

## CLINICAL AND LABORATORY OBSERVATIONS

# Uncommon leukemic case of anaplastic large cell lymphoma diagnosed through a typical chromosomal abnormality t(2;5) with a null phenotype and aberrant expression of NG2 and CD13

Kolenova A<sup>1</sup>, Mejstrikova E<sup>4</sup>, Fidlerova K<sup>2</sup>, Fronkova E<sup>4</sup>, Plank L<sup>3</sup>, Szepe P<sup>3</sup>, Jindrova J<sup>1</sup>, Sejnova D<sup>1</sup>, Zakovicova A<sup>2</sup>, Kaiserova E<sup>2</sup>

Department of Pediatric Oncology, Comenius University, Faculty of Medicine, University Children's Hospital, Bratislava, Slovakia. [sasa.kolenova@gmail.com](mailto:sasa.kolenova@gmail.com)

**Abstract:** Anaplastic large cell lymphoma represents approximately 10–15 % of pediatric non-Hodgkin lymphomas. Leukemic presentation is very rare, and in particular, the null phenotype ALCL without typical anaplastic morphology together with aberrant expression of CD13 and/or CD11b represents a diagnostic challenge. We report a case of a 9 year-old boy with leukemic presentation of ALCL with the typical translocation t(2;5) (p23;q35); in this patient, the only positive antigens identified by immunophenotyping were CD13, NG2 HLA-DR, and CD38. To our knowledge, aberrant expression of NG2 has never been reported in ALCL cases (*Tab. 1, Fig. 6, Ref. 20*) Full Text in PDF [www.elis.sk](http://www.elis.sk).

**Key words:** anaplastic large cell lymphoma, NG2 – chondroitin sulphate proteoglycan neuron-glia antigen 2, ALK- anaplastic lymphoma kinase protein.

Anaplastic large cell lymphoma (ALCL) represents approximately 10–15% of pediatric non-Hodgkin lymphomas (NHLs) (1, 2). ALCL cells typically express the antigen CD30 (Ki-1). Most pediatric cases are also characterized by anaplastic lymphoma kinase (ALK) expression (3). The ALK protein is a result of the t(2;5) (p23;q35) chromosomal translocation that juxtaposes the ALK locus at 2p23 to the nucleophosmin (NPM) gene locus at 5q35 (3); its presence can be routinely proved by immunohistochemistry.

Clinically, ALCL is characterized by a high incidence of systemic symptoms, typically fever, and spreading into extranodal sites. The most common sites involved include skin, bone, soft tissue, and lungs (4). Presence of the circulating lymphoma cells in the peripheral blood or bone marrow even on a submicroscopic level is associated with increased risk of relapse (5, 6). Leukemic presentation is very rare, and in particular, the null phenotype ALCL without typical anaplastic morphology together with aberrant expression of CD13 and/or CD11b represents a diagnostic challenge (7, 8). The prognosis of the leukemic form is extremely poor according to the published data (7–9). Here, we report a case of a patient with leukemic presentation of ALCL with the typical translocation t(2;5)(p23;q35); in this patient, CD13, NG2 (chondroitin sulphate proteoglycan neuron-glia antigen 2), HLA-DR, and CD38 were

the only positive antigens identified by immunophenotyping using peripheral blood and bone marrow samples, and the expression of CD7 and CD56 was identified on a minority of atypical cells. To our knowledge, aberrant expression of NG2, which is typically associated with both acute myeloid leukemia (AML) and B-cell precursor acute lymphoblastic leukemia (BCP-ALL) with the rearrangement of the mixed lineage leukemia (MLL) gene (10), has never been reported in ALCL cases.

## Methods

### Morphology and cytochemistry

Peripheral blood and bone marrow smears were stained with May-Grunwald-Giemsa stain. Cytochemistry analysis included myeloperoxidase (MPO), Sudan, and periodic acid-Schiff (PAS) staining.

### Immunophenotyping

The following spectrum of antigens, as proposed by European Group for the Immunological Characterization of Leukemias (EGIL) (11), was evaluated from the peripheral blood: CD2, CD3, intracellular CD3, CD4, CD5, CD7, CD8, CD10, CD13, intracellular CD13, CD14, CD15, CD19, CD20, intracellular CD22, CD33, CD34, CD38, CD41, CD42, CD56, CD61, CD65, CD99, CD117, MPO, intracellular TdT (terminal nucleotidyl transferase), glycophorin A (CD235a), HLA-DR, intracellular CD79a, and NG2.

### Cytogenetic, fluorescence in situ hybridization (FISH), and polymerase chain reaction (PCR) analyses

Peripheral blood, bone marrow, pleural effusion, and lymph nodes were evaluated as follows:

<sup>1</sup>Department of Pediatric Oncology, Comenius University, Faculty of Medicine, University Children's Hospital, Bratislava, Slovakia, <sup>2</sup>Department of Oncology and Genetics, National Institute of Oncology, Bratislava, Slovakia, <sup>3</sup>Department of Pathology, Jessenius Faculty of Medicine, Martin, Slovakia, and <sup>4</sup>Department of Pediatric Hematology and Oncology, Charles University in Prague, Second Medical School, Prague, Czech Republic

**Address for correspondence:** A. Kolenova; MD, Zahumenska 3, SK-846 01 Bratislava, Slovakia. Phone: +421.905111827

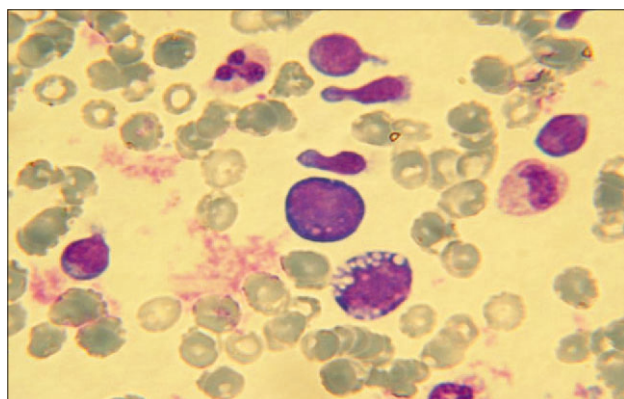


Fig. 1. Cytomorphologic picture of blasts in peripheral blood.

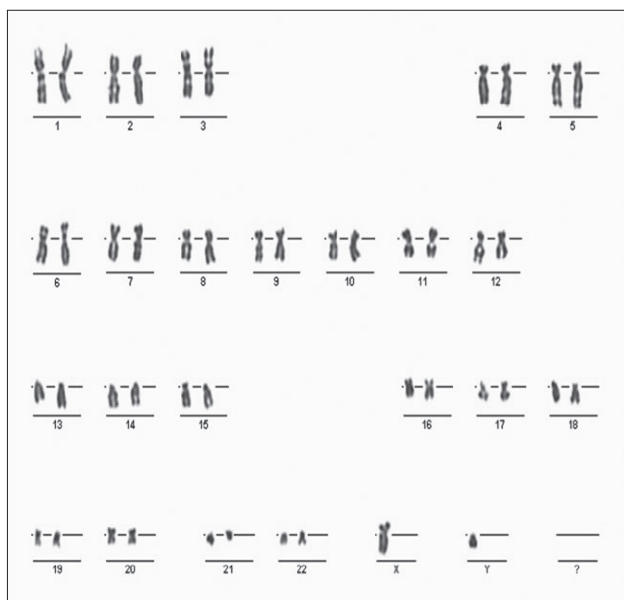


Fig. 2. Karyotype from peripheral blood (PB) 46, XY,t(2; 5)(p23; q35)(9).

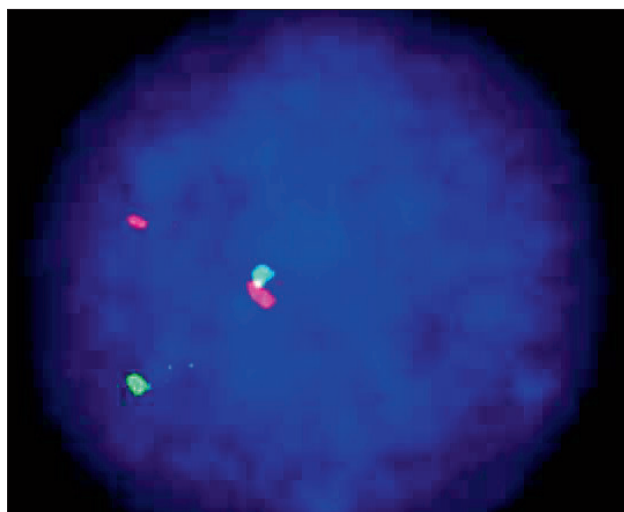


Fig. 3. FISH Peripheral blood negat.

*Conventional karyotyping using G-banding:* Twenty metaphases were analyzed.

*FISH studies:* At least 200 interphase nuclei were analyzed by the following probes (obtained from Vysis, Abbott Molecular Illinois, USA); Locus specific identifier (LSI) MLL, dual color, break-apart probe; LSI TEL/AML1 dual color translocation probe DC, ES and LSI ALK dual color DC, break-apart probe.

*PCR:* (bcr/abl, bcr1/abl, mll/af4, e2a/pbx1, tel/aml1, sil/tal1, plzf/rara, pml/rara, aml1/eto and cbf/myh 1) (12).

#### Histopathology

The formalin-fixed and paraffin-embedded lymph node and bone marrow biopsy specimens were processed with hematoxylin and eosin. The processing of bone marrow biopsy was preceded by an overnight decalcification in EDTA.

The immunohistochemical examination of paraffin tissue sections included examination of following antigens: CD45, CD30, CD15, CD3, CD2, CD20, CD79a, ALK1, and granzyme B.

#### Immunoglobulin (Ig)-T-cell receptor (TCR) clonality

Detection of immunoglobulin heavy chain (IGH) and TCR rearrangements

DNA-based PCR for IGH/TCR clonality was performed using BIOMED I primers (12).

The clonality of PCR products was assessed by heteroduplex analysis (13) and verified by sequence analysis.

#### Treatment

*AML BFM 1998:* Interim phase 2003: 1 day cytoreductive prophase with 6-thioguanine (40 mg/m<sup>2</sup>/d p.o.) and cytarabine (40 mg/m<sup>2</sup>/d).

*AIE induction:* cytarabine, 100 mg/m<sup>2</sup>/d 48 hours infusion; cytarabine, 100 mg/m<sup>2</sup> 12 × 30 min infusion; etoposide, 150 mg/m<sup>2</sup>/d 3 × 60 min infusion; idarubicin, 12 mg/m<sup>2</sup>/d 3 × 4 hours infusion; and cytarabine, i.th. 40 mg.

*ALCL 99, the first cycle:* methotrexate (MTX), 1 g/m<sup>2</sup>; ifosfamide; vinblastine (VBL); dexamethasone (DXM); Cytosar; and VP16.

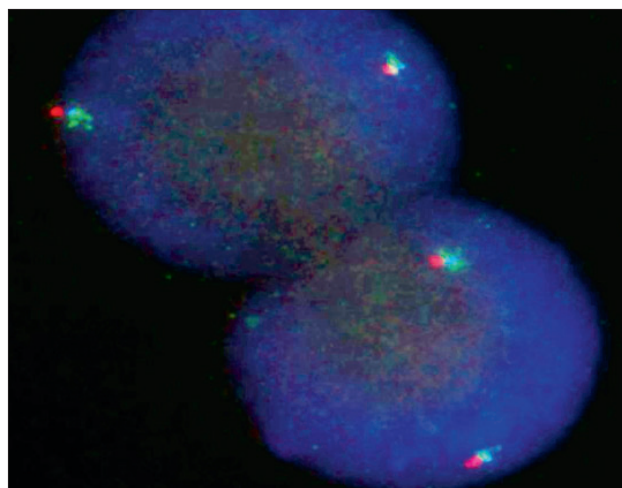
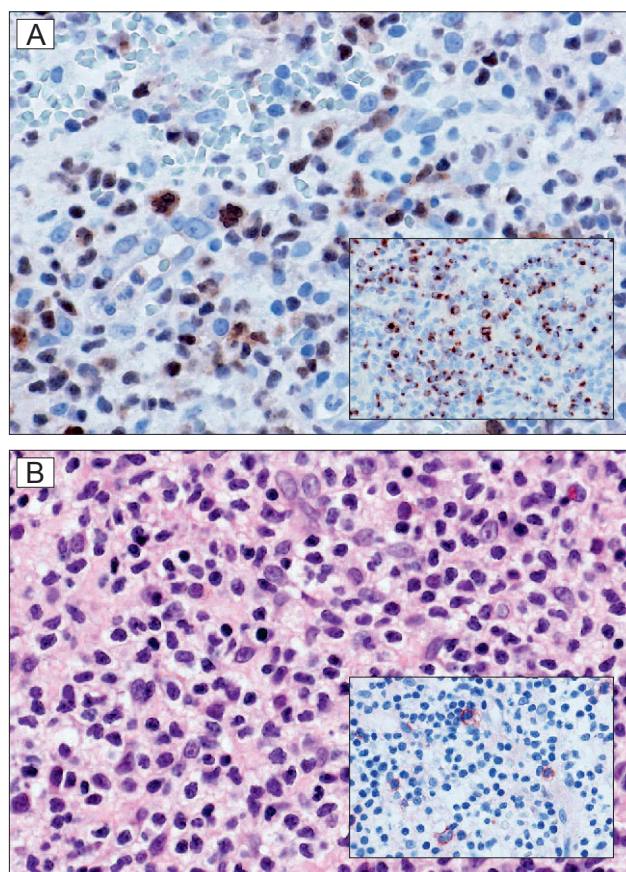


Fig. 4. FISH Peripheral blood: nuc ish 2p23 (3'ALKx2, 5'ALKx2) (3'ALK sep 5'ALKx1) [24/200].



**Fig. 5. Histopathology (A) HE: Predominant population of small to medium tumor cells with irregular nuclei and sparse anaplastic cells + CD30: Weak CD30 positivity in a few tumor cells. (B) ALK-1: Nuclear, nucleolar and cytoplasmic positivity associated with t(2; 5) translocation Granzyme B: Granular positivity of the tumor cells.**

*Modified second cycle: VBL, 6 mg/m<sup>2</sup> and DXM, 6 mg/m<sup>2</sup>.*

## Case

A previously healthy 9 year-old boy was treated with oral antibiotics because of a brief history of fever, sore throat, and neck lymphadenopathy. After 4 days of antibiotherapy, fever still persisted and new deterioration of clinical status appeared; leukocytosis (white blood cell [WBC] count of  $16.0 \times 10^9/L$ ) was noted. He was admitted to a local hospital with suspected infectious mononucleosis. The WBC count gradually increased to  $57.0 \times 10^9/L$ , and he was transferred to University Children's Hospital in Bratislava for further workup.

He presented with fever, malaise, respiratory distress, enlargement of neck, axillary and inguinal lymph nodes up to 3 cm, and hepatomegaly (10 cm below costal margin) and splenomegaly (9 cm in diameter). Complete blood count (CBC) revealed marked hyperleukocytosis ( $57.0 \times 10^9/L$ ), anemia (hemoglobin [Hgb] level, 101 g/L), and thrombocytopenia (platelet [Plt] count,  $78.0 \times 10^9/L$ ). Differential count revealed that 55 % of the cells were atypical, 42 % of the cells were neutrophils, 2 % of the cells were eosinophils,

**Table 1. Bone marrow: immunophenotype results at initial diagnosis**

Antigen	Classification (negative/partly positive/positive)
CD2	negative
CD3	negative
intra-CD3	negative
CD4	negative
CD5	negative
CD7	partly positive, <10%
CD8	negative
CD10	negative
CD13	positive
intra-CD13	positive
CD14	negative
CD15	negative
CD19	negative
CD20	negative
intra-CD22	negative
CD33	negative
CD34	negative
CD38	positive
CD41	negative
CD42	negative,
CD56	partly positive, 13%
CD61	negative
CD65	negative
CD99	positive
CD117	negative
MPO	negative
intra-TdT	negative
CD235a	negative
HLA DR	positive
intra-CD79a	negative
NG2	positive

and 1 % were lymphocytes. Biochemical analysis showed that the patient had elevated levels of liver enzymes (aspartate aminotransferase [AST], 1.63  $\mu\text{kat/L}$  and alanine aminotransferase [ALT], 1.48  $\mu\text{kat/L}$ ), elevated lactate dehydrogenase (LDH) level (11.83  $\mu\text{kat/L}$ ), and high C-reactive protein (CRP) level (180 nmol/L). Chest scan showed that he had bilateral pneumonia with pleural effusion. Ultrasonography confirmed hepatosplenomegaly, abdominal lymphadenopathy, and ascites. Presence of atypical cells in the peripheral blood and bone marrow aspiration was suspicious of acute leukemia (Fig. 1). Immunophenotyping of peripheral blood and bone marrow revealed an atypical population of small cells located mainly in the lymphocyte region and partially overlapping with the monocytic region by forward/side scatter (FSC/SSC) diagram; bright expression of CD45, expression of CD13, and partly aberrant expression of NG2 were identified on atypical cells (complete immunophenotype in Table 1); see also Figure 6.

Both surface and intracellular CD3 was completely negative. Cerebrospinal fluid was negative for tumor cells. Because the patient required rapid treatment and although the patient did not fulfill the complete EGIL criteria for AML (expression of at least 2 myeloid markers), we first classified the malignancy as suspected AML-M0 with suspected MLL gene rearrangement owing to NG2 positivity; thus, the patient received therapy for AML according to protocol AML BFM Interim 2003 (14). Treatment began with cytoreductive prophase (cytarabine [ara] and 6-thioguanine), and



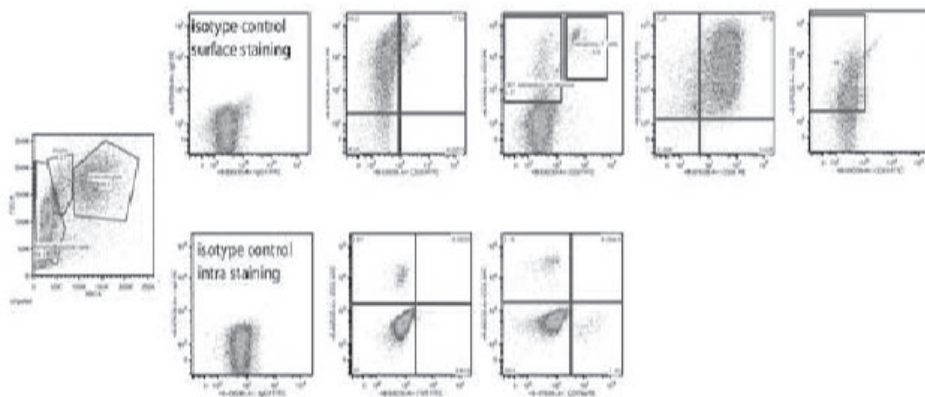


Fig. 6. Bone marrow: immunophenotype results at initial diagnosis.

leukapheresis was performed. The clinical status of the patient worsened; thus, induction regimen AIE was administered, which was followed by severe pancytopenia and multiple infectious complications.

Targeted FISH evaluation did not identify the MLL gene rearrangement, but surprisingly, conventional karyotyping (confirmed by FISH) identified a typical translocation t(2;5)(p23;q35) for ALCL (Figs 2, 3 and 4). According to this finding, we reclassified the immunophenotypic results as a suspected null phenotype ALCL. Clonality analysis revealed an incomplete monoclonal rearrangement of TCR delta (Dd2-Dd3).

Lymph node biopsy was performed and histopathology showed predominant population of small to medium sized tumor cells with irregular nuclei and sparse anaplastic tumor cells and both CD30 and granzyme B positivity was identified in part of the tumor cells.

Nuclear, nucleolar, and cytoplasmic positivity of ALK, which is associated with t(2;5) translocation, was identified by immunohistochemistry (Fig. 5). The patient initially responded to AML induction (3 weeks after AIE chemotherapy, bone marrow aspiration showed no blasts detected by flow cytometry). After the correct diagnosis was established, treatment was changed to ALCL 99 protocol. The first cycle of ALCL therapy was administered, which was followed by pancytopenia and reoccurrence of 5 % of the blasts in the bone marrow and peripheral blood. VBL at 6 mg/m<sup>2</sup> and DXM at 6 mg/m<sup>2</sup> were administered, but the patient exhibited rapid deterioration and died of severe infection during bone marrow depression within 5 months of the initial diagnosis.

## Discussion

ALCL is a highly malignant neoplasm characterized by a clinically pleomorphic appearance, different immunophenotypes, and variable sites of involvement. ALCLs are believed to be of cytotoxic T-cell origin; relatively recent data suggest that at least some ALCLs are derived from cytolytic CD4<sup>+</sup> T cells,  $\gamma\delta$  T cells, or natural killer-like T cells (CD56 or CD57pos) (15). Peripheral

blood and bone marrow involvement is a typical finding in NHL of B- or T-cell lineage; however, it has been only rarely reported in ALCL. Owing to the rarity of this type of presentation and wide morphologic spectrum of ALCL, such cases may represent a diagnostic challenge, especially at initial diagnosis. In case of peripheral blood and bone marrow involvement, ALCL may be difficult to diagnose; this is because it may be difficult to differentiate ALCL from other T-cell lineage leukemias or, as in our case, from acute myeloid leukemia owing to the expression of myeloid markers. It can occur in cases associated with the classic t(2;5) chromosomal translocation or with other variant translocations. Common clinical features include significant respiratory distress, lung infiltrates or pleural effusions, and hepatosplenomegaly. Unique immunophenotypic features (positivity of CD30, ALK1, and presence of cytotoxic granules) together with aberrant T-cell immunophenotype are helpful for making a correct diagnosis. The cytomorphology and flow cytometric analysis of the bone marrow and peripheral blood samples from our patient showed lymphoma cells expressing CD45 and the myelomonocytic markers CD13. T-cell markers were completely negative (only weak expression of CD7). These features are concordant with the findings of previously published cases although a majority of them exhibited more Tcell markers than our case (7–9, 16, 17). In our patient, aberrant expression of NG2 was also identified by immunophenotyping. NG2 is typically associated in both AML and BCP-ALL with MLL gene rearrangement (10), and to our knowledge, it has never been reported in ALCL cases.

Cytogenetic and FISH analyses of peripheral blood in our patient showed the pathogenomic t(2;5) translocation. Histologically, the most common ALCL variant associated with leukemia presentation at diagnosis was the small cell variant described and reviewed by Onciu et al in 9 of 12 patients (7), and it was also detected histologically in the lymph node in our patient. TCR clonality analyses showed an incomplete monoclonal rearrangement of TCR delta (Dd2-Dd3). This rearrangement occurs at very early stages of lymphocyte development and can be found in T-cell precursors as well as in B and NK cells (18). This rearrangement

should not be identified in mature T cells, and in this case, it might indicate that this tumor originated from NK cells.

If ALCL presents with a leukemia phase, it is usually a small cell variant with aggressive clinical course and a poor prognosis (7, 19). Although ALK positivity is associated with a favorable outcome, leukemia phase is characterized by a very aggressive course (7).

Patients with a high number of circulating tumor cells defined by more than 10 normalised copy numbers NPM-ALK in the bone marrow or peripheral blood have an extremely poor prognosis. These patients might constitute a group amenable to alternative therapy in future studies (5).

## Conclusion

Leukemic ALCL is an extremely rare disease and is typically associated with the small cell histological subtype; as is the case with other forms of ALCL, it is characterized by the translocation t(2;5)(p23;q35). In some cases, all the lymphoid immunophenotypic features are absent and the only positive antigens are aberrant myeloid markers, typically CD11b and/or CD13 (7, 9). Presence of malignant lymphoma cells in the peripheral blood and bone marrow was accompanied with myelomonocytic cells with changed reactivity in our case (20). These reactive infiltrates together with minimally differentiated atypical cells bearing only 1 or 2 myeloid antigens might lead to misdiagnosis with minimally differentiated AML. Our case did not fulfill the criteria for AML (at least 2 myeloid antigens; in our case, only CD13 was positive). In such case, the leukemic variant of ALCL must be considered and t(2;5)(p23;q35) together with TCR clonality should be analyzed as soon as possible.

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