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Clinical significance of complex karyotype at diagnosis in pediatric and adult patients with de novo acute promyelocytic leukemia treated with ATRA and chemotherapy

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ABSTRACT

Although additional cytogenetic abnormalities (ACA) do not affect the prognosis of patients with t(15;17) acute promyelocytic leukemia (APL), the role of a complex karyotype (CK) is yet to be clarified. We aimed to investigate the relationship of CK with relapse incidence in 1559 consecutive APL patients enrolled in three consecutive trials. Treatment consisted of AIDA induction followed by risk-adapted consolidation. A CK (CK) was defined as the presence of ≥2 ACA and a very CK (CK+) as ≥3 ACA. Eighty-nine patients (8%) had a CK, of whom 41 (4%) had CK+. The 5-year cumulative incidence of relapse (CIR) in patients with CK was 18%, and 12% in those with <2 ACA (p = .09). Among patients with CK+, the 5-year CIR was 27% vs 12% (p = .003), retaining the statistical significance in multivariate analysis. This study shows an increased risk of relapse among APL patients with CK+ treated with ATRA plus chemotherapy front-line regimens.

Introduction

Additional cytogenetic abnormalities (ACA) have been found in about 30% of de novo t(15;17) acute promyelocytic leukemia (APL) [1–15]. Some large studies have reported a lack of prognostic impact of ACA in patients with t(15;17) APL treated with all-trans retinoic acid (ATRA) and chemotherapy-based front-line therapies [1,4,5,11,12,14], while others have observed a negative impact on outcome [2,6–9,13,16]. On the other hand, recent randomized trials have shown less relapse and improved survival using.
front-line arsenic trioxide (ATO) plus ATRA regimens [17,18], making less relevant the impact of prognostic indicators [14,19]. However, a non-significant higher relapse rate was described among patients with ACA treated with ATO plus ATRA regimens [10], and Poire et al. recently reported that a complex karyotype (i.e. two or more ACA) was associated with more relapses and significant inferior survival in patients receiving chemotherapy– or ATO-based consolidation schedules [15].

In order to clarify the role of a complex karyotype in APL patients, we analyzed the characteristics, and prognostic impact of complex karyotype in a large cohort of successfully karyotyped patients with mature follow-up enrolled in three successive studies carried out by the Spanish Programa Español de Tratamientos en Hematología (PETHEMA) group.

Material and methods

Patients and eligibility

Between November 1996 and 2012, 1559 consecutive adult and pediatric patients were enrolled in the PETHEMA LPA 1996, 1999, and 2005 consecutive trials from the institutions of Spain, the Netherlands, Belgium, Argentina, Uruguay, and the Czech Republic (see "Appendix"). All patients had de novo genetic diagnosis of PML/RARA APL. Eligibility criteria and protocol design have been reported elsewhere [20–22]. Informed consent was obtained from all the patients. According to the Declaration of Helsinki, the protocol was approved by the Research Ethics Board of each participating hospital.

Treatment

Treatment consisted of AIDA induction consisting of oral ATRA (45 mg/m²/d), divided into two daily doses, which was maintained until complete remission (CR), and intravenous idarubicin (12 mg/m²/d) on days 2, 4, 6, and 8. For patients of age 20 years or younger, the ATRA dose was adjusted to 25 mg/m²/d. After CR achievement, therapy was followed by three monthly courses of risk-adapted consolidation chemotherapy [20–23]. In all trials, patients who tested negative for PML/RARA at the end of consolidation were started on maintenance therapy with oral mercaptopurine (50 mg/ m²/d), intramuscular methotrexate (15 mg/m²/week), and oral ATRA (45 mg/m²/d for 15 days every 3 months) over 2 years (Figure 1). Details of the supportive therapy have been described elsewhere [20–22].

Cytogenetics and FISH

Bone marrow samples for cytogenetic analysis were processed after short-term culture (24 or 48 hours) following standard procedures. The chromosomes were stained by G-banding and the karyotypes reported according to the International Standing Committee on Human Cytogenomic Nomenclature (ISCN 2016) recommendations in force [24]. Whenever possible at least 20 metaphases were analyzed in each case. Studies were considered normal diploid if no clonal abnormalities were detected in a minimum of 20 mitotic cells analyzed. In most of the patients with apparently normal karyotype and PML/RARA rearrangement demonstrated by reverse transcriptase-polymerase chain reaction (RT-PCR), fluorescence in situ hybridization (FISH) studies were additionally carried out in metaphase and interphase nuclei. Two-color FISH was performed using a PML/RARA translocation probe (Abbott, Wiesbaden, Germany).

The majority of cytogenetic analyses were performed at reference laboratories. The original cytogenetics reports were requested from the centers for central review. For patients of the HOVON Group, the Dutch Working Party on Cancer Genetics and Cytogenetics performed review of the cytogenetic results. Appropriate karyotype nomenclature (ISCN 2016) was centrally reviewed (J.C.).

RT-PCR studies

Details on processing bone marrow samples for RNA extraction and RT-PCR protocols for PML/RARA amplification used by the participating laboratories have been described elsewhere [20].

Definitions and study endpoints

Remission induction response was assessed according to the recently revised criteria by Cheson et al. [25]. Molecular remission was defined as the disappearance on an ethidium bromide gel of the PML/RARA specific band visualized at diagnosis, using an RT-PCR assay with a sensitivity level of $10^{-4}$. Molecular persistence was defined as PCR positivity in two consecutive bone marrow samples collected at the end of consolidation therapy. Molecular relapse was defined as the reappearance of PCR-positivity in two consecutive bone marrow samples at any time after consolidation therapy. Risk of relapse was established at diagnosis according to a predictive model based on patient leukocyte and platelet counts at diagnosis, as reported elsewhere [26].
For the purpose of this study, patients with a normal karyotype or with a t(15;17) as the sole cytogenetic detected abnormality were classified as having no ACA. The presence of multiple rearrangements (i.e. triple rearrangements involving chromosome 15/17 and other) were accounted as 1 ACA. All additional abnormalities detected in FISH were considered at a sensitivity level >8–10%. A complex karyotype was defined as the presence of two or more ACA. A very complex karyotype was defined as the presence of three or more ACA.

**Data collection and prognostic factors**

The following patient and disease characteristics documented at initial evaluation were examined to establish their relationship to the complex karyotype: age, gender, ECOG score, fever, and total body surface as well as liver and spleen enlargement, creatinine, uric acid, lactate dehydrogenase (LDH), alkaline phosphatases, total bilirubin, albumin, fibrinogen, hemoglobin level, platelet count, WBC count, blood blast cell percentage, bone marrow blast cell percentage, M3 subtype, FLT3-internal tandem duplication (FLT3-ITD) mutations, PML–RARα isoforms, and surface antigen markers CD2, CD7, CD11b, CD13, CD14, CD15, CD19, CD34, CD56, CD117.

**Statistical analysis**

Chi-square test, with Yates correction if necessary, was used to analyze differences in the distribution of categorical variables between patient subsets. The t-test and Mann–Whitney U test were used to detect differences in the distribution of continuous parametric and nonparametric variables, respectively. Unadjusted time-to-event analyses were performed by use of the Kaplan–Meier estimate [27] and, for comparisons, log-rank tests [28]. The probability of relapse was estimated by the cumulative incidence method (for marginal probability) [29,30]. Overall survival (OS) was calculated from the start date of induction therapy, whereas cumulative incidence of relapse (CIR) was calculated from the date of CR. For the CIR analyses, development of secondary myelodysplastic syndrome or acute leukemia, and death in CR were considered as a competing cause of failure. For all estimates in which the event “relapse” was considered as an end point, hematologic and molecular relapse, as well as molecular persistence at the end of consolidation, were each considered as uncensored events. Patient follow-up was updated on 15 September 2017. All p values reported are 2-sided. Multivariate analyses were performed by use of the Fine and Gray model for CIR [31]. Computations were performed using R 2.14.2 software package.
Results

Frequency of additional chromosomal abnormalities

A total of 1559 de novo genetically confirmed APL patients were enrolled in the three consecutive trials. Karyotype studies were available in 1128 patients (72%). The remaining patients lacked cytogenetic data in the case report form (n = 167, 11%), or had unsuccessful karyotype with no growth (n = 264, 17%). All these patients were genetically diagnosed of PML/RARA APL by double color FISH studies, RT-PCR or anti-PML staining (anti-PGM3).

Overall, 842 out of 1128 patients with an available karyotype (75%) showed no additional abnormalities (i.e., normal karyotype or single t(15;17)), 197 patients (17%) showed 1 ACA, and 89 patients (8%) had a complex karyotype (2 or more ACA). In addition, 41 patients (4%) had a very complex karyotype with 3 or more ACA (Figure 2).

The frequency of patients with complex karyotype was similar across the three consecutive PETHEMA trials (6/127, 6% in the LPA96, 40/404, 10% in the LPA99, and 43/597, 7% in the LPA2005, p = .11).

Baseline characteristics of patients according to presence of complex karyotype

The main clinical and biological characteristics of APL patients with complex karyotype and those with either t(15;17) alone or single ACA are shown in Table 1. Baseline characteristics were similar between patients with complex karyotype and those with 0 to 1 additional abnormality. The only clinical or biological characteristic associated with a complex karyotype was a higher percentage of patients with CD34 antigen negativity in leukemic blasts (86% versus 75%, p = .04). No significant differences were observed between patients with very CK and those without CK+.

Influence of complex karyotype on outcome in APL

There was not a higher induction death rate in patients with a complex karyotype (6/89, 7% versus 87/1039, 8%; p = .74), and CR rate was also similar (93% versus 92%). Median follow-up of patients alive at the time of the analysis were 82 months (range, 2–236 months) from diagnosis. The 5-year CIR was 18% in patients with complex karyotype, compared with 12% in those with no or one ACA (p = .09) (Figure 3(A)). The CIR at 5 years was higher among patients with a very complex karyotype (3 or more ACA) compared with others (27% versus 12%) (p = .003) (Figure 3(B)). In the multivariate analysis,
the presence of three or more additional abnormalities retained the statistical significance \( p < 0.001 \), together with higher relapse-risk score \( p < 0.001 \), male gender \( p = 0.008 \), and PETHEMA LPA96&99 trials \( p = 0.05 \). Univariate and multivariate analysis for CIR are showed in Table 2.

Table 2. Multivariate analysis for cumulative incidence of relapse.

<table>
<thead>
<tr>
<th>Variable</th>
<th>( p ) – multivariate</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female gender</td>
<td>0.008</td>
<td>0.6 (0.4–0.9)</td>
</tr>
<tr>
<td>Higher relapse-risk group</td>
<td>&lt;0.0001</td>
<td>2.1 (1.5–2.9)</td>
</tr>
<tr>
<td>Very complex karyotype ( \geq 3 ) ACA</td>
<td>0.0009</td>
<td>2.7 (1.5–4.9)</td>
</tr>
<tr>
<td>PETHEMA LPA96&amp;99 trials</td>
<td>0.05</td>
<td>1.4 (1.0–2.1)</td>
</tr>
</tbody>
</table>

ACA: additional cytogenetic abnormalities; CI: confidence interval.
Figure 4. OS in the series according to the presence of complex karyotype (Panel A) or very complex karyotype (Panel B).
The 5-year overall survival was not different between patients with complex karyotype and those with no or one additional abnormality (83% versus 84%, \( p=.56 \)) (Figure 4(A)). The 5-year overall survival was not statistically different between patients with very complex karyotype and those with 0 to 2 additional abnormality (76% versus 83%, \( p=.48 \)) (Figure 4(B)).

Discussion

The analysis of this large series of APL patients with very mature follow up of more than 6.5 years (median) enrolled in three consecutive front-line PETHEMA protocols with a schedule based on ATRA plus chemotherapy combination shows that patients with three or more ACA have a much higher relapse rate compared to those with two or less ACA. Four percent of patients had three or more ACA, indicating that the subset of very CK APL represents a specific subset of the disease. The adverse prognostic impact for relapse of a very complex karyotype was confirmed in the multivariate analysis. Very CK did not impact on OS.

To the best of our knowledge, this is the largest study analyzing the frequency and the impact of a complex karyotype in genetically diagnosed de novo APL patients. Interestingly, roughly one quarter of patients failed the cytogenetic analysis, mainly because of absence of sufficient evaluable metaphases. Although there might be a trend towards disregarding conventional karyotyping from the diagnostic screening for APL, in our protocols this technique was recommended for all patients, which is reflected by an adherence of 72% of enrolled patients.

It appeared that 25% of APL patients had at least one ACA at diagnosis, consistent with previous observations [1,4,11,14]. Our data presented here on complex karyotypes are novel. The frequency of a complex karyotype has been rarely reported in APL [4,11,14,15]. We found that 8% had a complex karyotype (2 or more ACA), as in two previously reported studies of much smaller numbers [14,15]. In addition, we observed that 4% had three or more additional abnormalities (defined as a very complex karyotype), which is in the range of that reported by Wiernick et al. in a relatively small series (6 out of 140 patients) [13].

Little is known about the characteristics of APL patients with complex karyotype at diagnosis. Several studies have suggested significant associations between the presence of ACA and lower platelet counts, less low relapse-risk score, presence of coagulopathy [11], less M3v morphology [3,12], breakpoint at BCR3 region [2], FLT3 gene mutations [32,33], and a trend towards younger age [4,11]. In our study, we explored the relationship between complex karyotype and the previously mentioned characteristics, but we were not able to confirm any of these associations.

Accordingly, Poire et al. showed that the presence of complex karyotype was not associated with age, sex, initial WBC, morphologic subset, specific isoform, or FLT3 mutations [15]. In our study, the only biological characteristic associated with a complex karyotype was CD34 antigen negativity in leukemic blasts, but the correlation was weak (\( p=.04 \)).

Regarding the clinical outcome, we found no effect of complex karyotype on CR rate, as previously described for ACA, both in the pre-ATRA, ATRA, and ATO era [8,14]. An earlier analysis in 495 patients from the PETHEMA LPA96&99 trials had demonstrated that the 5-year relapse-free survival showed a non-significant trend that correlated with the number of ACA, (90% without ACA; 86% with 1 ACA; 83% with 2 ACA; and 78% with 3 ACA). Our data suggests that, as in other AML subtypes [34], an increasing number of genetic aberrations identified by conventional karyotyping/FISH confers a higher risk of relapse in APL. In line with the randomized intergroup trial C9710 [15], more relapses and less OS was observed among patients with complex karyotypes as compared to those with sole t(15;17) or with one ACA (5-year OS 53% versus 81%), regardless of the post-remission therapy (ATO versus ATRA plus chemotherapy). However, Poire et al. did not report the characteristics of complex karyotype patients, and a multivariate analysis was not performed.

We believe that it is premature to make any recommendation for risk-adapted strategies or tight molecular monitoring in APL patients with more complex cytogenetics [35], especially when the paradigm of front-line therapy for APL is moving to chemotherapy-free regimens and the prognostic factors should be revisited under the new circumstances. Nonetheless, our study supports the continued routine use of cytogenetics in the diagnostic work-up of APL [36].

In conclusion, this study shows, for the first time, APL with very complex karyotype (at least three ACA) represents a distinct subgroup of the disease with a significantly enhanced risk of relapse.

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References


**Appendix**

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