Aberrant positivity for CD79a in erythroid lineage cells – a finding observed in a subset of re-staging bone marrow trephine biopsies after treatment

Aberrant expression of CD79a has been reported in neoplastic cells in peripheral T cell lymphoma, T-cell acute lymphoblastic leukemia and acute myeloid leukemia (especially those with t(8;21)). In this report, we document the first report of CD79a positivity in erythroid precursor cells in bone marrow. In all, we document this finding in five of 18 re-staging bone marrow trephine samples in patients of lymphoma treated with chemotherapy (one index case and 17 additional validation cases). It is important to appreciate this finding especially in rituximab treated patients where one tends to rely on CD79a to identify minimal marrow disease.

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CD79a and CD79b form disulphide-linked heterodimers, which associate with surface immunoglobulin to form the B-cell antigen receptor complex on mature B-cells. Among the numerous markers of B cell differentiation that can be applied on paraffin sections, CD79a has the advantage of being expressed from pre-B-cell stage to plasma cells.[1] This is attributed to its major functional role in the immunoglobulin-antigen complex. However, aberrant expression of CD79a has been reported in neoplastic cells in peripheral T cell lymphoma, T-cell acute lymphoblastic leukemia and acute myeloid leukemia. A good proportion of acute myeloid leukemia with t(8;21) show CD79a expression.[2-5] To the best of our knowledge, aberrant positivity for CD79a has not been reported in non-neoplastic cells and in erythroid precursors. We document the first report of CD79a positivity in erythroid precursor cells in bone marrow following chemotherapy for peripheral T cell lymphoma, unspecified (PTCL-U). Following the identification of the index case, we have reviewed 17 additional re-staging bone marrow trephines on patients who received treatment for lymphoma, and identified four other cases with similar findings.

Case report. We report a case of a 72 year old man who presented with generalised bulky lymphadenopathy in the mediastinum, axilla, retrocrural, periportal and peripancreatic areas, hepatosplenomegaly, night sweats and weight loss amounting to one stone in the preceding month. Patient underwent excision of right axillary lymph node (LN). Sections of the LN showed a diffuse infiltrate of lymphoid cells with typical features of PTCL-U. The lymphoid cells expressed CD45, CD3, CD2, CD5 and CD7. A majority of these cells expressed CD4. In addition, there were EBV-positive (*in situ* hybridization with EBER1 probe) B-immunoblasts that were polytypic on light chain immunohistochemistry. PCR analysis on paraffin sections showed evidence of a monoclonal T-cell population and absence of a clonal B-cell population. The staging bone marrow showed involvement. Treatment with 6 cycles of CHOP chemotherapy resulted in complete response. A bone marrow biopsy was performed after 6 cycles of CHOP chemotherapy for re-staging. The trephine biopsy was processed and immunostained as per our published protocols.[6]

Immunohistochemical evaluation of the bone marrow trephine showed scanty groups of small T cells suspicious for scanty residual disease. In addition, some of the erythroid precursors showed megaloblastoid morphology. A proportion of such erythroid cells showed positivity for CD79a. The positivity was seen as granular cytoplasmic staining with a Golgi accentuation. The CD79a antibody clone used was JCB117 (1:10, DAKO, Cambridgeshire, UK). The same foci of cells also showed cytoplasmic membrane expression of glycophorin-C, thereby confirming that the cells were indeed erythroid precursors. These larger cells were distinct from the lymphoid cells and the plasma cells that expressed CD79a (Figure 1). These cells did not express CD20, Tdt, CD10 or Pax-5 (hence the possibility of haematogones was excluded).

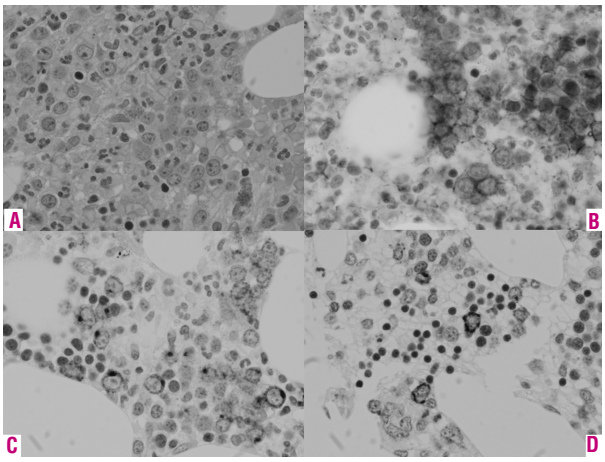

Figure 1. Groups of erythroid precursor cells (A: H&E ×400) showing expression of glycophorin-C (B: ×400) and CD79a (C: ×400). Plasma cells in the background show CD79a expression (D: ×400).

Validation and review of additional cases. Seventeen additional bone marrow trephine samples from patients of B-cell non Hodgkin's lymphoma (n=13), classical Hodgkin's lymphoma (n=3) and angioimmunoblastic lymphoma (n=1) were reviewed by one of the authors (KN). These samples had been obtained following chemotherapy for the purposes of re-staging and all samples represented biopsies with no evidence of marrow involvement. In all samples, immunostaining for CD79a had been carried out at the time of diagnosis in addition to other immunostains as per the requirements for each case. In three of the 17 samples, morphologically identifiable erythroid precursors were seen to be positive for CD79a. In one other sample there was doubtful/possible positivity for CD79a in the erythroid precursors. This was not seen in the other 13 cases. One of the cases was a markedly hypocellular marrow with only few regenerating erythroid islands, and CD79a positivity was noted in these cells (Figure 2).CD79a is a membrane glycoprotein with extracellular, transmembrane and intracytoplasmic domains, which is coded for by the MB1 gene located on 19q13.2. It is a 22kd protein, which in the B-cell ontogeny is first expressed in the cytoplasm prior to μ heavy chain expression. In the normal B-cell differentiation programme, CD79a expression is regulated by Pax-5 and PU.1, both of which are known to bind to the regulatory sequences of the MB1 gene.[7,8] CD79a is expressed as two alternatively spliced transcripts. The smaller transcript codes for an immature protein, which does not form disulphide-linked dimers with CD79b. However, the immature protein has the transmembrane and cytoplasmic portions and is detected by the CD79a antibody on immunohistochemistry.[9] Aberrant expression of CD79a is a recognised phenomenon in neoplastic myeloid cells and T-cells.[2-5] CD79a expression among AMLs is almost restricted to cases with translocation t(8;21), which results in expression of AML1-ETO fusion

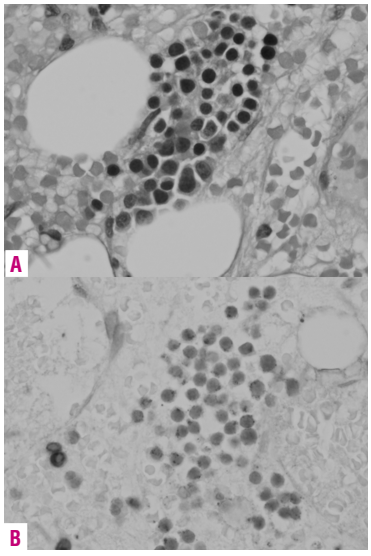

Figure 2. Scanty regenerating erythroid islands in a hypocellular marrow. **A.** H&E ×600. showing CD79a expression **B.** ×600.

protein. Most of such cases also express other the B-cell molecules, such as CD19 and Pax-5. In *in vitro* experiments, the Runt domain of AML1-ETO fusion protein binds to PAX5. Hence, it possible that Pax-5 expression, and through it the expression of CD79a could be a consequence of t(8;21). Furthermore, nearly 40-50% of T-cell acute lymphoblastic leukemia (T-ALL) shows CD79a expression, mostly cytoplasmic and weak; however, they do not express Pax-5. Both CD79a expression and IgH DJ rearrangement are preferentially associated with T-ALLs expressing T-cell receptor γδ, though CD79a expression occurs independent of IgH rearrangement. The significance of CD79a expression in T-ALLs is not fully clear. However a link to PU.1 is possible, as early precursor T cells and a proportion of T-ALL express PU.1.[4,10] In cases described in this report, we suspect that the aberrant positivity for CD79a in the erythroid precursors could be possibly related to the effects of chemotherapy and/or regeneration. Earlier studies have not shown Pax-5 expression among erythroid precursors, similar to the index case in this report.[11] Furthermore, CD79a positivity in erythroid precursors cannot be due to PU.1, as PU.1 expression is incompatible with the erythroid program. PU.1 and GATA-1 are two mutually exclusive sequence-specific DNA binding proteins, which play a central role in decisions, related to lineage commitment. While PU.1 commits the common myeloid-erythroid progenitors to the myeloid programme, GATA-1 commits the cells to megakaryocytic-erythroid programme. Enforced expression of either factor results in inhibition of the programme dictated by the other.[12] Hence, our finding suggests that there are probably other mechanisms for positivity of CD79a apart from Pax-5 and PU.1. We believe, the aberrant CD79a positivity represents aberrant expression. In a relatively large number of bone marrow trephines (about 1500 per year), that are evaluated in our institution (a good proportion of which are immunostained for CD79a), we have not noticed such aberrant positivity among benign erythroid or myeloid cells, apart from the cases described in this paper. Hence, we do not think that this positivity is due to non-specific unmasking of other proteins during the antigen retrieval procedure employed.

To conclude, CD79a positivity can be induced in erythroid precursors possibly under *stress* and the underlying molecular mechanisms are likely to be different from those understood till date. In practical terms, it is important to appreciate this finding while interpreting re-staging bone marrow trephine biopsies, especially those of rituximab-treated B-cell lymphoma. In patients who have been treated on rituximab based regimens, CD20 immunostaining is unhelpful possibly due to masking of antigen or loss of CD20.[13] In this subset of patients, we tend to rely on CD79a immunostain to identify minimal marrow disease, and we should be careful not to misinterpret erythroid precursors as residual neoplastic B-cells.

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