A rare case of de novo CD5⁺ diffuse large B-cell lymphoma in leukemic phase and positive for CD13

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Abstract

We report a case of de novo diffuse large B-cell lymphoma (DLBCL) in leukemic phase, positive for both CD5 and CD13. Morphologic evaluation, flow cytometric immunophenotyping, karyotyping and polymerase chain reaction studies were performed. Neoplastic lymphocytes appeared as blast-like cells, positive for CD19, CD20, CD5, CD13, CD79α, HLA-DR, and with restriction for surface immunoglobulin K light chains. Rearrangement of IgH gene, BCL2/IgH translocation and complex karyotype were found. The patient was treated with R-COMP regimen and achieved complete remission. However, only one month after the first restaging of disease, the patient presented with symptoms attributable to central nervous system involvement and her clinical conditions worsened rapidly. While both CD5 expression and leukemic presentation are uncommon findings in DLBCL, positivity for CD13 is very rare. The outcome of our patient shows the poor prognosis of CD5⁺ DLBCL with leukemic presentation. The possible role of CD13 co-expression is discussed.

Case Report

A Caucasian 65-year-old female was transferred to our Institution from a peripheral hospital because of the suspicion of acute leukemia. She was suffering from fever and mild hemorrhagic manifestations. A complete blood count showed: Hb 11.4 g/dL; platelets 58×10⁹/L; WBC 6.63×10⁹/L; manual differential count with 48% neutrophils, 21% lymphocytes, 6% monocytes, blasts 25%, which appeared as undifferentiated, large cells.

At our first observation, peripheral blood smears showed medium- or large-sized blasts (30% of nucleated cells) with dispersed nuclear chromatin; nucleoli; agranular, mildly basophilic; scanty cytoplasm. Such cells were negative at myeloperoxidase staining (Figure 1A,C). On physical examination, splenomegaly was found. CT scans confirmed the presence of enlarged spleen (17 cm longitudinal axis) along with enlarged lymph nodes on both sides of the diaphragm.

Myelosupirate samples showed marked infiltration by blast-like cells with similar morphologic and cytochemical features as the peripheral counterpart (Figure 1B,D). Samples from both peripheral and bone marrow blood were subjected to immunophenotyping by using a FacsCanto II cytometer (Becton Dickinson, Palo Alto, CA, USA) equipped with 3 lasers (405, 488 and 633 nm) and assisted by the FacsDiv software (Becton Dickinson). A 6-7-color method was applied. Because of the very low probability of an acute myeloid leukemia, we used a panel which included several MoAbs: CD45, CD13, CD33, CD117, HLA-DR, CD14, CD64, CD36, CD11b, CD16, CD3, CD4, CD5, CD8, cyCD3, CD19, CD10, CD20, CD81, CD71, CD41, CD61, CD15, CD38, CD56, surface K and λ immunoglobulin light chains (s-K and s-λ), CD79a, TdT, MPO, cyCD3, CD79a. MoAbs were purchased from Becton Dickinson; rabbit F(Ab’)2 polyclonal antibodies purchased from Dako (Agilent Technologies, Santa Clara, California, USA) were used for detecting s-K and s-λ. For CD79a, cyCD3 and TdT, blasts were permeabilized by using the Fix & Perm kit; for MPO, the Intrasure kit (Becton Dickinson) was used.

Blasts resulted to be positive for: CD45, CD19, CD20, CD5, CD13, HLA-DR, CD79a and s-K. The expression of CD19 and CD5 was very low. CD13 was expressed with the same intensity as residual neutrophils (Figure 2). Similar results were found in samples from both peripheral blood and in bone marrow aspirate. Therefore, we were oriented for a mature B-cell lymphoid neoplasm.

Bone marrow samples were investigated...
ed also for rearrangement of immunoglobulin heavy chain (IgH) and for BCL1/IgH and BCL2/IgH rearrangements.

Bone marrow mononuclear cells were isolated by density gradient (Lymphoflot, Bio-Rad, Dreieich, Germany) and nucleic acids were extracted using the EZ1 DSP DNA Blood kit (Quiagen, Hilden, Germany) and quantified with NanoDrop system (2000 Spectrophotometer – Thermo Fisher Scientific, Waltham – MA, USA). PCR assays were carried out using aliquots of 200 ng of nucleic acid.

IgH gene rearrangement was investigated by two primers: VH region primer (FR3, 5'- ACA CGG CYS ATT ACT GT -3’) and JH consensus primer (5'- ACC TGA GGA GAC GGT GAC C -3’), which was 5'-labelled with 6-carboxyfluorescein (6-FAM). The PCR-amplified product was analyzed by capillary electrophoresis on an ABU PRISM 3100 Genetic Analyzer. The GeneMapper v3.5 software (Thermo Fisher Scientific, Waltham – MA, USA) was used to analyze PCR fragments. The positive fragment was 65 to 130 bp long. BCL1/IgH and BCL2/IgH rearrangements were studied according to the protocol established by the European network (BIO-MED-2 Concerted Action).7

PCR assays showed clonal rearrangement of IgH gene and positivity of BCL2/IgH translocation. BCL1/IgH rearrangement were negative.

Bone marrow samples were subjected to karyotyping by using the Q-banding technique and a complex karyotype was obtained: 46,X, -X, -4, +7, -8, -19, -22, der(3)t(3;?), der(6)del(6)(?q14), der(16)t(q22;?), +r, +4m, inc[2].

Bone marrow trephines were fixed in Myelodec reagent A (Bio-Optica), decalcified in EDTA, embedded in paraffin, and cut into 3-5 µm sections. Morphological evaluations were carried out on hematoxylin-eosin, Giemsa, and Gordon-Sweet for reticulin-stained sections. Immunohistochemical stainings were performed using a peroxidase-based system including antibodies specific for: CD20/L6, CD3/PS1, CD5/4C7, CD23/1B12, DBA44, BCL2/100-D5, bcl6/PG-B6p, cyclin-D1 (DSC-6), MUM-1/RF-4, MPO, p53, BCL-6, CD10, CD23, c-MYC. A BenchMark automated Slide Stainer (Ventana Medical Systems, Tucson, AZ, USA) was used.

The histological sections were characterized by cellularity of about 95%, largely represented by medium- and large-sized cells with blast morphology, with one or more nucleoli, scanty cytoplasm. Infiltrating cells were positive for CD20, CD5, MUM-1/RF-4, BCL-2 and BCL-6, and negative for CD10, CD23, p53, MPO, cyclin-D1 (Figure 3); c-MYC was positive in only 15% of neoplastic cells.

The final diagnosis of activated B cell (ABC)-like subtype of DLBC, with CD5 and CD13 positivity and leukemic presentation, stage IVB, IPI 4, was established.

Our patient underwent chemotherapy with six courses of R-COMP regimen. After completion of therapy, whole body CT showed complete remission of lymph node and splenic localization of disease. Immunophenotyping of bone marrow showed disappearance of the pathological clone. Mature B-lymphocytes were undetectable, and mild increase in hematogone percentage was detected. The histologic sections obtained by trephine biopsy confirmed the complete

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**Case Report**

**Figure 1.** Morphology of peripheral blood (A) and bone marrow (from aspirate, B) samples. May-Grunwald-Giemsa staining shows blast-like morphology of neoplastic lymphocytes. Myeloperoxidase staining of peripheral blood (C) and bone marrow (D) cells.

**Figure 2.** Immunophenotyping results. A: weak expression of CD19 and CD5. B: co-expression of CD20 and CD5 (red dots and red arrow); the green dots and the green arrow show the pattern of CD5 expression by the residual T-lymphocytes. C: restriction for surface immunoglobulin K light chains. D: pattern of CD13 expression of neoplastic lymphocytes (red dots and red arrow) compared with neutrophils (blue dots and blue arrow). E: positivity of HLA-DR; F: CD45: back-gating: the neoplastic population shows different CD45 and SSC properties compared with residual normal lymphocytes.
clearance of the pathologic cell population. Finally, normal 46,XX karyotype was found in a myeloaspirate sample. Despite this apparent good outcome, one month after disease re-staging, our patient complained of neurologic symptoms (convulsion, confusion, limb weakness, headache). Gadolinium-enhanced MRI of the CNS was in agreement with central nervous system involvement. Cytologic examination of cerebrospinal fluid was negative. Nevertheless, the clinical conditions worsened rapidly, the patient was transferred to a hospice and was lost to our follow-up.

Discussion and Conclusions

CD5+ DLBCL represents a peculiar subtype of DLBCL, accounting for 5-10% of all cases of DLBCL. Various studies have shown that this subtype of DLBCL has heterogeneous morphological features (monomorphic, giant cell-rich, polymorphonuclear, immunoblastic), frequent expression of BCL2 protein, no derivation from germinal center, more advanced clinical stage at diagnosis, worse general condition, higher LDH level and more frequent central nervous system involvement, as well as a worse response to chemotherapy.9,10

A leukemic phase of DLBCL seems to be less frequent and no information is available about its prevalence, since only sporadic reports or small patient series can be found in the literature.11 Therefore, a leukemic phase of a CD5+ positive DLBCL seems to occur very rarely.10,12,13 Yamaguchi et al.10 reported 4 cases of CD5+ DLBCL in leukemic phase in a series of 120 patients: thus, a prevalence of about 3.3% might be calculated.

We describe herein a very rare case of DLBCL characterized by combination of expression of CD5, leukemic presentation and co-expression of the myeloid marker CD13. After revision of the literature, we found only one previous case with the same features at presentation.9 In that case, a 61-year-old male presented with leucocytosis with blast-like cells, splenomegaly, no lymphadenopathy, massive bone marrow infiltration, and immunophenotyping and immunohistochemistry of the bone marrow clot sections characterized by neoplastic cells which were positive for CD19, CD10, CD20, CD5 and CD13. The outcome of that case was poor, since the patient died after the third course of CHOP regimen.

Our patient presented with poor prognostic indexes: CD5 expression, leukemic phase, advanced clinical stage, high IPI score. The presence of complex karyotype might be an additional negative feature. In one previous study,11 among 29 cases of DLBCL in leukemic phase, six showed complex karyotype. Nevertheless, such a feature did not appear to impact on the response to induction or duration of remission.

Our patient showed very good compliance towards chemo-immunotherapy and, despite achieving complete remission, experienced neurological complications interpreted as central nervous system involvement by disease.

As far as other aggressive lymphomas are concerned, single cases or small series of cases with CD13 expression have been reported in anaplastic large cell lymphomas (ALCL), including CD30+ ALCL, ALK+ ALCL, CD68+ anaplastic lymphoma.14-16

The significance of the presence of the CD13 molecule in such cases, as well as in other neoplastic diseases of lymphocytes, is unclear. However, it is known that, under experimental conditions, CD13 can be expressed by cultured lymphocytes by providing a direct contact with CD13-positive cells such as epithelial cells, and monocytoid or macrophages,17 and that CD13 expression by blasts from acute lymphoblastic leukemia can be dependent on interaction with bone marrow stromal cells.18 Therefore, we can hypothesize that CD13 expression by neoplastic cells from aggressive lymphomas, DLBCL included, may be due to an origin from a pluripotential stem cell, a misprogramming during malignant transformation, or a peculiar interaction between stromal cells and neoplastic lymphoid cells.

The actual prevalence of CD13 expression in aggressive B-cell lymphomas is not known, probably because this marker is not present in the MoAb panels which are used routinely in the diagnostic approach of chronic lymphoid diseases. Similarly, the clinical relevance of such finding is not known. Some previous studies are consistent with a possible role of CD13 in facilitating proliferation of both neoplastic B-lymphoblasts and non-neoplastic B-cell precursors.19 Therefore, it might be hypothesized that CD13 expression could represent an additional negative prognostic factor in cases of CD5+ DLBCL.

We think that extending immunophenotyping of B-cell non-Hodgkin lymphomas with the use of a few myeloid antigens (such as, for example, CD13) could provide additional information about the biology of these diseases and might improve our knowledge of B-cell differentiation and/or B-cell-microenvironment interaction. In addition, because of the relative paucity of cases, multicentric studies should be encouraged in order to establish the frequency of CD13 expression in DLBCL, independently from its leukemic presentation. The positivity of CD5 and CD13 may lead to a misdiagnosis of pleomorphic mantle cell lymphoma, mostly because of blast-like morphology of neoplastic lymphocytes. Therefore, a wide MoAb panel should be used, along with PCR, cytogenetic and/or

Figure 3. Bone marrow trephine biopsy results. Morphology and immunohistochemistry. A) Hematoxylin-Eosin (40x). B) Hematoxylin-Eosin (1000x). C-D) neoplastic lymphocytes are positive for CD20 (C, 400x) and CD5 (D, 200x). E) BCL2 expression (200x). F) neoplastic lymphocytes are negative for Cyclin D1 (200x).
FISH assays, in order to distinguish such a peculiar subset of DLBCL from rare cases of CD13+ mantle cell lymphoma.20

References
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