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Acute Myeloid Leukemia

The incidence of submicroscopic deletions in reciprocal translocations is similar in acute myeloid leukemia, BCR-ABL positive acute lymphoblastic leukemia, and chronic myeloid leukemia

We compared the incidence of submicroscopic deletions accompanying balanced translocations using interphase fluorescence *in situ* hybridization (FISH) in 245 patients with chronic myeloid leukemia (CML), 79 patients with acute lymphoblastic leukemia (ALL) and BCR-ABL (n=70) or MLL rearrangements (n=29), and 412 patients with acute myeloid leukemia (AML) with CBF β -MYH11 (n=122), PML-RAR α (n=108), AML1-ETO (n=112), or MLL rearrangements (n=98). The incidence of submicroscopic deletions was 2-9% depending on the entity.

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Submicroscopic deletions adjacent to the breakpoints of balanced translocations were identified in 9%-16% of all cases of chronic myeloid leukemia (CML) by interphase fluorescence *in situ* hybridization (FISH).^{1,2} The rate of hematologic and cytogenetic responses to imatinib was statistically significantly lower in patients with deletions.¹

So far only a few studies, with limited numbers of cases, have examined the incidence and prognostic impact of submicroscopic deletions in balanced translocations in acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) (Table 1). We, therefore, determined the incidence of submicroscopic deletions in the most common balanced rearrangements in leukemia using interphase FISH. This study was based on 245 cases with CML, 79 patients with ALL, and 412 patients with AML, who were referred to our laboratory from January 2000 to April 2004. The cohort of patients with AML comprised 112 patients with AML1-ETO, 108 patients with PML-RAR α , 122 patients with CBF β -MYH11, and 96 patients with different MLL rearrangements. The cohort with ALL comprised 70 patients with BCR-ABL positive ALL and 29 patients with different MLL rearrangements. Cytogenetic analysis was performed in all cases. FISH was performed on interphase nuclei and/or metaphases on bone marrow smears or blood smears. We used a BCR-ABL two color/two fusion probe (Cancer Genetics, CGPT 07), a LSI AML1-ETO dual color, dual fusion translocation probe, a LSI PML-RAR α dual color, dual fusion translocation probe, a LSI CBF β dual color, break apart rearrangement probe (Core Binding Factor β -subunit), and a LSI MLL dual color, break apart rearrangement probe (Abbott, 5J 63-01). At least 100 interphase nuclei were viewed for each case. The analyzing system, ISIS[®] (MetaSystems, Altusheim, Germany), was used for documentation.

In all cases the leukemia-specific fusion transcripts were also amplified by reverse transcription polymerase chain reaction (RT-PCR). For analysis of MLL fusions with partner genes in AML and ALL the respective RT-PCR was performed. In CML we found submicroscopic deletions in 9% of cases (22/245) with interphase FISH. In BCR-ABL positive ALL the incidence was 6% (4/70) and in ALL with MLL rearrangements it was 3% (1/29). In the different subgroups of AML the incidence of deletions was between 2% and 8% (AML1-ETO: 4% (4/112); CBF β -MYH11: 2% (3/122); PML-RAR α : 6% (7/108); MLL rearrangements: 8% (8/96) (χ^2 , n.s.) (Table 1).

Submicroscopic deletions occur in 9-16% of patients with CML and are clearly associated with an inferior prognosis.^{1,2} We determined the incidence of submicroscopic deletions in the most frequent reciprocal translocations in acute leukemias, as well as in CML. Our results in CML (deletions in 9%) and in BCR-ABL positive ALL (deletions in 6%) were in the ranges reported in the literature.¹⁻³ The incidence that was found in ALL with different MLL rearrangements was 3%, which is lower than published so far.⁴ We observed deletions in 3% of AML with PML-RAR α and in 4% of AML with AML1-ETO. Kolomietz *et al.* did not find deletions in subtypes of AML; to our knowledge their study is the only other examination of submicroscopic deletions in these subtypes.² The incidence of submicroscopic deletions in AML with CBF β -MYH11 was 2% in our study.

These data provide a strong indication that the frequency of these deletions is much lower than previously published (10-33%).^{2,5,6} We found submicroscopic del-

Table 1. Comparison of our data and previously published data: incidence of submicroscopic deletions in leukemias with reciprocal translocations.

Disease	Reference
CML	22/245 (9%): this study 23/250 (9.2%): Kolomietz <i>et al.</i> , 2001 39/241 (16.2%): Huntly <i>et al.</i> , 2001
BCR-ABL positive ALL	4/70 (6%): this study 1/13 (7.7%): Kolomietz <i>et al.</i> , 2001 4/45 (9%): Specchia <i>et al.</i> , 2003
ALL with MLL rearrangements	1/29 (3%): this study 3/18 children (17%): Barber <i>et al.</i> , 2004
AML with AML1-ETO	4/112 (4%): this study 0/14 (0%): Kolomietz <i>et al.</i> , 2001
AML with CBFβ-MYH11	3/122 (2%): this study 5/15 (33%): Martinet <i>et al.</i> , 1997 2/20 (10%): Kolomietz <i>et al.</i> , 2001 6/42 (14%): Marlton <i>et al.</i> , 1995
AML with PML-RARA	3/108 (3%): this study 0/30 (0%): Kolomietz <i>et al.</i> , 2001
AML with MLL rearrangements	8/96 (8%): this study 0/22 children (0%): Mathew <i>et al.</i> , 1999
ALL and AML with MLL rearrangements	7/43 (16%): Kolomietz <i>et al.</i> , 2001

tions in 8% of our cohort with AML with different *MLL* rearrangements. This is in contrast to the results of Mathew *et al.*, who did not find submicroscopic deletions in 22 children with AML and *MLL* rearrangements.⁷

In conclusion, we found a similar incidence of 2-9% of submicroscopic deletions in a variety of leukemias. Although we analyzed a high number of patients we were unable to determine the prognostic impact of these deletions in acute leukemias (*data not shown*) because of the limited number of patients with submicroscopic deletions and the specific individual prognoses: AML with favorable balanced translocations have a good prognosis; *MLL* rearrangements in acute leukemias and *BCR-ABL* positive ALL show a poor outcome.

Submicroscopic deletions could represent a non-specific event in different types of leukemia without an association with a specific entity.² Polymerase chain reaction and Southern blot analyses of reciprocal breakpoints in ALL with *MLL* rearrangements and in CML showed a high rate of submicroscopic deletions at the molecular level.^{8,9} The loss of tumor-suppressor genes due to submicroscopic deletions in CML might play a role in the worse prognosis of these patients.¹ Given the very low incidence of cases with deletions accompanying reciprocal translocations in AML and ALL, definitive conclusions on prognosis cannot be drawn.

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Key words: submicroscopic deletions, interphase fluorescence *in situ* hybridization, balanced translocations, AML, BCR-ABL positive ALL.

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Multiple Myeloma

Global real-time quantification/reverse transcription-polymerase chain reaction for detecting proto-oncogenes associated with 14q32 chromosomal translocation in multiple myeloma

A global real-time quantitative/reverse transcription-polymerase chain reaction technique for detecting the expression of six 14q32 chromosomal translocation-associated proto-oncogenes in marrow plasma cells was established and applied to myeloma specimens. This technique is an alternative method of detecting 14q32 rearrangements and allows investigation of the relationship between proto-oncogene expression and clinical features.

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Chromosomal translocations involving the immunoglobulin heavy chain gene (*IGH*) locus (14q⁺) play important roles in multiple myeloma (MM).^{1,2} The transcriptional acti-