good, intermediate, poor and very poor risk category [8].

2. Design and methods

2.1. Eligibility

Patients were eligible for inclusion if they were newly diagnosed with MDS according to the WHO 2001 classification [6] and had a low or intermediate-1 risk score according to the IPSS [7]. Patients with post-cytotoxic MDS have been excluded from this Registry. The ethics committees of all participating countries and centers have approved the EUMDS registry (trial number NCT00600860). Patient-specific (including bone marrow morphology, histology and cytogenetics), intervention and outcome data were collected at baseline and at each 6-monthly out-patient follow-up visit for the routine clinical care of patients with MDS. All subjects were prospectively followed until death, progression to higher-risk MDS or leukemia, loss to follow-up or withdrawal of informed consent.

2.2. Assignment of IPSS(-R) score

Both the IPSS cytogenetic score and the IPSS-R cytogenetic score were determined from the diagnostic cytogenetic reports at registration. The local investigator assigned the IPSS cytogenetic scores. The IPSS-R cytogenetic scores were retrospectively assigned by one of the investigators of the EUMDS registry and verified by an independent expert of the international IPSS working group (D. Haase). IPSS and IPSS-R cytogenetic risk score were calculated and the IPSS-R cytogenetic risk category of patients with only nMP was assigned as good-risk. In these cases no abnormal MP were reported. Patients with abnormal MP were categorized to the IPSS-R cytogenetic risk score: very good, good, intermediate, poor and very poor risk category [8].

2.3. Statistical analysis

Standard descriptive techniques were used to assign the distribution of baseline patient characteristics including chi squared test and Wilcoxon rank sum test. Overall survival (OS) was defined as the time from date of diagnosis to death, or for subjects still alive at the date of the last follow-up visit. Time to disease progression (TDP) was measured from date of diagnosis to date of disease progression to either higher-risk MDS or acute leukemia. Patients without disease progression were censored at date of death or date of last follow-up visit. Standard methods were used to test time to event, namely Cox proportional hazards regression models and Kaplan–Meier survival curves. Hazard ratios (HR) and 95% confidence intervals (95% CI) are reported for univariate analyses, unadjusted and adjusted for sex and age at diagnosis. All analyses were undertaken in Stata 14 (StataCorp, College Station, TX).

3. Results

In total 2196 patients were registered to the study between 1st April 2008 and 31st March 2017 and patients were followed-up to the 1st June

Table 1

Number of metaphases by age at diagnosis and cytopenias for subjects with a normal karyotype only or an abnormal karyotype.

<table>
<thead>
<tr>
<th>Metaphase category (n)</th>
<th>Normal karyotype</th>
<th>Abnormal karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>Median age years (range)</td>
</tr>
<tr>
<td>Total</td>
<td>1225 (100)</td>
<td>73 (18–93)</td>
</tr>
<tr>
<td>1–9</td>
<td>42 (3.4)</td>
<td>72 (47–89)</td>
</tr>
<tr>
<td>10–19</td>
<td>137 (11.2)</td>
<td>72 (21–90)</td>
</tr>
<tr>
<td>20–24</td>
<td>634 (51.8)</td>
<td>74 (21–93)</td>
</tr>
<tr>
<td>25–29</td>
<td>164 (13.4)</td>
<td>73 (42–93)</td>
</tr>
<tr>
<td>30+</td>
<td>72 (5.9)</td>
<td>74 (57–90)</td>
</tr>
<tr>
<td>Not recorded</td>
<td>176 (14.4)</td>
<td>74 (18–93)</td>
</tr>
</tbody>
</table>

In total 2196 patients were registered to the study between 1st April 2008 and 31st March 2017 and patients were followed-up to the 1st June
2017. The majority of patients had conventional cytogenetics performed (95%) and 1999 had a karyotype recorded. Sixty-one percent had a normal karyotype (1225/1999) at diagnosis and 39% an abnormal karyotype (774/1999). Patients were subdivided into six categories based on the number of reported MP (Table 1). The median number of MP examined was 20 for patients with normal and abnormal karyotypes, however, the distributions were very different as can be seen in Fig. 1 (p = .01). Whilst some patients with an abnormal karyotype had over 40 MP assessed, 35% had less than 20 MP assessed (suboptimal), this is in contrast to those with a normal karyotype where only 17.1% had less than 20 MP assessed (p < 0.0001). Patients with an abnormal karyotype were, on average, older (75 vs 73 years, p = .03), and in terms of cytopenias they lower hemoglobin values than those with a normal karyotype (p < .0001). However, there were no differences in hemoglobin, platelets or neutrophil counts by number of metaphases examined (Table 1).

Table 2 shows the number of normal MP were evenly distributed within the various categories with the exception of the participating countries. The median number of analyzed MP was significantly higher (p < .0001) in the two Scandinavian countries compared to the number of MP in Israel, Italy, Serbia, Croatia and Romania. As expected, there were differences between the normal and abnormal karyotypes in terms of WHO diagnosis and IPSS-R score; patients with an abnormal karyotype were more likely to have been transfused at diagnosis compared to those with a normal karyotype.

### 3.1 Overall survival and progression-free survival

Median follow-up was 2.1 years (range of 0.1–8.7 years) and 33% (669 of 1999) of patients had died during the observation period; median survival for patients with a normal karyotype was 5.2 years (95% Confidence Intervals (95% CI): 3.3–4.5) and abnormal karyotype (4.0 years (95% CI: 4.0 (4.8–5.9)) log rank test = 15.63, p = .0001. The univariate overall survival (Fig. 2A and B) and progression-free survival estimates, as depicted in Fig. 3A and B, showed a similar outcome in the six categories throughout the whole observation period.

Multivariate analyses were performed to adjust for the various relevant prognostic components: age at diagnosis, MDS WHO category, blast count, hemoglobin levels, platelets and neutrophil count, RBCT-dependency (> 1 unit/month for 6 months) and country. IPSS-R cytogenetic risk category was also included in the model in patients with an abnormal karyotype. The largest category of MP (20–42 MP) was used as the reference category. The number of MP, analyzed both as a continuous variable or as a categorical variable did not significantly influence survival nor progression-free survival. The group with MP not recorded in the database were included in all analyses (Table 3 and Figs. 2 and 3).
4. Discussion

The analyses of this study were focused on the impact of analyzable metaphases in patients with a normal karyotype on outcome, including estimated overall survival and progression-free survival. Our recently published study on the first 1000 patients within the EUMDS registry confirmed established prognostic factors, such as age, gender and World Health Organization 2001 classification [5] in addition, with low health-related quality of life (EQ-5D visual analogue scale score) and a high co-morbidity index predicted poor outcome. The IPSS-R was superior to the original IPSS for predicting both disease progression and survival [5]. We identified 1225 patients with normal conventional
Table 3 Multivariate analyses on impact of number of normal and combined normal/abnormal metaphases on survival and progression-free survival.

<table>
<thead>
<tr>
<th>Metaphase category (n)</th>
<th>Normal karyotype</th>
<th>Abnormal karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Survival</td>
<td>Progression-free survival</td>
</tr>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>Adjusted HR (95% CI)</td>
</tr>
<tr>
<td>Continuous</td>
<td>1225</td>
<td>1.00 (0.98-1.02)</td>
</tr>
<tr>
<td>1-9</td>
<td>42</td>
<td>0.83 (0.42-1.61)</td>
</tr>
<tr>
<td>10-19</td>
<td>137</td>
<td>0.85 (0.60-1.22)</td>
</tr>
<tr>
<td>20-24</td>
<td>634</td>
<td>1.00 (0.66-1.23)</td>
</tr>
<tr>
<td>25-29</td>
<td>164</td>
<td>0.90 (0.66-1.23)</td>
</tr>
<tr>
<td>30+</td>
<td>72</td>
<td>0.87 (0.56-1.36)</td>
</tr>
<tr>
<td>Not Recorde-d</td>
<td>176</td>
<td>1.00 (0.74-1.35)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Metaphase category (n)</th>
<th>Survival</th>
<th>Progression-free survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>Adjusted HR (95% CI)</td>
</tr>
<tr>
<td>Continuous</td>
<td>774</td>
<td>1.00 (0.98-1.02)</td>
</tr>
<tr>
<td>1-9</td>
<td>44</td>
<td>0.83 (0.53-1.33)</td>
</tr>
<tr>
<td>10-19</td>
<td>180</td>
<td>1.01 (0.81-1.26)</td>
</tr>
<tr>
<td>20-24</td>
<td>276</td>
<td>1.03 (0.82-1.29)</td>
</tr>
<tr>
<td>25-29</td>
<td>104</td>
<td>0.81 (0.64-1.29)</td>
</tr>
<tr>
<td>30+</td>
<td>45</td>
<td>0.91 (0.94-1.75)</td>
</tr>
<tr>
<td>Not Recorded</td>
<td>125</td>
<td>1.13 (0.79-1.57)</td>
</tr>
</tbody>
</table>

Abbreviations: WHO, World Health Organization; MP, metaphases.

Acknowledgements

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References


