Chemotherapy alone may lead to a PCR negative stem cell harvest in transformed lymphoma refractory to rituximab

We report a case of patient with transformed low-grade lymphoma who was treated with chemotherapy alone for her 4th relapse and achieved polymerase chain reaction (PCR) negative stem cell harvest. Based on our observations we would like to discuss the strengths and weaknesses of PCR monitoring in lymphoma patients. Chromosomal translocation t(14;18)(q32;q21) between the BCL-2 protooncogene and the JH immunoglobulin gene region can be detected in 85% to 90% of follicular lymphoma (FL). Up to 70% of low-grade lymphomas tend to convert into an aggressive lymphoma with diffuse large cell architecture over time.[3, 4] The histological shift is generally accompanied by the accumulation of secondary genetic changes that lead to poor outcome (p53 mutations, mutations of BCL-6 etc.).[5] The BCL-2/JH translocation remains stable even after the transformation and thus is used as a clonal marker. Polymerase chain reaction (PCR) followed by sequencing is a rapid technique for diagnosis confirmation. PCR monitoring is of prognostic value when used for minimal residual disease (MRD) evaluation during the follow-up.[6]

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The DNA was extracted from bone marrow (BM) and peripheral blood stem cells (PBSC) using standard precautions to avoid cross-contamination.[7] The presence of the BCL-2/JH translocation in the major breakpoint region (MBR) of the BCL-2 gene was examined using a touchdown PCR (TD-PCR)[8] modified for our conditions (for details contact author).

Each reaction contained positive and negative control. The sensitivity was routinely better then 10 positive cells in 10^5 normal cells. PCR products were purified with Microcon-100 (Millipore) purification columns, sequenced with BigDye Terminator Cycle Sequencing kit (Applied Biosystems) and analyzed on ABI Prism 310 Genetic Analyzer (PE BioSystems).

The patient was a 58-year-old female diagnosed with nonhodgkin's lymphoma (NHL) in 1992. The histology was concluded as clinical stage IVB of small lymphocytic lymphoma (cervical, axilary, inquinal nodes, spleen and BM involvement). She received 10 cycles of COP and 3 cycles of chlorambucil with prednisone and reached partial remission (PR; BM morphologically negative, spleen borderline). She was asymptomatic untill 1st progression (Pr) occurred in 1997 (in BM, paraaortic, mesenterial and all external lymphnodes). She received chemotherapy (6xCHOP then 6xM2) with radiotherapy of spleen and reached PR again. The 2nd Pr was treated by radiotherapy of involved lymph nodes (axillary) and spleen again (II/1999). In IX/1999 the patient was first time evaluated by PCR. BCL-2/JH translocation was confirmed in BM. The histology diagnosis has been changed into follicular lymphoma.

The patient was also positive for immunoglobulin heavy chain gene rearrangement (CDRIII) by PCR. PCR for BCL-2/JH translocation was used for further monitoring. The 3rd Pr (IV/2000; in BM, spleen, liver, paraaortic and mesenterial lymphnodes) was accompanied by transformation into aggressive diffuse large cell lymphoma (partially expressing also CD30). The patient received 4 doses of rituximab (monoclonal antibody anti-CD20) in combination with ESAP chemotherapy and achieved CR including PCR negativity in BM. In XII/2000 she presented with her 4th relapse in chest and abdominal lymphnodes, BM histology was negative, but PCR positive with a molecular confirmation of the malignant clone. The patient received 2 cycles of salvage chemotherapy (IVE) and was able to collect a stem cell harvest that was negative by PCR. However, being heavily pretreated she developed profound pancytopenia complicated by sepsis and renal failure. The patient succumbed to these complications.

Using TD-PCR and sequencing we obtained a BCL-2/JH translocation of approximately 200 bp (within published range) both prior and at the time of 4th relapse. (see Table 1)

Thus the initial diagnosis was changed into follicular lymphoma. It is obvious that a PCR technique should be attempted in order to establish a precise diagnosis especially in cases when the histology or other laboratory tests are not conclusive.

Median survival of patients with transformed low-grade lymphomas is less than 1 year despite further conventional therapy.[9] Therefore patients with chemosensitive transformed NHL should be seriously considered for high-dose therapy and autologous stem cell support.[10] Numbers of patients in reports showing the outcome after treatment with rituximab for transformed malignancies are low, especially those evaluating the molecular features of malignant cells.[11, 12] We observed that it is possible to obtain a PCR negative autograft by using salvage chemotherapy even in patients with a 4th relapse of transformed NHL.

We would like to highlight some issues of PCR monitoring in NHL. Our patient's BM was PCR negative (VIII/2000) and CT studies were also negative shortly

Table 1A: MBRA-JH sequence obtained from PCR positive BM samples: MBRA (blue)- major breakpoint region A, NDN (black)- sequence between break of MBRA and JH genes (blue); D segment (green), primer sequences, D segment and break sequence are underlined.

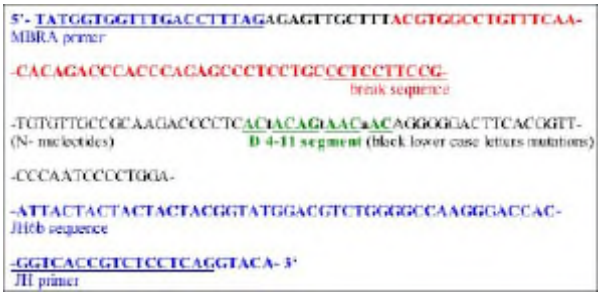
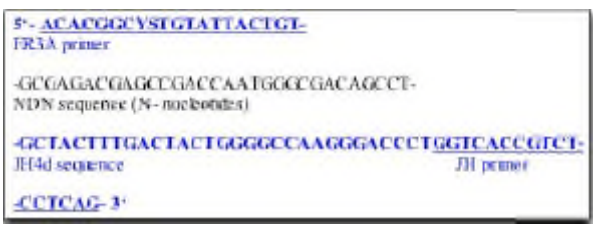


Table 1B: CDRIII rearrangement sequence obtained from PCR positive BM samples: FR3A (blue)- farmework region 3, NDN (black)- sequence between rearranged VH genes and JH genes; JH gene (blue), primer sequences underlined.



before the 4th relapse.

That relapse occurred in lymph nodes allows us to reason that residual tumor cells in lymph nodes were responsible for the recurrence. More frequent PCR evaluation has a prognostic impact in patients with acute lymphocytic leukemia. [13] Our experience suggests that more frequent PCR testing especially in patients with aggressive lymphomas should be warranted during the follow up. Secondly, unlike in leukemia where the disease typically presents within easy-to-sample tissues (BM, PB), in NHL the most reliable tissue for PCR follow up has not yet been clearly established. We recently presented that rituximab alone or in a combination with chemotherapy may induce PCR negativity in the BM of high percentage of NHL patients and that is connected with a better prognosis. [6, 14] However, patient with PCR negativity in BM may still have active disease e.g. in lymph nodes. [15] These observations bring to our attention other methods for early disease detection such as positron emission tomography (PET). [16] We believe that PET imaging may have a prognostic impact in patients without molecular marker and may be also considered in PCR informative patients as the disease may recur in sites or organs that are difficult to sample regularly. Initiation of randomized trials is needed to validate this hypothesis.

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