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Acute Myeloid Leukemia

Multiplex reverse transcription polymerase chain reaction screening in acute myeloid leukemia detects cytogenetically unrevealed abnormalities of prognostic significance

A commercial multiplex reverse transcription polymerase chain reaction screening assay, covering 28 leukemic fusion transcripts, was applied in 143 samples obtained from patients with acute myeloid leukemia at primary diagnosis. In five patients, a cytogenetically unrevealed fusion gene of prognostic importance was detected, while the assay failed to detect one case of t(15;17).

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Current prognostic stratification of acute myeloid leukemia (AML) at diagnosis is based on conventional cytogenetics. Recognition of patients with the abnormalities t(8;21), t(15;17) and inv(16) is of particular interest in AML prognostication because these abnormalities are associated with a relatively favorable prognosis.[1-4] Conventional karyotyping may be hampered by insufficient quality or number of metaphases, low sensitivity or selective outgrowth. Furthermore, translocations of prognostic significance may be cytogenetically undetected if they involve regions with similar band patterns.[5-8] The aim of this study was to evaluate the additional prognostic information obtained by multiplex reverse transcription polymerase chain reaction (RT-PCR) screening for leukemia-associated genes with a commercially available assay. Multiplex RT-PCR screening for translocations is independent of dividing cells, has a high level of sensitivity, and may identify translocations that are not detected by conventional karyotyping. The HemaVision® Multiplex-RT-PCR Screen Test (DNA Technology, Aarhus, Denmark) detects 28 of the most common leukemic fusion genes and more than 80 splice variants[7,9,10] (Table 1). In brief, reverse transcription is performed with a mixture of translocation-specific primers. PCR amplification is performed in two steps: a master PCR amplification followed by nested PCR which screens for the presence of fusion transcripts and a split-out PCR amplification followed by nested PCR which identifies the specific fusion transcript(s). Each of the 8 parallel nested multiplex master PCR reactions contains a mixture of primer pairs for the detection of several fusion transcripts and two primer pairs for an internal control gene product of 911 base pairs. When the presence of one or more fusion transcripts is detected by one or more master PCR reactions, the corresponding split-out reactions with individual primer pairs are performed.

To assess the additional prognostic information obtained by the multiplex RT-PCR screening test, we performed a retrospective study of 143 patients with a median age of 63 years (range 0-85)(132 adults, 11 children). Inclusion criteria were: (i) adults diagnosed with AML at Herlev Hospital, Denmark, during a 12-year period from

Table 1. Fusion transcripts detected in 143 samples by the multiplex RT-PCR screen test.

Balanced translocations covered by the multiplex RT-PCR screen test		*No. of fusion transcript positive cases*	
Translocation	*Fusion transcript*	*Adults (N=132)*	*Children (N=11)*
t(1;11)(p32;q23)	MLL1/AF1p	0	0
t(1;11)(q21;q23)	MLL1/AF1q	0	0
t(1;19)(q23;p13)	E2A/PBX1	0	0
t(3;21)(q26;q22)	AML1/EAP/MDS1/EVI1	0	0
t(3;5)(q25.1;q34)	NPM/MLF1	0	0
t(4;11)(q21;q23)	MLL1/AF4	0	0
t(5;12)(q33;p13)	TEL/PDGFRβ	0	0
t(5;17)(q35;q21) /(q34;q21)	NPM/RARα	0	0
t(6;11)(q27;q23)	MLL1/AF6	1	0
t(6;9)(p23;q34)	DEK/CAN	0	0
t(8;21)(q22;q22)	AML1/MGT8	3	1
t(9;11)(q22;q23)	MLL1/AF9	2	0
t(9;9)(q34;q34)	SET/CAN	0	0
t(9;12)(q34;p13)	TEL/ABL	0	0
t(9;22)(q34;q11)*	BCR/ABL*	4**	0
t(10;11)(p12;q23)	MLL1/AF10	0	2
t(11;17)(q23;q21)	MLL1/AF17	0	0
t(11;17)(q23;q21)	PLZF/RARα	0	0
t(11;19)(q23;p13.1)	MLL1/ELL	0	0
t(11;19)(q23;p13.3)	MLL1/ENL	0	1
t(12;21)(p13;q22)	TEL/AML1	0	0
t(12;22)(p13;q11)	TEL/MN1	0	0
t(15;17)(q21;q22)	PML/RARα	3	1
t(16;21)(p11;q22)	TLS/ERG	0	0
t(17;19)(q22;p13)	E2A/HLF	0	0
inv(16)(p13;q22)	CBFβ/MYH11(A) or (B)	2***	1
t(X;11)(q13;23)	MLL1/AFX1	0	0
TAL1deletion(p34)	SIL/TAL1	0	0
Total		**15**	**6**

**The screening test covers the BCR/ABL variants: b2ab, b3a2, c3a2, b2a3, b3a3, e1a2, e6a2, e1a3. **t(9;22)(q34;q11) BCR/ABLb3a2 and t(9;22)(q34;q11)BCR/ABLe1a2 were detected in one case, t(9;22)(q34;q11)BCR/ABLb2a2 and t(9;22)(q34;q11) BCR/ABL e1a2 were detected in one case , and t(9;22)(q34;q11)BCR/ABLb3a3 and t(9;22)(q34;q11) BCR/ABL e1a2 were detected in one case. ***CBFα/MYH11(A) and CBFβ/MYH11 (B) were detected in one case.*

Table 2. Results of karyotyping and multiplex RT-PCR screening in the six cases for which the two methods gave discordant results.

Case	*Sex/age(years)*	*Karyotype*	*Multiplex RT-PCR*
1	M/24	50,XY,+8,+9,+13,+14 [15][1]	CBFβ/MYH11(A)*[1]
2	M/42	45,X,-Y [15][1]	AML1ex5/MGT8(ETO)*[1]
3	M/3	47,XY,del(8)(q12q22),del(9)(q12q22),der(11), add(11)(p14)del(11)(q23),+13,del(20)(p11) or q(11) [26][1]	AML1ex5/MGT8(ETO)**[1]
4	F/1	46,XX, add(5)(p14-15),del(15)(q15q22),add(17)(q11) [25], suspected abnormality of PML/RARA***[1]	PML/RARα****[1]
5	F/4	46,XX [25][1]	MLL1/AF10**[2]
6	F/62	46,XX,t(15;17)(q22;q12) [15][1], verified by FISH	No fusion transcript detected[1]

**The translocations were retrospectively recognized by re-evaluation of specimens for cytogenetic analyses. **The translocations were retrospectively confirmed by FISH with loci-specific probes. ***By spectral karyotyping at diagnosis the following karyotype was described: 46,XX, der(5)t(1;5)(p14-15;?),del(15)(q15;q22), both chromosomes 17 normal. ****A t(15;17)(q21;q22) was detected at primary diagnosis by RQ-PCR. [1]Analysis performed on bone marrow. [2]Analysis performed on blood.*

1992 to 2003 or children diagnosed during a 3-year period from 2001-2004 at the two departments of pediatric oncology that treat all cases of childhood AML in Denmark; (ii) cytogenetic analysis had been performed at the time of primary diagnosis; and that (iii) that multiplex RT-PCR screening either had been performed at primary diagnosis or could be performed retrospectively using cryopreserved cells obtained at diagnosis. All samples (126 bone marrow/17 blood) were obtained at the time of diagnosis prior to therapy. By multiplex RT-PCR, a total of 26 fusion transcripts were detected in 21/143 samples (15%) while no abnormality was detected in 122/143 samples (85%).

In 137/143 samples there was agreement between the results of RT-PCR and conventional karyotyping: In 121/143 samples, no abnormality was detected by either method. In 16/143 samples a total of 20 fusion transcripts were detected by multiplex RT-PCR in agreement with the karyotype, in four of these samples, two fusion transcripts were detected by multiplex RT-PCR in each sample (Table 1). In 6/143 cases, the results of RT-PCR screening and conventional karyotyping did not agree (Table 2). In one adult case out of the 143 samples, a t(15;17) was detected by cytogenetics and verified by fluorescence *in situ* hybridization (FISH) while the fusion transcript was not detected by multiplex RT-PCR analysis. Five cytogenetically unrevealed abnormalities were detected in 5/143 samples: t(8;21) and inv(16) in two adult samples; t(8;21), t(15;17) and t(10;11) in three pediatric samples. All cytogenetically unrevealed abnormalities were retrospectively confirmed by molecular methods or recognized by re-evaluation of the specimens for conventional karyotyping (Table 2). The cytogenetically unrevealed abnormalities detected by the screening assay were all of prognostic significance.

In conclusion, the present report supports the belief that molecular methods such as RT-PCR or FISH for detection of t(8;21), t(15;17) and inv(16) should complement conventional karyotyping at diagnosis. It remains to be evaluated whether cytogenetically unrevealed abnormalities detected by RT-PCR have the same prognostic significance as the corresponding translocations detected by conventional karyotyping. Consequently, translocations identified by RT-PCR should be confirmed by other methods, such as conventional karyotyping or FISH if they have implications on treatment. Although the number of pediatric patients and younger adults in this study was low, the results suggest that additional prognostic information obtained by this screening assay may predominantly be found in children and younger adults with AML.

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Acute Myeloid Leukemia

Treatment of elderly acute myeloid leukemia with valproic acid and all-trans retinoic acid

Valproic acid (VPA) has been demonstrated to be able to inhibit histone deacetylase activity and to synergize with all-trans retinoic acid (ATRA) in inducing the differentiation of acute myeloid leukemia (AML) cells. A pilot study of the VPA/ATRA combination was performed in 11 elderly patients with *de novo* AML (median age, 82 years). Complete marrow response was observed in 3 patients, including 1 complete remission. Two additional patients had hematologic improvement.

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Acute myeloid leukemia (AML) is characterized by arrested differentiation of leukemic cells at an early stage of maturation. Revival of the maturation process and reversal of the immature phenotype have been obtained with all-*trans* retinoic acid (ATRA) in acute promyelocytic leukemia (APL) cells (AML-M3 in the French-American-British classification). *In vitro*, ATRA is also capable of inducing differentiation of non-M3 AML cells, such as HL60 cells. The differentiation process is dramatically increased in the presence of cyclic adenosine monophosphate (cAMP).[1] However, *in vivo*, ATRA-induced differentiation of AML cells has only been observed in APL patients. Apart from the PML-RARα fusion oncoprotein target, one explanation of this selective effect might be that the ATRA-receptor complex is unable to bind target gene promoters because of epigenetic chromatin modifications, such as hypermethylation of promoter CpG islands, recruitment of co-repressors, and histone amino acid deacetylation. Some studies using sodium phenylbutyrate as a histone deacetylase (HDAC) inhibitor in patients with AML or myelodysplastic syndromes (MDS) have already been reported.[2]

In the present study, we administered ATRA in combination with another HDAC inhibitor, namely valproic acid (VPA),[3] in elderly patients with AML. Of interest, VPA has been demonstrated to be able to synergize with ATRA in inducing the differentiation of non-M3 AML cells and more recently to be capable, as a single agent, of inducing clinical responses in patients with MDS or secondary AML.[4,5] All consecutive patients aged 70 years or more with *de novo* non-M3 AML unsuitable for intensive chemotherapy were eligible after informed consent had been obtained. Between November 2001 and August 2003, 11 patients (median age, 82 years) were treated (Table 1). Two eligible patients diagnosed during the same period were not included because of white blood cell counts (WBC) > 30×10^9/mm^3. Oral VPA was administered daily at the dosage required to reach therapeutic serum concentrations for treatment of seizures (between 50 and 100 μg/mL). ATRA was given daily at the dosage of 45 mg/m^2/day, beginning a week after the initiation of VPA. In order to increase intracellular cAMP levels, patients also received theophylline orally at the dosage required to reach therapeutic serum concentrations for treatment of asthma (between 10 and 15 μg/mL), at least when well tolerated. Patients with WBC > 10×10^9/mm^3 (patients nos. 1, 2, 5, 7, and 9) initially received low-dose hydroxyurea