Expression of recombination activating genes-1 and-2 immunoglobulin heavy chain gene rearrangements in acute myeloid leukemia: evaluation of biological and clinical significance in a series of 76 uniformly treated patients and review of the literature

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Background and Objectives. Early lymphoid differentiation is characterized by antigen receptor gene rearrangements; the rearrangement process is governed by two lymphoid-specific genes, RAG (recombinase activating gene)-1 and -2. The available data on the incidence and prognostic significance of clonal immunoglobulin heavy chain (IgH) gene rearrangements in acute myeloid leukemia (AML) are rather contradictory. The aim of this study was to evaluate the incidence and prognostic significance of RAG-1 and -2 mRNA transcripts and clonal IgH gene rearrangements in a cohort of uniformly treated AML patients; the available literature is also reviewed.

Design and Methods. The study was performed on 76 AML patients, newly diagnosed between August 1996 and November 1999. RAG-1/-2 gene expression was analyzed by a reverse transcriptase polymerase chain reaction technique and IgH gene rearrangements were detected with variable region (V_H) family-specific and consensus framework region (FWR)-2 and/or-3 primers. Statistical associations were explored between IgH monoclonality/ RAG mRNA expression and: (i) age, gender, FAB subtype, immunophenotype, cytogenetic risk groups; (ii) response variables (response/relapse incidence, survival).

Results. In total, 38/75 samples (50.6%) were RAG-1 and/or -2 positive; 30/76 samples (39.5%) carried clonal IgH genes, whereas 13/30 IgH-positive samples (43.3%) were RAG-1/2-negative. Significant associations were detected only for RAG-2 positivity and unfavorable karyotype and IgH monoclonality and FAB subtypes M4/ M5; no association was identified with response outcome and survival.

Interpretation and Conclusions. Lymphoid-specific molecular markers are detected in a significant proportion of AML patients, regardless of differentiation status (assessed morphologically/ immunophenotypically); however, in our experience, they do not seem to constitute an adverse prognostic factor.

Key words: AML, immunoglobulin gene rearrangements, RAG.

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Cells belonging to the lymphoid lineage recognize antigen through highly specific membrane receptor molecules. The extraordinary diversity of antigen receptors rests on a highly specialized process of DNA rearrangement, which brings together one each of several discontinuous gene segments and creates the exon encoding for the variable region of the antigen receptor molecules. Antigen receptor gene rearrangements [V-(D)-J recombination] are guided by an enzymatic complex (the *recombinase*)[1] regulated by at least two lymphoid-specific genes [recombinase activating gene (RAG)-1 and -2].[2] RAG-1 and -2 appear to be coordinately transcribed: however, this is not always the case.[3]

Acute myeloid leukemia (AML) represents the clonal outgrowth of cells arrested while undifferentiated or in early stages of myeloid differentiation.[4] However, a proportion of AML patients display surface antigens associated with lymphoid development;[5,6] furthermore, rearrangements of antigen receptor genes have also been detected in AML, albeit at varying frequencies.[7,8] It is difficult to draw firm conclusions from these data due to significant differences not only in study populations (e.g., children versus adults, selection for specific AML subtypes) but also, and perhaps more importantly, in the methodology applied.

Over the last decade, several reports have addressed the prognostic impact of lymphoid antigen expression in AML with rather discrepant results.[5,9] The experience on the biological consequences and possible prognostic significance of lymphoid-specific gene expression in AML is even more limited: the only two comprehensive studies addressing the prognostic impact of clonal immunoglobulin heavy chain gene rearrangements in AML patients reached markedly different conclusions.[10,11]

In this study, we report on the incidence of RAG-1 and -2 mRNA transcripts and monoclonal heavy chain variable region gene rearrangements in 76 AML patients, newly diagnosed and uniformly treated between August 1996 and November 1999. We also present the results of statistical analyses aimed at assessing the prognostic impact of these variables and possible associations with specific disease characteristics.

Design and Methods

Patients and cell samples

Bone marrow (BM) samples were collected from 76 patients with *de novo* AML diagnosed between August

Table 1. Patients' characteristics.

Characteristics	*N*	*(%)*
Age (years)		
>60	25/76	33
<60	51/76	67
Gender		
Male	38/76	50
Female	38/76	50
FAB subtypes		
M0	4/76	5
M1	15/76	20
M2	24/76	31
M3	9/76	12
M4 / M4 Eo	15/76-1/76	20/1
M5	5/76	7
M6	3/76	4
Cytogenetics		
Favorable	16/70	23
Intermediate	30/70	43
Unfavorable	24/70	34
Immunophenotype		
CD2	3/68	4
CD3	1/68	1
CCD3	5/68	7
CD7	18/68	26
CD10	1/68	1
CD19	11/68	16
CD20	1/68	1
CD56	10/68	15
Response*	48/76	63
Relapse	21/48	44

**attainment of complete remission.*

Table 2. Oligonucleotide primers used for RT- PCR in the present study.

RAG-1A	5'-CCAAATTGCAGACATCTCAAC-3'
RAG-1B	5'-CAACATCTGCCTTCACATCGATCC-3'
RAG-228	5'-TTGGCATACCAGGAGACAAT-3'
RAG-229	5'-ACTATTTGCTTCTGCACTGA-3'
RAR6	5'-GGTGCCTCCCTACGC CTTCT-3'
RAR8	5'-GGCGCTGACCCCATAGT GGT-3'
VH1	5'-CAGGTGCAGCTGGTGCAGTCTGG-3'
VH2	5'-CAGGTCAACTTAAGGGAGTCTGG-3'
VH3	5'-GAGGTGCAGCTGGTGGAGTCTGG-3'
VH4	5'-CAGGTGCAGCTGCAGGAGTCGGG-3'
VH5	5'-GAGGTGCAGCTGTTGCAGTCTGC-3'
VH6	5'-CAGGTACAGCTGCAGCAGTCAGG-3'
JH1-2	5'-TGAGGAGACGGTGACCAGGGTGCC-3'
JH3	5'-TGAAGAGACGGTGACCATTGTCCC-3'
JH4-5	5'-TGAGGAGACGGTGACCAGGGTTCC-3'
JH6	5'-TGAGGAGACGGTGACCGTGGTCCC-3'
FWR2	5'-TGG[A/G]TCCG[A/C]CAG[G/C]C[C/T][C/T]C[A/C/G/T]GG-3'
FWR3	5'-ACACGGC[C/T][G/C]TGTATTACTGT-3'

1996 and November 1999. Clinical and hematologic laboratory data at diagnosis before treatment were available in all 76 patients (Table 1). Patients were consecutively enrolled in two different treatment protocols according to age (> or < 60 years old); within each group, patients were treated uniformly.

Briefly, patients younger than 60 received 2 courses of induction therapy consisting of idarubicin 12 mg/m^2 i.v and cytarabine 200 mg/m^2 in 24-hour continuous i.v. infusion (course 1: days 3+7, course 2: days 2+5). Patients achieving a CR were assigned or randomized to one of three post-remission therapies. Those with a histocompatible sibling donor were assigned to allogeneic hematopoietic cell transplantation (HCT). Those without matched donors were randomized to either intensive consolidation chemotherapy (two courses of cytarabine at 3 g/m^2 over 3 hours in an i.v. infusion twice daily over days 1, 3, 5) or autologous HCT with stem cells collected on recovery from a consolidation course as described above. Patients older than 60 were treated with two identical induction courses with oral idarubicin (20mg/m^2, days 1-3) and etoposide (160mg/m^2, days 1-5), followed by two identical courses administered as consolidation therapy; patients were then given maintenance treatment with 6-mercaptopurine and methotrexate. Granulocyte colony-stimulating factor (G-CSF) was routinely administered to all patients, starting with the second induction course. In both age groups, AML-M3 cases received specific treatment containing all-*trans* retinoic acid.

FAB morphologic classification was based on morphology and cytochemistry. Flow cytometry was used to confirm FAB M0 cases. Immunological marker analysis was performed on freshly isolated mononuclear cells. Cytogenetic analysis of bone marrow unstimulated mononuclear cells (obtained prior to induction treatment) was conducted by standard G-banding; karyotypes were interpreted using International System for Cytogenetic Nomenclature criteria (1995).[12] Samples were considered normal diploid if no abnormalities were detected in a minimum of twenty mitotic cells examined. Three cytogenetic risk categories were defined;[13,14] favorable [(inv(16)/t16;16/del(16q), t(15;17), t(8:21) with/without secondary aberrations], intermediate (normal, +8), unfavorable (all other abnormalities).

RNA extraction and cDNA preparation

Total cellular RNA was isolated by the guanidinium isothiocyanate method.[15] *In vitro* reverse transcription of 3 μg of total cellular RNA to cDNA was performed using Moloney Murine Leukaemia Virus reverse transcriptase (Gibco-BRL, Gaithersburg, MD, USA) and random hexamers as primers (Gibco-BRL, Gaithersburg, MD, USA). After an initial denaturation of 5 min at 65°C, the reaction mixture was incubated at 37°C for 60 min.

As a control for the presence of amplifiable RNA, 5 μL of the reverse transcription cDNA product were amplified by polymerase chain reaction (PCR) using primers specific for the retinoic acid receptor α (RARα) gene (Table 2). Amplification consisted of an initial denaturation step of 5 min at 94°C, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 1 min and extension at 72°C for 1 min with a final extension step of 10 min at 72°C.

PCR amplification of RAG-1 and RAG-2 transcripts

Five microliters of the reverse transcription reaction product were amplified by PCR using 40 pmol each of primers specific for the RAG-1 (RAG-1A/ RAG-1B)[16] and RAG-2 (RAG-228/ RAG-229)[17] genes (Table 2). PCR was carried out in a final volume of 100 μL with 40 pmol of each primer, 200 μM each of dNTP, 2.5 U Taq polymerase (Gibco-BRL, Gaithersburg, MD, USA) in PCR buffer (50mM KCL, 10 mM Tris-HCl pH 8.0, 1.5 mM $MgCl_2$, 0.01% gelatin). Amplification consisted of an initial denaturation step of 5 min at 94°C, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min for RAG-1 and 55°C for 1 min for RAG-2 and extension at 72°C for 1 min with a final extension step of 10 min at 72°C.

PCR amplification of immunoglobulin heavy (V_H) chain variable region genes

Five microliters of the reverse transcription reaction were amplified by PCR using a mixture of oligonucleotides specific for each of the V_H leader sequences of the V_H1-6[18] gene families together with a mixture of oligonucleotides complementary to all possible JH gene segments J_H1-6[19] (Table 2). PCR was carried out in a final volume of 100 μL with 40 pmol of each equimolar mixture of oligonucleotide primers, 200 μM each of dNTP, 2.5 U Taq polymerase (Gibco-BRL, Gaithersburg, MD, USA) in PCR buffer (50mM KCL, 10 mM Tris-HCl pH 8.0, 1.5 mM $MgCl_2$, 0.01% gelatin). Amplification consisted of an initial denaturation step of 5 min at 94°C, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 61°C for 1 min and extension at 72°C for 1 min with a final extension step of 10 min at 72°C. PCR products were analyzed on 6% polyacrylamide gels.

In the second round (nested PCR), 1 μL of amplified DNA (1st round product) was reamplified using as primers oligonucleotides representative of framework regions (FWR)-2 and -3 (FWR2/ FWR3)[19] (Table 2) together with the mixture of J_H1-6 oligonucleotides. PCR was carried out as described above. Amplification consisted of an initial denaturation step of 5 min at 94°C, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min with a final extension step of 10 min at 72°C. PCR products were analyzed on 8% polyacrylamide gels.

PCR sensitivity was performed with dilution experiments (RNA in RNA); the dilution was performed in 10-fold steps, from undiluted to 10^{-6}. We used a bone marrow sample of a patient with pre B-ALL and almost 100% infiltration at diagnosis as the positive control and peripheral blood lymphocytes from healthy volunteers as the negative control. The first round PCR reached a sensitivity level of $10^{-3}/10^{-4}$, while the second round of amplification reached a reproducible sensitivity level of $10^{-4}/10^{-5}$.

Both in the reverse transcription reaction and in the ensuing amplification reactions, recommended measures to prevent cross-contamination of samples were followed.[20] In addition, for each experiment, a control with no template was used to check for the presence of any contaminant. In positive cases, controls with all reagents including RNA albeit without the reverse transcriptase enzyme were used to rule out the possibility of cDNA contamination of the RNA samples.

Statistical evaluation

The categorical variables taken into account in this analysis are as follows: (i) age (two levels, <60 years and >60 years) (ii) gender; (iii) FAB subtype (seven levels, M0 through to M6) (iv) *lymphoid-specific* (B/T) surface markers (two levels, presence and absence); special emphasis was given to CD56 expression; (v) karyotype (three levels: favorable, intermediate and unfavorable risk groups); (vi) RAG-1, RAG-2, RAG-1/-2 mRNA (two levels, existence and non-existence); (vii) IgH monoclonality (two levels, presence and absence); (viii) response [two levels, complete remission (CR) and otherwise]; (ix) relapse (two levels, occurrence of relapse, no relapse). Survival time was measured with the related censoring information. The analysis of the relationships between categorical variables was split in two basic stages: i) in the first stage, the bivariate relationships between the existence of RAG (-1/-2) mRNA transcripts and IgH monoclonality with the remaining categorical variables were explored with the use of Fisher's exact test at a signicance level of 0.1 so as to allow for potential associations. The same test was used for the study of the relationships between response and relapse incidents with the remaining variables; ii) in the second stage, in cases showing significant bivariate relationships, a log-linear model was built to explore the existing associations, adjusting for other variables. Similarly, the analysis of the differences in survival time was implemented in two basic stages: i) in the first stage, the Kaplan-Meier procedure was conducted and a log-rank test was calculated to identify significant differences in survival

Table 3. RAG mRNA and IgH monoclonality data by certain *lymphoid-specific* surface markers.

	CD2	*CD3*	*cCD3*	*CD7*	*CD10*	*CD19*	*CD20*	*CD56*
RAG-1 (+)	0/14	0/14	0/14	4/14	0/14	3/14	1/14	0/14
RAG-2 (+)	0/32	0/32	3/32	8/32	1/32	5/32	1/32	3/32
RAG-1/2 (+)	0/36	0/36	3/36	9/36	1/36	6/36	1/36	3/36
IgH (+)	2/29	1/29	3/29	8/29	1/29	5/29	1/29	6/29
TOTAL*	3/68	1/68	4/68	18/68	1/68	11/68	1/68	10/68

**number of AML cases positive for a given marker (regardless of IgH clonality/RAG expression status)/number of analyzed cases.*

Table 4. RAG mRNA and IgH monoclonality data by cytogenetic risk group.

	Favorable	*Intermediate*	*Unfavorable*
RAG-1 (+)	5/13	4/13	4/13
RAG-2 (+)	3/32	13/32	16/32
RAG-1/2 (+)	6/36	13/36	17/36
IgH (+)	7/28	10/28	11/28
TOTAL*	16/70	30/70	24/70

**number of AML cases positive for a given marker (regardless of IgH clonality/RAG expression status)/number of analyzed cases.*

time for all variables at a significance level of p=0.1; ii) in the second stage, a Cox's proportional hazards model for survival data was implemented to investigate the relationship between the prognostic variables detected in stage A and survival time. All analyses were implemented with the use of the statistical package SPSS version 10.

Results

Detection of RAG-1 and RAG-2 transcripts and monoclonal Ig heavy variable region gene rearrangements

RAG-1 mRNA transcripts were detected in 15/75 samples (20%) while RAG-2 was found in 34/75 samples (45.3%). In total, 38/75 samples (50.6%) were RAG-1 and/or -2 positive; 30/76 samples (39.5%) carried clonally rearranged IgH variable region gene sequences, whereas 13/30 IgH-positive samples (43.3%) were RAG-1/2 negative. Interestingly, the vast majority of IgH-positive samples (28/30) tested positive already from the first round of amplification (with a sensitivity level of 10^{-3}/10^{-4}). RAG mRNA and IgH monoclonality data in specific karyotype and surface marker-positive AML subgroups are shown in Tables 3 and 4.

Table 5. Statistical associations between RAG-1 and –2 mRNA expression/presence of clonal IgH gene rearrangements and various clinical and biological features of the patients analyzed.

p-values	*RAG-1 mRNA*	*RAG-2 mRNA*	*RAG-1/-2*	*IgH*
Age	0.55	0.33	0.63	0.63
Gender	0.39	1.00	0.36	0.48
FAB*	0.77	0.79	0.92	0.01
Lymphoid markers	1.00	0.15	0.22	0.15
Karyotype°	0.28	0.04	0.34	0.95
Response	0.56	0.32	0.62	0.80
Relapse	1.00	1.00	0.77	0.37

**Monoclonal IgH rearrangements were found at a higher extent among patients with FAB subtypes M4 and M5. **RAG-2 mRNA transcripts were detected at a significantly higher rate among patients with unfavorable cytogenetics.*

Statistical analysis

The analysis was based on measurements of 76 patients. When data were missing, the analysis was focused on cases with complete records. All variables were recorded at a percentage exceeding 95% except for expression of lymphoid markers (89.5%) and karyotype (92%). Table 5 shows the results of Fisher's exact tests between the existence of the particular genetic markers and all other categorical variables. It can be seen that monoclonally rearranged IgH genes were present at a statistically significant higher extent among patients with AML FAB-M4 and M5. Furthermore, RAG-2 mRNA transcripts were detected at a significantly higher rate in patients with unfavorable cytogenetics.

Concerning outcome, none of the genetic markers under study (IgH monoclonality, RAG mRNA transcripts) was significantly associated with response and relapse incidences. In order to focus on the relationship between all other variables and the response outcomes (response and relapse), all bivariate significance Fisher's exact tests were calculated. Significantly higher percentages of complete remission were recorded among patients aged less than 60 years and patients falling in the favorable cytogenetic subgroup. Log linear model analysis between response and each of the categorical variables, after adjusting for the remaining variables, showed that a higher complete remission rate was recorded among patients of younger age (<60) (χ^2=21.8 at 1 d.f., p<0.01). Survival time was recorded for all 76 patients who entered the study. At the time of completion, 26 patients were still alive. With the use of the Kaplan-Meier procedure, survival time was calculated for each subgroup of patients and the log-rank test was used to explore survival differences between groups. None of the genetic markers under study was significantly associated with survival time (IgH monoclonality: p =

Table 6. Survival data: significant prognostic variables.

Groups	*Mean survival**	*Median survival**
Age: < 60/> 60	*34.6/8.3*	*38.0/2.5*
• FAB		
M0/M1/M2/M3/M4/M5/M6	14.7/13.0/30.7/42.2 21.3/21.0/2.7	2.0/3.5/21.0/°/9.0 6.0/2.0
• Cytogenetics		
Fav/Interm/Unfav#	36.0/28.1/15.2	38.0/21.0/6.0

**In months; °Median survival time was not calculated due to high percentage of censored data; #Fav: favorable; interm: intermediate; unfav: unfavorable.*

0.88; RAG-1 mRNA expression: $p = 0.79$; RAG-2 mRNA expression: $p = 0.98$). On the contrary, a significantly worse outcome was seen in patients of older age ($p < 0.01$), non-M2/M3 FAB subtypes ($p < 0.01$), and unfavorable cytogenetics ($p < 0.02$). Mean and median survival times for each subgroup are shown in Table 6. A Cox's proportional odds model investigating the simultaneous prognostic effect of age, FAB subtype and karyotype on survival time showed that age and FAB subtype were significant prognostic variables.

Discussion

The precise frequency of immunoglobulin gene rearrangements in AML is difficult to calculate from previously reported data[7,10,11] not only because several studies selected particular AML subtypes but also, and perhaps more importantly, different experimental protocols with markedly different sensitivities were adopted (Southern blot, various forms of PCR: RT-PCR, one- or two-round PCR, *whole VH region* or *FWR2* or *FWR* PCR).

To our knowledge, this is the largest study of IgH rearrangements in uniformly treated AML patients. Based on past experience both by our group and by others,[19,21,22] we adopted a sensitive RT-PCR approach (sensitivity level 10^{-5}) and performed two round amplifications using primers derived from VH family leader sequences (first round)[18] and FWR2 or FWR3 sequences (second round).[19] Our results indicate that immunoglobulin heavy chain genes are rearranged in a significant proportion of AML cases (30/76; 39.5%); similar results were obtained in the studies by Kyoda *et al.*[10] (14/35 cases; 40%) and Yen *et al.*[11] (11/39 cases; 28%). Of course, one cannot rule out the possibility that by adopting a sensitive RT-PCR protocol one may detect monoclonal IgH rearrangements in minor blast cell subpopulations; nevertheless, even if this were true in some cases, it would point to the propensity of the malignant clone to behave in an *illegitimate* way and would be evidence for some biological difference (with as yet undefined prognostic significance) from cases lacking clonally rearranged IgH genes. Importantly, we did not find any case positive for monoclonal IgH rearrangements among 20 healthy individuals; in the same context, it is of interest that monoclonal IgH gene rearrangements were detected in a previous study by our group in only 6/23 patients with chronic myeloid leukemia in chronic phase.[23] Altogether, the aforementioned results indicate that the relatively high incidence of clonally rearranged genes in AML is not very likely to result from amplification of minor clones or non-clonal reactive cells and probably represents a *bona fide* feature of AML. However, no associations have been detected between IgH monoclonality and either specific cytogenetic abnormalities (in analogy to what has been reported for lymphoid-associated surface marker expression in AML)[5,6] or immunophenotypic patterns. The possible correlation with morphology (FAB subtypes M4/M5) is intriguing given that, exclusively among FAB subtypes, M4 and M5 have also been described to express significantly more frequently the lymphoid-associated markers CD4[5] (perhaps not surprisingly, as normal monocytes are known to express CD4) and (perhaps) CD19.[24]

In the present study, RAG-1 mRNA transcripts were detected in 15 cases and RAG-2 in 34. There are several explanations for this discrepant finding: (i) RAG genes appear to be expressed and act coordinately to activate the recombinase;[2] however, exceptions to this rule are known to occur naturally;[25] (ii) this rather discrepant result may be attributed to technical reasons: generally, RAG-1 transcripts appear to be more difficult amplification targets; (iii) a final explanation might be that when the enzymatic recombination system functions *inappropriately* some of its components might be missing.[26]

Our analysis failed to detect any correlation between RAG mRNA expression in AML blasts and the presence of a clonally and completely rearranged V(D)J gene complex (at least in the sense that it could be amplified by the PCR protocols applied here). Of interest, a similar result was reported in the largest of the published series:[8] in that study, although RAG-1 mRNA expression was detected in a significant proportion of AML cases, it was not correlated with immunoglobulin gene rearrangement. In general, RAG mRNA expression was not associated with any biological feature of the AML blasts. The possible association of RAG-2 mRNA expression with unfavorable cytogenetics is of uncertain biological significance.

The *recombinase* normally recognizes highly conserved recombination signal sequences (RSS) (the heptamer CACAGTG and the nonamer ACAAAAACC) that flank one or both sides of all recombining gene segments.[1] Interestingly, a com-

puter-aided search detected typical RSSs in many of the genes participating in the most common chromosomal translocations in AML [t(8;21), inv(16), t(15;17), t(6;9)]. Taken together, this finding and the data presented herein concerning RAG mRNA expression allude to the possible role of the *recombinase* in mediating *AML-specific* chromosomal translocation events.

The prognostic significance of clonal antigen receptor gene rearrangements in AML remains unresolved, not only because the available data are limited but also because the two largest published series (35 and 39 patients, respectively) addressing this issue have reached conflicting results.[10-11] Regarding the prognostic impact of RAG mRNA expression in AML, to our knowledge, the present analysis is the first to address this issue. Importantly, in our study, the presence of either monoclonally rearranged IgH genes or RAG mRNA transcripts was not found to affect remission and relapse incidence or duration of remission. Our series included almost twice as many cases as either of the aforementioned studies, had a longer follow-up and was the only one to discriminate patients for treatment according to age; furthermore, we adopted a significantly more sensitive approach[22] (two-round, *nested* PCR vs. single-round, FWR3, PCR vs, Southern blot) for the detection of clonal IgH gene rearrangements.

Terminal deoxynucleotidyl transferase (TdT) was initially considered as a marker of immature lymphoid cells; however, many studies have conclusively demonstrated TdT expression in AML cases.[27,28] The reported incidence of TdT expression in AML varies significantly (0-55%, average of combined adult data: 21%). In our study, with a 20% cut-off level for positivity, 9/76 cases (11.8%) were found to express TdT; on multivariate analysis, the expression of TdT was not correlated with any other variable. As in most studies (except, importantly, the study by Venditti *et al.*)[28] no association was found between TdT positivity and immunoglobulin gene rearrangements; this is not biologically surprising given that, although TdT plays an important role in lymphoid ontogeny by inserting random nucleotides at the junctions of rearranging antigen receptor genes and thus increasing immune diversity, this enzyme is not essential for the gene rearrangement process.

In conclusion, the prognostic significance of *lymphoid-specific* genetic markers in AML remains unresolved and should be evaluated in further studies. However, an important parameter that must be taken into account in assessing the impact of a molecular marker is the methodology applied, which varies significantly between studies thus precluding direct comparison of the results. Finally, the results presented herein are in agreement with data from studies of hematopoietic development: thus, lymphoid-marker-positive AML might derive from an expansion of abnormal cells blocked at a particular stage of differentiation of a common lymphoid/myeloid precursor.[29] However, one might argue that they fit an alternative model whereby primitive leukemic cells differentiate and acquire lineage markers depending upon the influence of specific transforming events.[4,30]

Appendix

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Pre-publication Report & Outcomes of Peer Review

Contributions

NS and CB were responsible for immunoglobulin gene rearrangement analyses; KS and CK performed the RAG RT-PCR experiments; GP was in charge of flow cytometry; DZ and CP undertook cytogenetic analyses; finally, XY supervised the whole project. Primary responsibility for the paper: NS; primary responsibility for all Tables: NS, CB, KS; primary responsibility for Figure 1: DB, NS, XY.

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Disclosures

Conflict of interest: none.

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Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Francesco Lo Coco, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Professor Lo Coco and the Editors. Manuscript received September 26, 2002; accepted January 25, 2003.

In the following paragraphs, Prof. Lo Coco summarizes the peer-review process and its outcomes.

What is already known on this topic

A number of studies have reported that lymphoid-related molecular markers are detectable in AML. Their clinical significance remains controversial, also in light of the heterogeneous clinical context in such analyses.

What this study adds

The study reports high frequency of either Ig gene rearrangements or RAG gene expression in AML. By analyzing a clinically homogeneous group, it also shows convincingly that these biological features have no significant impact on prognosis.

Caveats

The detection of these molecular markers in AML should not influence therapeutic decisions.