Clinico-biological features of 5202 patients with acute lymphoblastic leukemia enrolled in the Italian AIEOP and GIMEMA protocols and stratified in age cohorts

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ABSTRACT

The outcome of children and adults with acute lymphoblastic leukemia is markedly different. Since there is limited information on the distribution of clinico-biological variables in different age cohorts, we analyzed 5202 patients with acute lymphoblastic leukemia enrolled in the Italian multicenter AIEOP and GIMEMA protocols and stratified them in nine age cohorts. The highest prevalence of acute lymphoblastic leukemia was observed in children, although a second peak was recorded from the 4th decade onwards. Interestingly, the lowest incidence was found in females between 14-40 years. Immunophenotypic characterization showed a B-lineage in 85.8% of patients: a pro-B stage, associated with *MLL/AF4* positivity, was more frequent in patients between 10-50 years. T-lineage leukemia (14.2%) was rare among small children and increased in patients aged 10-40 years. The prevalence of the *BCR/ABL1* rearrangement increased progressively with age starting from the cohort of patients 10-14 years old and was present in 52.7% of cases in the 6th decade. Similarly, the *MLL/AF4* rearrangement constantly increased up to the 5th decade, while the *ETV6/RUNX1* rearrangement disappeared from the age of 30 onwards. This study shows that acute lymphoblastic leukemia in adolescents and young adults is characterized by a male prevalence, higher percentage of T-lineage cases, an increase of poor prognostic molecular markers with aging compared to cases in children, and conclusively quantified the progressive increase of *BCR/ABL*$^+$ cases with age, which are potentially manageable by targeted therapies.

Introduction

Acute lymphoblastic leukemia (ALL) is the most frequent neoplasm in children, whereas it is relatively rare in adults. Over the last decades, there has been a considerable improvement in the outcome of children, with complete remission and long-term survival rates reaching 95% and 80%, respectively.[1,2] Contrariwise, in adults the survival rates generally do not exceed 40%.[1,4-6] Several factors can explain this marked difference, including the more intensive regimens used in children, comprising high-dose methotrexate, asparaginase and reinduction therapy, fewer toxic effects, greater compliance to high doses of chemotherapy, and increased "physician and parent compliance" in pediatric oncology/hematology wards.[7] As a proof of principle, several studies[8-13] showed a superior outcome for adolescents and young adults treated with pediatric-like regimens. In adults, the most important risk factors are age, white blood cell (WBC) count, a pro-B ALL and poor prognosis molecular markers.[6]

Some biological characteristics are partly responsible for the different clinical scenarios, such as the increased incidence of the *BCR/ABL1*[14,15] and *MLL/AF4* transcripts, negatively affecting prognosis, and a decreased incidence of *ETV6/RUNX1* rearrangement, associated with a favorable outcome, in older patients.[16] Only few studies have focused on a detailed analysis of clinico-biological features among various age cohorts and those that have done so usually took into account only few age groups in heterogeneous popula-

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tions.

In this study, we retrospectively evaluated the clinical-biological features of 5202 ALL patients, enrolled in Italian multicenter protocols by AIEOP (*Associazione Italiana di Ematologia ed Oncologia Pediatrica*) and GIMEMA (*Gruppo Italiano Malattie EMatologiche dell'Adulto*) for the treatment of pediatric and adult ALL, respectively. All patients were uniformly characterized at presentation. Although comparison of outcomes in the various age cohorts considered was not a purpose of this study, event-free survival was not different from that previously reported:[2-5] it decreased proportionally with age and was below 50% in patients >18 years, who, at the time of data collection, were treated with non-intensive regimens (*Online Supplementary Figure S1*).

Methods

Patients' enrollment

Between April 1995 and June 2009, 5202 patients were included in the Italian pediatric (AIEOP) and adult (GIMEMA) multicenter protocols, and evaluated for clinico-biological features at presentation. Of 3753 children, 1711 were enrolled in the ALL 95 and 2042 in the ALL 2000 AIEOP trials - designed for children aged between 1-18 years. Of 1449 adults, 457 were enrolled in the GIMEMA LAL 0496 (age 14-60 years), 565 in the GIMEMA LAL 2000 (age 14-60 years), 385 in the GIMEMA LAL 0904 (age 15-60 years), and 42 in the GIMEMA LAL 1205 protocols; the last protocol, enrolling patients ≥18 years harboring the *BCR/ABL1* rearrangement, was included because partly simultaneous with the LAL 0904 trial; these trials were previously approved by local ethical committees. All patients, parents or guardians gave their informed consent to blood/marrow collection and to biological analyses, in agreement with the Declaration of Helsinki.

For this study, patients were stratified into nine age cohorts: 1-5, 5-10, 10-14, 14-18, 18-25, 25-30, 30-40, 40-50, and 50-60 years. Infants were excluded from the analysis.

Clinico-biological features

Clinical parameters included gender, WBC count, platelet count and hemoglobin (Hb) levels, mediastinal, spleen and liver enlargement, and central nervous system (CNS) involvement. For WBC and platelet counts and Hb levels, the following cut-points were considered: $50x10^9$/L, $100x10^9$/L and 10 g/dL, respectively. The mediastinum, spleen and liver were considered enlarged if >3 cm. CNS involvement was defined as described previously.[17]

The diagnosis of ALL was based on May-Grünwald-Giemsa smears and immunophenotyping: the latter allowed definition of the lineage derivation and degree of differentiation of the leukemic cells. The cut-off for positivity was ≥20% for surface antigens and 10% for intracytoplasmatic antigens.

Cases of B-lineage ALL (B-ALL) were subdivided into B1 (pro-B ALL, $CD10^-$), B2 [common-ALL, $CD10^+$ and intracytoplasmic (cy) $Ig\mu^-$] and B3 (pre-B ALL, $CD10^+$ and $cyIg\mu^+$), and NC if not further classified (cyIgμ not tested).[18] T-lineage ALL (T-ALL) cases were subdivided into T1 (pro-T and pre-T ALL, $cyCD3^+$ and $CD7^+$, $CD2^+$ and/or $CD5^+$ and/or $CD8^+$, respectively); T2 (cortical T-ALL, $CD1a^+$) and T3 (mature T-ALL, surface $CD3^+$ and $CD1a^-$).[18]

Molecular analysis of adults[19] included *BCR/ABL1*, *E2A/PBX1*, *ETV6/RUNX1* and *MLL* rearrangements (i.e. *MLL/AF4* and *MLL/ENL*) screening for B-ALL patients, and *BCR/ABL1* and *MLL* rearrangements for T-ALL cases; *SIL/TAL1*, *NUP298/RAP* and *NUP214/ABL1* were investigated only in more recent trials, and since these data were not consistently available, they were not considered. Children were screened for *BCR/ABL1*, *ETV6/RUNX1*, *MLL/AF4* and partly for *E2A/PBX1*.[20]

Since karyotyping data were not uniformly available, this parameter was not considered. Flow-cytometry and molecular analyses were centrally performed in two laboratories, one for pediatric cases and the other for adult cases.

Statistical analysis

Patients' characteristics were summarized by cross-tabulation (categorical variables) and quantiles (ordinal factors). Non-parametric tests were applied, as appropriate, for comparisons between groups (Pearson's χ^2, Mantel-Haenszel χ^2 or Fisher's exact test for categorical variables, Mann-Whitney or Kruskal-Wallis test for continuous variables). Analyses were performed using SAS v9.2 software. All tests were two-sided, accepting P values ≤0.05 as statistically significant.

Results

Incidence of acute lymphoblastic leukemia

The distribution of ALL among the different age cohorts is illustrated in Figure 1A. The majority of ALL cases was included within the 1-5 year age cohort (37% of the whole cohort) and the prevalence progressively decreased up to the 3rd decade; however, a slight increase in ALL was again recorded starting from the 4th decade onwards (>5% in the 30-40, 40-50 and 50-60 age groups).

Immunophenotye

Flow cytometry analysis revealed an overall predominance of B-ALL in the whole cohort (85.8%), while T-ALL was much less frequent (14.2%). The distribution of B- and T-ALL was of interest (Table 1A, Figure 1B). The incidence of T-ALL increased from the 10-14 age cohort up to the 4th decade of life, with a tendency to decline thereafter. Conversely, B-ALL was less frequent in the same age framework (P<0.0001).

When looking at the differentiation stage of B-ALL, the most significant (P<0.0001) finding was a constant and significant increase in the percentage of pro-B up to the 5th decade of life: in small children (age cohorts 1-5 and 5-10 years) the incidence of pro-B ALL was 3.27% and 3.88%, in older children (10-14 years) and adolescents (14-18 years) it was 8.9% and 10.5%, while in adults it was 17.58%, 13.92%, 17.89% and 18.78% for the 18-25, 25-30, 30-40, 40-50 age cohorts, respectively (*Online Supplementary Figure S2*).

In T-ALL, a peak of pro-T/ pre-T ALL cases was recorded in the 5th and 6th decades, with an incidence of 68.18% and 58.33% of cases, respectively; consistently, the percentage of cortical T-ALL was very low in adults and young elderly (*Online Supplementary Figure S3*).

Gender distribution

The cohort evaluated included 2889 males and 2313 females, with a male-to-female ratio of 1.25. Gender distribution was of interest: in fact, while there was an overall prevalence of the male gender in almost all age cohorts, this phenomenon was particularly evident between the age of 14 and 40, started to revert in the 5th decade of life and females were prevalent over the age of 50 (P<0.0001; Figure 2A). Furthermore, a striking association was found between lineage derivation and gender, T-ALL being more frequently diagnosed in male subjects: this association

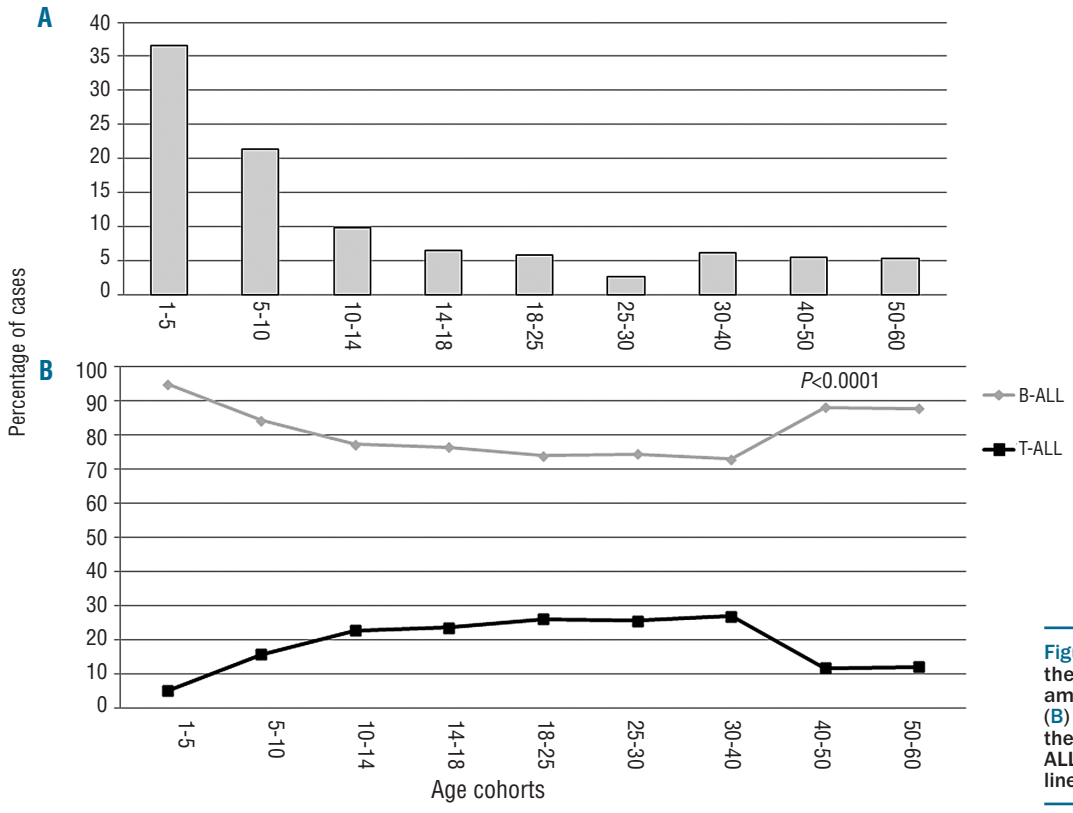

Figure 1. (A) Percentage of the distribution of ALL among various age groups; (B) Lineage derivation in the various age cohorts. B-ALL: gray line; T-ALL: black line.

was statistically significant up to the 4th decade of life (Figures 1B, 2A and 2B).

Hematologic parameters and correlation with immunophenotype

A WBC count >50x10^9/L was significantly less frequent in children, being recorded in 18.87% and 16.34% of patients aged 1-5 and 5-10, while it progressively increased from pre-adolescence onwards (P<0.0001); a platelet count <100x10^9/L was more frequently detected in patients older than the age of 30 years (P<0.0001); finally, Hb levels <10g/dL were more frequently detected in younger patients and even more in small children (1-5 years, P<0.0001) (*Online Supplementary Figure S4A*, Table 1B).

A WBC count >50x10^9/L was more frequently recorded in T-ALL than in B-ALL patients (53.41% *versus* 16.56%, P<0.0001), regardless of the age cohorts considered. However, in B-ALL a WBC count >50x10^9/L was relatively rare up to 25 years, while it was more recurrent form 25 years onwards. At variance, in T-ALL the scenario was almost opposite, with a WBC count >50x10^9/L detected in >45% of patients aged <30 years, and tended to decrease thereafter (P<0.0001, *Online Supplementary Figure S4B*).

Finally, a significantly higher percentage of cases with platelet count <100x10^9/L and Hb levels <10g/dL was observed in B-ALL compared to T-ALL (platelet count 70.12% *versus* 64.94% and Hb levels 77.14% *versus* 42.94%; P=0.0053 and P<0.0001, respectively; *Online Supplementary Figure S4C,D*).

Organ involvement

Organ involvement was considered; since reactive adenopathies are a frequent event in healthy children, this parameter was not taken into account.

Mediastinal, spleen and liver enlargements were prevalently recorded in T-ALL (P<0.0001). The first two were more frequently detected between 10 and 25 years (P<0.0001 and P=0.028, respectively; Table 1B, *Online Supplementary Table S1*, Figure 3A,B), while liver enlargement decreased with age (P<0.0001; Table 1B, *Online Supplementary Table S1* and Figure 3C).

Finally, CNS involvement was a rare event (2%) and was more frequently detected in T-ALL than in B-ALL (5.95% *versus* 1.47%, P<0.0001); furthermore, in T-ALL, it was associated with hyperleukocytosis (P<0.0001). When patients were stratified according to age, a peak incidence of CNS positivity was recorded in the groups aged 10-14, 25-30 and 50-60 (3.91%, 5% and 4.91%, respectively). Interestingly, if patients were stratified for both lineage derivation and age, in T-ALL CNS positivity was a more frequent event in children, particularly in the age groups 5-10, 10-14 and 14-18 (8.67%, 10.34% and 5.41%, respectively), and then progressively decreased, whereas in B-ALL meningosis was more frequent in older patients, particularly in the 25-30 (5.48%) and 50-60 age groups (5.56%) (Table 1B, *Online Supplementary Table S1* and Figure 3D): notably, in the latter age group, a significant association was found with the *BCR/ABL1* rearrangement (10% of patients with *BCR/ABL1* had CNS involvement, P=0.028).

Molecular analysis in B-lineage acute lymphoblastic leukemia

The overall frequency of molecular aberrations is reported in Table 1C and Figure 4.

ETV6/RUNX1 was the most frequent alteration in small children (age cohorts 1-5: 26.14%; 5-10: 23.96%), being detected in more than 20% of cases; its incidence

Table 1. Immunophenotypic features (A), clinical parameters (B) and molecular features (C) among different age cohorts. Median counts and *P*-values are also indicated.

	Age cohorts (years)									*P*
	1-5	5-10	10-14	14-18	18-25	25-30	30-40	40-50	50-60	
A. Immunophenotypic features										
B-lineage ALL (%)	1793 (94.97)	932 (84.27)	397 (77.24)	259 (76.4)	224 (73.93)	103 (75.18)	235 (72.98)	254 (88.19)	245 (87.81)	<0.0001
T-lineage ALL (%)	95 (5.03)	174 (15.73)	117 (22.76)	80 (23.6)	79 (26.07)	34 (24.82)	87 (27.02)	34 (11.81)	34 (12.19)	
B. Clinical parameters										
WBC count>50x10^9/L (%)	359 (18.87)	182 (16.34)	138 (26.74)	79 (23.24)	77 (25.50)	43 (31.16)	93 (28.97)	85 (29.62)	73 (26.16)	<0.0001
Median WBC count (Range)	12.59 (0.35-1000)	8.99 (0.01-761)	10.45 (0.18-940)	9.62 (0.2-708)	13.20 (0.5-848)	20.05 (0.8-321)	17.30 (0.4-700)	17.90 (0.4-872)	15.00 (0.5-597)	
Platelet count<100x10^9/L (%)	1352 (71.61)	685 (61.88)	320 (62.99)	236 (71.08)	201 (69.79)	92 (69.17)	232 (75.08)	217 (76.41)	216 (80)	<0.0001
Median platelet count (Range)	47 (1-982)	72 (2-875)	73 (2.8-582)	54.5 (1-730)	56 (4-461)	48 (1.4-290)	48 (4-517)	46 (1-376)	37 (1-298)	
Hemoglobin<10 g/dL (%)	1600 (84.75)	812 (73.35)	314 (61.57)	188 (57.32)	163 (62.45)	68 (55.28)	169 (59.93)	142 (57.26)	147 (62.82)	<0.0001
Median Hb concentration (Range)	7.40 (2.3-15)	8.30 (3-15)	9.10 (1-17.1)	9.5 (2.9-16.8)	9 (3.4-16.7)	9.5 (3.1-17.5)	9.4 (3-17)	9.45 (4.3-16)	9.2 (4-16.6)	
Mediastinum enlargement (%)	79 (4.16)	90 (8.11)	58 (11.26)	35 (10.74)	28 (11.07)	10 (8.93)	26 (9.59)	10 (4.12)	7 (3.14)	<0.0001
Spleen enlargement (%)	516 (27.26)	331 (29.79)	174 (33.92)	104 (32)	75 (30.99)	31 (27.43)	73 (28.85)	61 (26.18)	46 (21.9)	0.028
Liver enlargement (%)	421 (22.25)	233 (20.92)	107 (20.86)	54 (16.93)	33 (14.29)	10 (8.85)	39 15.48	34 (14.19)	22 (10.33)	<0.0001
CNS involvement (%)	20 (1.05)	26 (2.34)	20 (3.91)	4 (1.29)	5 (2.36)	5 (5.0)	6 (2.58)	5 (2.5)	8 (4.91)	n.a.
C. Molecular features										
ETV6/RUNX1 (%)	337 (26.14)	162 (23.96)	20 (7.04)	8 (4.71)	2 (1.79)	1 (1.71)	0 (0)	0 (0)	0 (0)	<0.0001
E2A/PBX1 (%)	14 (2.12)	13 (3.99)	8 (7.08)	6 (5.71)	6 (3.28)	1 (1.22)	10 (5.08)	5 (2.22%)	5 (2.26)	0.06
MLL/AF4 (%)	8 (0.48)	6 (0.70)	10 (2.78)	5 (2.15)	8 (3.86)	6 (6.45)	17 (7.94)	27 (11.74)	12 (5.24)	<0.0001
BCR/ABL (%)	28 (1.68)	24 (2.79)	20 (5.52)	14 (5.81)	32 (14.41)	26 (26)	84 (37.33)	102 (42.86)	125 (52.74)	<0.0001

decreased progressively in pre-adolescents, adolescents and young adults (age cohorts 10-14: 7.04%; 14-18: 4.71%; 18-25: 1.49%; 25-30: 1.41%), and it disappeared in adults from 30 years onwards (*P*<0.0001).

The *E2A/PBX1* aberration was relatively infrequent, being detected in ≤7% of cases in all age cohorts, without a specific trend; it was slightly more frequent in the 10-14 and 14-18 groups, reaching incidences of 7% and 5.7% respectively, but showed no significant variation (*P*=0.06).

The *MLL/AF4* rearrangement was virtually absent in the 1-5 and 5-10 age groups (0.48% and 0.7%, respectively), while it progressively increased up to the 5th decade of life, being detected in 2.78%, 2.15%, 3.86%, 6.45%, 7.94% and 11.74% of patients in the 10-14, 14-18, 18-25, 25-30, 30-40, and 40-50 age cohorts, respectively, and decreased again to 5.24% in the 50-60 age cohort (*P*<0.0001).

Finally, the *BCR/ABL1* rearrangement showed a striking behavior: in fact, it was very rare in small children (1.68% and 2.79% in the 1-5 and 5-10 age groups, respectively), while its frequency progressively increased with age (age cohorts 10-14: 5.52%; 14-18: 5.81%; 18-25: 14.41%, 25-30: 26%; 30-40: 37.33%; 40-50: 42.86%) reaching a frequency of 52.74% in patients of the 50-60 age group (*P*<0.0001).

Correlation between molecular aberrations, white blood cell count and flow cytometry in B-lineage acute lymphoblastic leukemia

To investigate whether specific aberrations might influence the WBC count at presentation, this parameter was correlated with the presence of *ETV6/RUNX1*, *E2A/PBX1*, *MLL/AF4* and *BCR/ABL1* rearrangements in the different age cohorts.

ETV6/RUNX1 was significantly associated with a lower WBC count when the whole cohort was considered (*P*=0.0001); the same trend was also observed in the 1-5, 5-10, 10-14 and 14-18 age groups, although it did not reach statistical significance. For the remaining groups, a statistical analysis was not feasible because of the small number/absence of positive cases (*Online Supplementary Table S2*).

No significant association was found between the

E2A/PBX1 rearrangement and WBC count, either in the global series or in the various age cohorts (*Online Supplementary Table S3*). At variance, the *MLL/AF4* rearrangement was associated with a higher WBC count in almost all age groups and in the whole cohort ($P<0.0001$, *Online Supplementary Table S4*).

Finally, the *BCR/ABL1* rearrangement displayed a peculiar behavior (*Online Supplementary Table S5*): this aberration was associated with a higher percentage of patients with a WBC count >50x10^9/L in the whole cohort ($P<0.0001$) and in younger children (1-5 years), adolescents and young adults (10-30 years) and older patients (>50 years). There was no statistical association between *BCR/ABL* positivity and WBC count >50x10^9/L in the remaining age groups. The overall results are shown in Figure 5A.

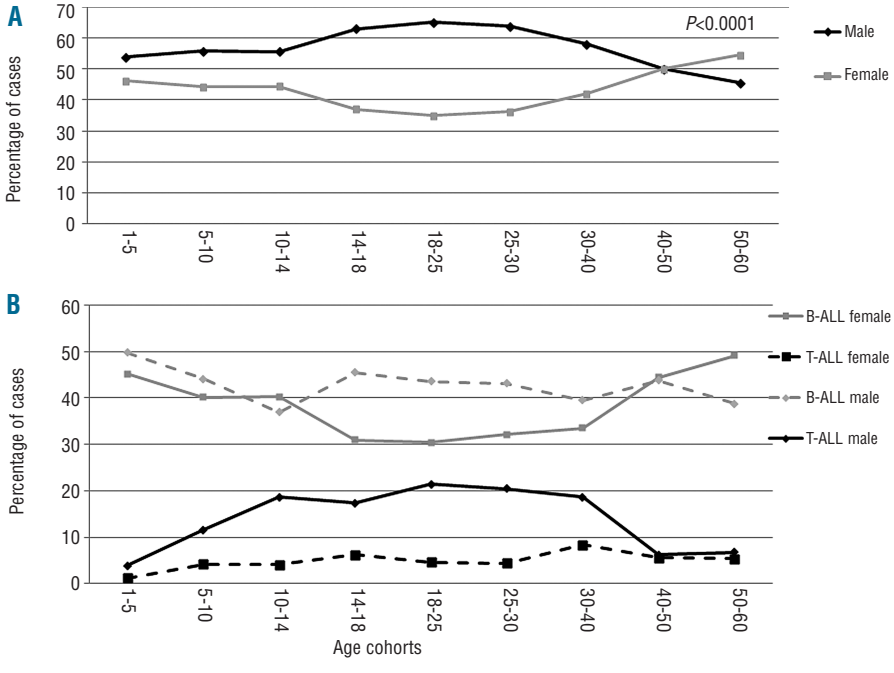

Figure 2. Gender distribution. (A) Gender distribution analysis among age cohorts. The analysis reveals a decreased incidence of females between 14 and 50 years. The gray line represents females, the black line males. (B) Gender distribution and lineage derivation among different age groups. The gray continuous line represents B-ALL females, the gray dotted line B-ALL males, the black continuous line T-ALL males and the black dotted line T-ALL females.

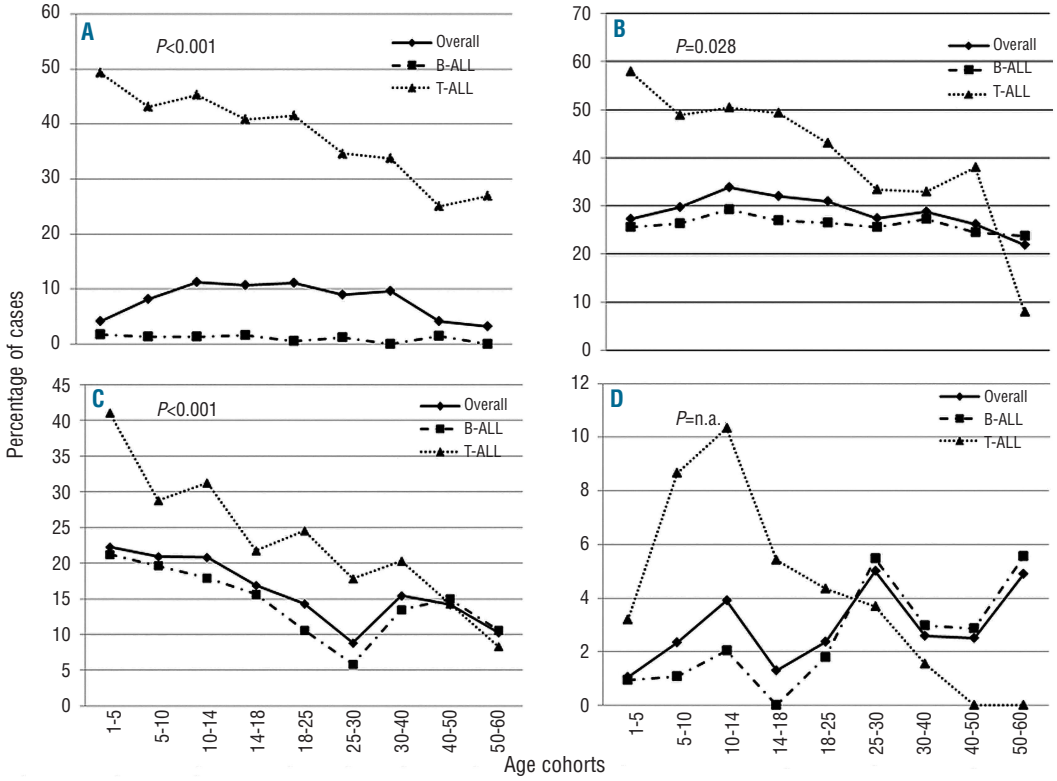

Figure 3. Organ involvement among age cohorts in the whole cohort (continuous line) and stratified according to the lineage derivation (B-ALL: dashed line, T-ALL: dotted line). (A) Mediastinal involvement; (B) spleen involvement; (C) liver involvement; (D) CNS involvement.

Next, a correlation between molecular aberrations and flow cytometry was carried out (*Online Supplementary Tables S6-S9*). The *ETV6/RUNX1* rearrangement was more frequently identified in common ALL (Figure 5B): this association reached statistical significance when the whole cohort was considered ($P<0.0001$) and in the age cohort 1-5 years old ($P=0.025$; *Online Supplementary Table S6*). The *E2A/PBX1* aberration was never detected in pro-B ALL cases, while it was more frequent in pre-B ALL cases (Figure 5C); nevertheless, this finding was significant only when the whole cohort was considered ($P<0.0001$), and in the 1-5, 5-10 and 18-25 year old age cohorts ($P=0.013$, $P=0.018$ and $P=0.012$, respectively), probably because of the small number of positive cases in the other subgroups (*Online Supplementary Table S7*).

Contrariwise, the *MLL/AF4* rearrangement was detected almost exclusively in pro-B ALL cases (Figure 5D) with this finding being highly significant in every age cohort (*Online Supplementary Table S8*).

Finally, the *BCR/ABL1* fusion product was rarely detected in pro-B ALL cases (Figure 5E): the association between a non-pro-B stage and the presence of *BCR/ABL* transcript was statistically significant when the whole cohort was considered ($P=0.0017$) and starting from the 4th decade of life onwards, ($P\leq0.0001$, *Online Supplementary Table S9*).

Discussion

ALL is a heterogeneous disease affecting children and adults with distinct incidences. According to the National Cancer Institute, Surveillance, Epidemiology and End Results (US-SEER) Program[21] approximately 60.3% of ALL cases are diagnosed in patients under the age of 20; 10.3% between the ages of 20 and 34, 5.9% between 35 and 44 years old , 6.7% between 45 and 54, 6.1% between 55 and 64, 5.0% between 65 and 74, 4.0% between 75 and 84 and 1.7% in people over 85 years of age.

Since the clinical scenarios differ profoundly among age groups, particularly in terms of outcome,[1-6] we considered data from a series of children and adults with ALL to evaluate the clinical, hematologic and biological features at diagnosis among the patients subdivided into nine age cohorts.

Few studies have looked at these features in a prospective, uniform manner. In children it was found that a hyperdyploid karyotype and the *ETV6/RUNX1* aberration decrease with age, whereas the presence of *BCR/ABL1* and an increased incidence of T-lineage ALL are more frequent in older children and young adults.[7,22] A study on a relatively small cohort of adults with ALL[23] found that the male/female ratio is decreased in older patients (>60 years) and that they have a decreased incidence of mediastinal involvement, adenopathy, and splenomegaly, a reduced median WBC count, and a lower percentage of T-lineage ALL.

The current study included 3753 children and 1449 adults enrolled between 1995 and 2009 in, respectively, two and four consecutive multicenter Italian protocols for children and adults. All cases were prospectively and uniformly characterized at the time of diagnosis.

The highest peak of incidence was detected in children between 1 and 5 years old; the occurrence of the disease increased again from the 4th decade, reaching a steady percentage of about 5.5%, as reported elsewhere[23] and similar to the US-SEER data; in fact, in our cohort, the overall percentage of ALL cases was 74% in patients aged <18 years, 8.5% in cases between 18 and 30, and 6.2%, 5.5% and 5.3% in patients aged 30-40, 40-50 and 50-60 years, respectively. Patients ≥60 years were, at that time, eligible for only one protocol enrolling exclusively *BCR/ABL1*$^+$ patients; we cannot, therefore, determine the incidence of ALL in these individuals.

Gender distribution was remarkable: starting from the 10-14 age cohort there was a striking decrease of the ALL rate in females; this tendency was evident up to the 4th

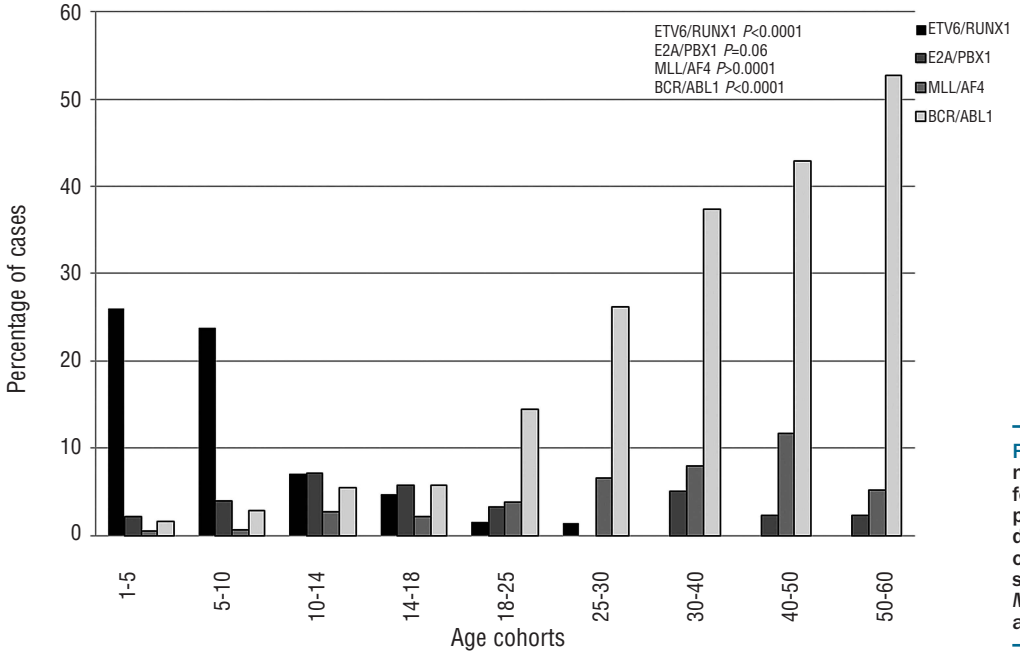

Figure 4. Incidence of the molecular aberrations in different age cohorts of B-ALL patients. A significant decrease of *ETV6/RUNX1* is observed with age progression, while *BCR/ABL* and *MLL1/AF4* rearrangements are more frequent in adults.

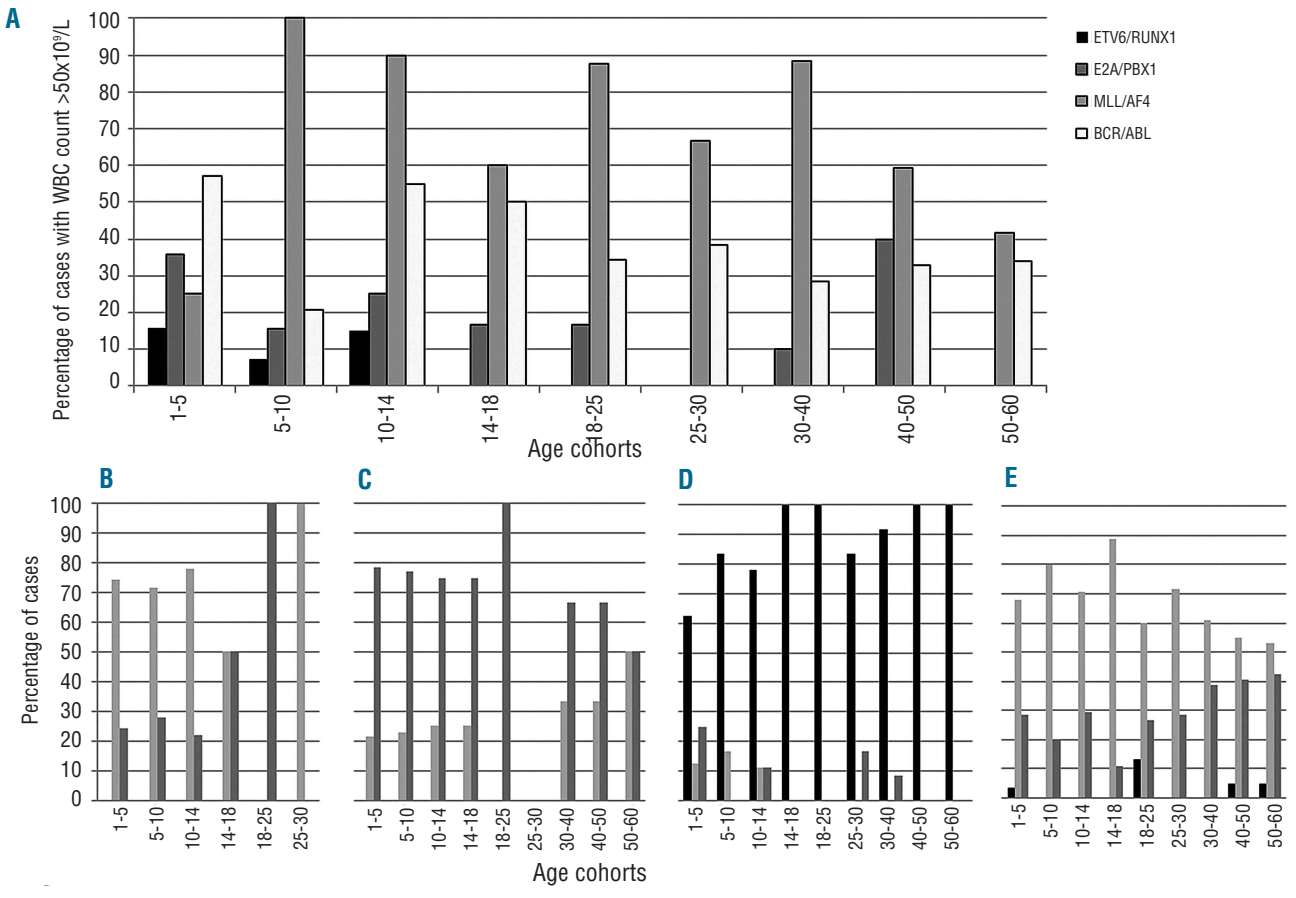

Figure 5. Hyperleukocytosis and stage of differentiation of B-ALL analyzed on the basis of the molecular aberrations. (A) Percentage of cases with hyperleukocytosis (WBC count >50x10⁹/L) in cases with *ETV6/RUNX1+* (black bars), *E2A/PBX1+* (dark gray bars), *MLL1/AF4+* (light gray bars) and *BCR/ABL+* (white bars). (B-E) Stage of differentiation within the different molecular aberrations [(B) *ETV6/RUNX1*; (C) *E2A/PBX1+*; (D) *MLL1/AF4*; (E) *BCR/ABL+*]; the black bars indicate a pro-B ALL, the light gray bars a common ALL and the dark gray bars a pre-B ALL. Detailed *P*-values are provided in *Online Supplementary Tables S2-S9*.

decade of life, and disappeared in the cohort aged 50-60 years. This phenomenon seems to be associated with lineage derivation: in fact, the incidence of ALL is higher in male adolescents and young adults with T-ALL and only to a lesser extent with B-ALL. Although these data were partly known,[24,25] the analysis on this broad cohort strongly suggests that age-related sex hormone levels may be a "protective factor" in ALL initiation in females and prompts further, prospective investigations of the contribution of the hormonal changes occurring during menarche and menopause.

Similarly, organ involvement was widely distributed: besides mediastinal enlargement, frequently detected in older children and young adults (age 10-25 years) and significantly associated with a T-lineage phenotype, spleen and liver enlargement, also associated with a T-lineage, were less frequent in older patients, in line with earlier reports,[24,26] thus suggesting a physiological atrophy of lymphoid organs with aging. As previously reported,[17,27] CNS involvement was rare, being found more frequently in adults than in children. In children, it was more frequently detected in T-ALL patients aged 5-18 years. The reason for the preferential contamination of CNS by leukemic T cells in children is not clear, but it might be postulated that adhesion and/or metastatic molecules are more expressed in pediatric leukemic T cells than in adult ones. This hypothesis is corroborated by the high levels of interleukin-15, a pro-inflammatory cytokine that promotes T-cell proliferation, detected in childhood ALL with CNS involvement.[28] Otherwise, this behavior might be sustained by more invasive properties of pediatric T-ALL, as also suggested by the association with a higher WBC count, and organ enlargement observed in pediatric, but not in adult T-ALL.

Immunophenotypic analysis confirmed the overall higher incidence of B-ALL *versus* T-ALL, but also showed that T-ALL is significantly more frequent in patients aged between 14 and 40 years, thus confirming that this immunophenotypic subset is more represented in adolescents and young adults. Moreover, hyperleukocytosis (WBC >50x10⁹/L) was infrequent in pediatric B-ALL and progressively increased with age in this group, whereas in T-ALL hyperleukocytosis was less frequent in patients >30 years. The study also showed that, among B-ALL, there is a progressive increase of pro-B cases up to the 5th decade of life, a finding significantly associated with the presence of the *MLL/AF4* rearrangement. As far as concerns T-ALL, T1 cases were more frequently detected in the 5th and 6th decades; this finding is of interest, since early T-ALL cases tend to have a more unfavorable prognosis.[29]

Molecular screening showed the disappearance of the *ETV6/RUNX1* rearrangement and the progressive increase of the *BCR/ABL1* and *MLL/AF4* fusion transcripts with age progression. The disappearance of *ETV6/RUNX1* with aging further supports the notion that this subset of ALL has a prenatal origin, as indicated by neonatal blood spots or Guthrie card screening.[30] Moreover, it is in agreement with the finding that a "second leukemogenic hit" must occur during childhood.[31,32] It could be speculated that, upon puberty, the pre-leukemic clone harboring the *ETV6/RUNX1* rearrangement is physiologically cleared out by other factors, such as hormonal changes or a normal degeneration of lymphoid development.

In our analysis the *MLL/AF4* rearrangement was virtually absent in the cohort of patients 1-5 years old and was detected more frequently with increasing age, suggesting that the rearrangement detected in these patients is probably different from that of infant ALL. To validate this hypothesis, the 1-5 age cohort was further subdivided into single years and we did not detect an increased incidence of the transcript in the 1-2 years' age group (*data not shown*).

It is, therefore, unlikely that the leukemogenic clone originates from *in utero* conditions, as opposed to infant *MLL*-rearranged leukemias:[33] it is intriguing to speculate that infant *MLL*-rearranged leukemias require the concomitant presence of residual cord blood and/or maternal cells to occur, whereas in pediatric and adult *MLL*-rearranged leukemias these "nurturing" cells are no longer required. Indeed, previous gene expression profiling studies showed that infant *MLL*-rearranged leukemias share a common expression pattern with *MLL*-germline infant leukemias.[34]

Finally, the constant and progressive increase of *BCR/ABL1* rearrangements in the elderly indicates that the genomic instability associated with aging leads to the appearance of the leukemic clone and the accumulation of additional oncogenic hits.

Overall, the variable incidence of these lesions suggests that in children harboring *ETV6/RUNX1* and, to a lesser extent, *MLL/AF4* the driver event is prenatal, whereas in *BCR/ABL1*$^+$ ALL, the driver event occurs during life-time and induces an overt leukemia only when several additional hits occur.

While *ETV6/RUNX1*$^+$ cases were characterized by a low WBC count in the whole cohort, a striking association between WBC count >50x10^9/L was found in all *MLL/AF4*$^+$ patients, but not in all patients harboring the *BCR/ABL1* rearrangement. This finding, particularly evident in patients aged 30 and 50 years, did not change when a different cut-off point was used (i.e. 30x10^9/L, *data not shown*). The fact that *BCR/ABL1*$^+$ patients have a different clinical presentation is in line with the well-documented biological heterogeneity of this subset at the transcriptional level[35] and with the presence of additional molecular lesions, such as *IKZF1* deletions.[36]

Overall, this study – involving the largest cohort of ALL cases uniformly characterized across ages – confirms and extends previous results on the incidence and distribution of clinico-biological characteristics.[7,14-16,22-26] Furthermore, it provides three important pieces of information. First, it conclusively shows that the subset of adolescents and young adults has a higher frequency of hyperleukocytosis and low platelet count, a predominance of males, a greater percentage of T-ALL, and an increase of unfavorable prognosis aberrations. Altogether, these data help to understand the worse outcome of this age group and support the use of more intensive regimens in it.[8-13]

Secondly, the study shows that the incidence of molecular aberrations varies with age. Nevertheless, the associated clinical characteristics do not change among the different age cohorts, indicating that the most important factor for leukemia initiation is the biological mechanism/s responsible for the emergence of the molecular lesion/s.

Finally, it shows that *MLL/AF4* and *BCR/ABL1* rearrangements constantly increase with age. This is particularly relevant for the *BCR/ABL1* fusion, detected in 52.7% of the 50-60 year-old subgroup. Given the profound changes in the management of Philadelphia-positive ALL following the advent of tyrosine kinase inhibitors,[37-39] less fit patients can also be successfully treated nowadays. Indeed, it is now possible to treat patients effectively with tyrosine kinase inhibitors plus steroids without systemic chemotherapy,[37,39] which is a viable option for elderly ALL patients.

In conclusion, this large retrospective study on adult and pediatric ALL characterized at presentation has allowed better definition of clinico-biological features in the various age subgroups, helps to unravel the differences in clinical behavior and outcome, and provides therapeutic indications for specific subgroups of patients.

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