B-cell acute lymphoblastic leukemia with t(4;11)(q21;q23) in a young woman: evolution into mixed phenotype acute leukemia with additional chromosomal aberrations in the course of therapy

Giovanni Carulli,1 Alessandra Marini,2 Maria I. Ferreri,3 Antonio Azzara,1 Virginia Ottaviano,1 Tiziana Lari,1 Melania Rocco,1 Stefano Giuntini,1 Mario Petriti1

1Division of Hematology, Department of Clinical and Experimental Medicine, University of Pisa; 2Laboratory of Clinical Pathology, Versilia Hospital, Lido di Camaiore; 3Laboratory of Cytogenetics, AOUP, Pisa, Italy

Abstract

About 5% of adult B-cell acute lymphoblastic leukemias (B-ALL) are characterized by t(4;11)(q21;q23), which confers peculiar features to this B-ALL subtype, including a very immature immunophenotype and poor prognosis. We describe the case of a 21-year-old female who presented with B-ALL carrying the t(4;11)(q21;q23) and blasts positive for CD19, TdT, CD34, CD79a, CD38, HLA-DR. Before completing induction therapy, probably accelerating the fatal outcome of the patient. Cytogenetics and in situ fluorescent hybridization (FISH) showed the co-existence of t(4;11)(q21;q23) with a complex karyotype, which was characterized by three trisomies and the presence of two derivatives of chromosome.

Therefore, the initial B-ALL with t(11;14)(q21;q23) showed evolution into a bilineal acute leukemia (lymphoid and myeloid) compatible with the 2008 WHO entity defined as mixed phenotype acute leukemia (MPAL) with t(v;11q23), MLL rearranged. To the best of our knowledge, a similar evolution of B-ALL with t(11;14)(q21;q23) has not been described so far.

Case Report

A Caucasian 21-year-old female presented at the Division of Hematology of the University of Pisa, Italy, with fever and anemia-related symptoms. A complete blood count showed hyperleukocytosis [white blood cell (WBC) 400 x 10^9/L], anemia and thrombocytopenia (9 g/dL and 50 x 10^9/L, respectively). Her clinical history was silent, but she declared intake of heroin and cocaine. Physical examination showed mild splenomegaly. Whole body computed tomography confirmed the spleen enlargement (15 cm longitudinal diameter) and did not show pathologic lymph nodes.

Manual WBC differential count of peripheral blood showed 90% blasts without morphologic differentiation (Figure 1A), 2% neutrophils, 8% small lymphocytes. Blasts resulted negative for myeloperoxidase stain. Flow cytometric analysis was therefore performed using a wide monoclonal antibody panel and a six-color method: blasts were positive for CD19, TdT, CD79a, CD38, CD56, CD15, CD4dim compatible with derivation from the myeloid/monocytic lineage. Karyotype showed the co-existence of three cell lines, with persistence of t(4;11)(q21;q23) and appearance of +8,+12,+13 and two der(4). The patient died because of disseminated intravascular coagulation. Our report describes a rare, possible evolution of such a subtype of B-ALL, with transformation into mixed phenotype acute leukemia in the course of therapy. This finding suggests a blast cell derivation from a common lymphoid/monocytic precursor leading to a final bilineal acute leukemia.

Introduction

Human acute lymphoblastic leukemia of the B lineage (B-ALL) involves clonal expansion of neoplastic B precursors at one of the stages of B-cell development. Some cases show rearrangements of the mixed-lineage leukemia (MLL) gene, located at 11q23. MLL is able to recognize at least 64 partner genes, giving rise to at least 104 different MLL rearrangements. For this reason, the most recent World Health Organization (WHO) classification of hematological myeloid neoplasms and acute leukemias identifies one sub-type of B-ALL [termed as B-lymphoblastic leukemia/lymphoma with t(v;11q23); MLL rearranged], which involves all translocations of MLL with one of the possible gene partners. About one third of B-ALL cases are characterized by t(4;11)(q21;q23), which produces the AF4/MLL fusion gene. This subtype of B-ALL accounts for about 5% of adult B-ALL, being more frequent in the pro-B-ALL subtypes.

A very recent review shows that this subtype of B-ALL, although rare because of the low incidence of B-ALL in adults, is of great clinical interest due to biological, phenotypic and clinical features.

In the present paper we describe a case of very immature B-ALL with t(4;11)(q21;q23) in a young woman. The disease was characterized by an uncommon evolution, since an additional leukemic clone, with myeloid/monocytic phenotypic features, appeared in the course of induction therapy, probably accelerating the fatal outcome of the patient. Cytogenetics and in situ fluorescent hybridization (FISH) showed the co-existence of t(4;11)(q21;q23) with a complex karyotype, which was characterized by three trisomies and the presence of two derivatives of chromosome.
Chemotherapy with the dose-intensive phase of hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (Hyper-CVAD) regimen was therefore given. Therapy included 8 cycles of dose-intensive therapy courses of Hyper-CVAD (courses 1, 3, 5, and 7) alternating with high-dose methotrexate and cytosine arabinoside (courses 2, 4, 6, and 8). Intrathecal central nervous system prophylaxis was also given with methotrexate or cytosine arabinoside, and recombinant human granulocyte colony-stimulating factor was administered as a supportive care.

After six of the established eight courses of therapy, although complete clearance of blasts from peripheral blood was obtained, minimal residual disease (0.2% by flow cytometry), was detected in the bone marrow, along with the presence of a small leukemic clone in cerebrospinal fluid samples. The final two cycles of therapy were programmed, but the patient refused further antiblastic therapies and was dismissed from our hospital.

At home, her clinical conditions showed a rapid worsening and two weeks later she was admitted at Versilia Hospital, Italy. The patient presented with hyperleukocytosis (220×10⁹/L), hemoglobin 7 g/dL, platelets 10×10⁹/L, cutaneous hemorrhages (petechiae, purpura), laboratory findings of disseminated intravascular coagulation (low fibrinogen, prolonged prothrombin time and a-PTT, high D-dimer levels, low antithrombin levels), fever.

The observation of peripheral blood smears showed blasts 95%, which consisted of two different clones, the former being represented by blast cells with lymphoid appearance (about 10%), the latter being represented by cells with a larger size, abundant cytoplasm, giant nucleus with irregular profile (about 90%) (Figures 1B and 1C). Some cells were classified as atypical monocytoid cells (arrowheads) and very few blasts showed cytoplasmic granulations (Figure 1B). Immunophenotyping of circulating blasts showed a peculiar CD45/SSC dot-plot, with the presence of two distinct blast cell populations. Blasts with small forward scatter (FSC) and side scatter (SSC), which accounted for 10%, showed the phenotypic characteristics observed at diagnosis and were classified as belonging to the B-cell lineage. The majority of blasts (90%) were characterized by larger FSC and SSC and appeared to derive from a myeloid/monocytic clone, being positive for CD13, CD33, CD64, CD15, CD56 and CD4 dim (Figure 3).

A new evaluation of karyotype confirmed the presence of the sole abnormality t(4;11)(q21;q23) in 10% of metaphases, and showed the appearance of two additional cell lines: one with 50 chromosomes, t(4;11)(q21;q23), trisomy of chromosomes number 8, 12 and 13, and a derivative of chromosome 4 [der(4)] (Figure 4B). This latter anomaly was confirmed by FISH, which was performed on metaphases using whole chromosome painting n. 4 and n. 11 probes (Cytocell Inc. UK) and detected two der(4) (Figure 5). It was not possible to perform other studies, with the exception of a new PCR for IgH rearrangement, which showed persistence of a clonal pattern. The patient died because of disseminated intravascular coagulation.

**Discussion**

The B-ALL subtype carrying t(4;11)(q21;q23) is a rare event, representing 5-10% of adult

![Figure 1](image1.png) Morphology of blast cells at diagnosis (A) and in the course of fatal evolution (B, C). A) blasts appear with lymphoid morphology; B, C) blasts consist of two different clones. Blasts with lymphoid morphology (arrows) show smaller size. The additional blast cell population consists of cells of larger size and more abundant cytoplasm (long arrows). Some monocytoid cells (arrowheads) and one myeloblast with evident cytoplasmic granulations (B) are also shown. Peripheral blood, May-Grünwald-Giemsa staining (1000x).

![Figure 2](image2.png) Immunophenotype of blasts at diagnosis in peripheral blood samples. A) CD45/SSC dot-plot. Blasts are included in P1 population. B) blasts are CD19-positive, with a minority of them (18%) being CD34-positive (Q2 quadrant). Blasts are CD10-negative (C) and CD20-negative (D) and CD58-positive (D). E) positivity of CD79a. F) blasts are negative for CD13.

![Figure 3](image3.png)

![Figure 4](image4.png)
cases of B-ALL which, in turn, is a disease with very low incidence (less than 1 per 100,000 persons/year). In an ample study published by the Medical Research Council and the Eastern Cooperative Oncology Group, which involved 1522 patients with acute lymphoblastic leukemia, only 54 showed a B-ALL with t(4;11)(q21;q23). This type of B-ALL shows peculiar features. From a clinical point of view, it is characterized by elevated WBC counts, high incidence of central nervous system involvement, frequent hepato-splenomegaly, poor clinical outcome both in children and in adults. Due to these reasons, B-ALL with t(4;11) are considered as high risk leukemias. The presence of t(4;11)(q21;q23) is more frequent in B-ALL deriving from a very immature B-cell precursor. This feature is suggested by the phenotype displayed by blast cells, which are generally positive for CD19 and markers associated with an immature immunphenotype, as demonstrated by the co-expression of CD19, TdT, CD79a and often CD34, and the absence of CD10 and CD20. Negativity for CD10 is always observed. Because of this peculiar immunphenotype, the B-ALL subtype carrying t(4;11)(q21;q23) represents about 40% of all forms of pro-B ALL in adults. Several but not all cases show co-expression of some myeloid markers, such as CD15 and CD65, with constant negativity of CD13 and CD33.

In our case findings of typical B-ALL with t(4;11)(q21;q23) were found at diagnosis. Central nervous system involvement was detected during the course of disease, in a phase of bone marrow minimal residual disease.

The poor outcome of our patient was favored by an unexpected event, represented by the appearance of an additional leukemic clone with both morphological and immunophenotypical typical properties which could be attributed to the myeloid/monocytic lineage. In fact, during the terminal and fatal phase of disease, two distinct blast cell populations were found, the former with a B-cell phenotype and the latter with a separate phenotype characterized by co-expression of markers frequently found in acute myeloid leukemias with prevalent monocytic differentiation, such as CD13, CD33, CD64, CD15, CD4. Co-expression of B-cell markers and myeloid markers was not detected. The appearance of this additional leukemic clone was accompanied by the preponderant presence of additional cytogenetic aberrations, such as trisomy of chromosomes 8, 12 and 13. Trisomy 8 is a relatively frequent (10-15% of cases) abnormality in acute myeloblastic leukemias (AML), in which is associated with a poor prognosis. Isolated trisomy 12 is an infrequent finding both in AML and in B-ALL and has been described in highly undifferentiated acute leukemias. Isolated trisomy 13 is another marker with a negative impact on prognosis and seems to be associated with the FAB M0 subtype and very low remission rates. Thus, the association of these three cytogenetic anomalies should be interpreted as biologic marker with an additional negative impact on disease. The presence of double der(4) [derivative of t(4;11)(q21;q23)] is likely to have given a synergistic effect to the leukemic phenotype as well as an attractive leukemic subtype with poor prognosis. The latter finding is consistent with the initial lymphoblastic clone, since a der(4) chromosome can be found in up to 65% of patients with t(4;11)(q21;q23).

There is evidence of an interesting association of MLL gene rearrangements and MPAL. The 2008 WHO classification has identified a particular subtype of MPAL termed mixed phenotype acute leukemia with t(v;11q23); MLL rearranged, which can show either the presence of blasts with simultaneous expression of markers of different lineages or the presence of two populations of blasts with distinct phenotypes. In a recent analysis of 100 cases of MPAL, Matutes et al. found only three cases with MLL gene rearrangement due to t(4;11)(q21;q23) at initial presentation. In a less recent report Johansson et al. analyzed 183 cases of t(4;11)(q21;q23) and found that 34% were children, 95% were B-ALL and that only one case was a biphenotypic acute leukemia. Rubnitz et al. reported a series of 35 children with MPAL and found four cases with MLL gene rearrangement: only two cases showed a bilineal pattern (myeloid and lymphoid B), but t(4;11)(q21;q23) was not detect-
ed. Sporadic additional cases of acute bipheno-
typic leukemia associated with complex MLL
gene rearrangement have been reported.

Thus, although MLL gene rearrangement
with t(4;11)(q21;q23) can be observed in cases
of MPAL, this peculiar subtype of acute
leukemia seems to be very rare at first presen-
tation, probably being more frequent in chil-
dren.

Another possible presentation of cases of B-
ALL with t(4;11)(q21;q23) is represented by
phenotypic shift. Indeed, phenotypic changes
of blast cells in this sub-type of B-ALL have
rarely been reported. Lineage switch from ly-
mphoid to myeloid phenotype was reported by
Trikalinos et al. in a young woman after allo-
geneic stem cell transplantation. Germano et
al. reported a pediatric case with two consec-
tutive phenotypic changes from lymphoid to
myeloid lineage and vice versa. The phenotyp-
ic switches were observed after lineage-target-
ed chemotherapy in both instances. Two addi-
tional pediatric cases of switch from acute lym-
phoblastic leukemia to acute myeloid
leukemia involving MLL gene rearrange-
ments, were reported by Jiang et al. and by
Stasik et al.

In all the above cases, it could be thought
that the lineage switch might represent the
expansion of a pre-existing minor population
of blasts of myeloid lineage following chemo-
therapy-induced suppression of the initial
major lymphoid blast population. Another
possible interpretation, according to a current
opinion about the existence of a common lym-
phoid-myeloid precursor, is that leukemic
clones carrying rearrangements of the MLL
gene are able to differentiate along the lym-
phoid or myeloid lineage depending on activa-
tion/deactivation of differentiation programs,
and that chemotherapy may play a role in this
scenario.

The pathogenetic interpretation of our case
may be facilitated by previous reports showing
a relationship between the t(4;11) transloca-
tion and a singular association of lymphoid
and monocytic lineage in human leukemic
cells lines. Stong et al. reported a study con-
cerning a cell line derived from the bone mar-
rrow of a female adult patient in first relapse.
The cell line, termed RS4;11, showed lymphoid
morphology, various B-associated markers,
HLA-DR expression, clonal rearrangement of
heavy chain and light chain genes of immunglobulins, and lacked the CALLA anti-
gen (corresponding to CD10). Such cells
acquired cytochemical and ultrastructural fea-
tures following treatment with the phorbol
ester TPA. Similar results had been observed
in two pediatric cases. In a more recent
report, Dunphy et al. described a case of a
CD15-positive, t(4;11)-positive B-ALL which
relapsed as acute leukemia with monocytoid
features after allogeneic bone marrow trans-
plant. The initial CD19-positive clone, howev-
er, was no longer present, although persist-
ence of clonal rearrangement of the IgH gene
could be detected.

The very few previous reports are consistent
with a possible relationship between the B-lin-
eage and the monocytic lineage in cases of acute
leukemia with t(4;11)(q21;q23) and sug-
gest the possible involvement of a common
lymphoid-myeloid precursor which, some-
times, might show a predominant monocytic
differentiation program.

Indeed, Zangrandc et al. employing gene
expression profiling assays, found that, in
cases of acute leukemia with MLL gene
rearrangements, a subset of genes identifies
MLL-specific rearrangements and is able to
distinguish ALL from AML. In addition, a small
subset of genes (MEIS1, ZEB2, SRGAP2P1,
TMEM30A, AK2, TMED2, HIPK3 and FAM62B)
showed marked up-regulation in patients with
MLL mutation, both in ALL and AML. These
findings appear of particular interest, because
they seem to emphasize the role of MLL gene in
the onset of cases of BPAL.

Conclusions

Because of the above considerations, it
appears that in our patient the initial B-ALL
with t(4;11)(q21;q23) showed, in the course of
therapy, an evolution into a MPAL with the
same MLL gene rearrangement, but with ad-
ditional chromosomal aberrations probably con-
ferred by the additional, non-lymphoblastic,
leukemic clone.

We think that the case described in the cur-
rent report adds new information about the
possible evolution of B-ALL with t(4;11)
(q21;23). Not only phenotypic switches are
possible after chemotherapy, but additional
leukemic clones, with myeloid/monocytoid
properties, may arise in the course of antiblas-
tic therapy, probably deriving from a common
precursor. Thus, a wide panel of MoAb should
be used when monitoring B-ALL positive for
t(4;11)(q21;q23), in order to detect eventual
phenotypic shifts and/or appearance of addi-
tional leukemic clones. Due to the poor out-
come which characterizes both B-ALL with
t(4;11)(q21;q23) and BPAL, early detection of
such uncommon evolution of this B-ALL sub-
type may have practical implications in terms
of follow-up strategies.

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