C14. **Acute leukemia with ambiguous triphenotypic lineage – a challenge for diagnosis and treatment**

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Background. Undifferentiated acute leukemias were best analyzed by immunophenotypic methods and in EGIL classification for the first time were found leukemias with more than one lineage. The scoring system of EGIL was integrated in the 2001 and 2008 edition of the WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues, which recognized a special category called "leukemias of ambiguous lineage." The vast majority of these rare leukemias are classified as mixed phenotype acute leukemia (MPAL), and acute undifferentiated leukemias and natural killer lymphoblastic leukemias are also included in this category.

The major immunophenotypic markers used by the WHO 2008 to determine the lineage for these proliferations are myeloperoxidase, CD19, and cytoplasmic CD3. However, extensive immunophenotyping is necessary to confirm that the cells indeed belong to 2 different lineages or coexpress differentiation antigens of more than 1 lineage. Specific subsets of MPAL are defined by chromosomal anomalies such as the t(9;22) Philadelphia chromosome BCR-ABL1 or involvement of the MLL gene on chromosome 11q23.

Other MPAL are divided into B/myeloid NOS, T/myeloid NOS, B/T NOS, and B/T/myeloid NOS. MPAL are usually of dire prognosis, respond variably to chemotherapy of acute lymphoblastic or acute myeloblastic type, and benefit most from rapid allogeneic hematopoietic stem cell transplantation.

A diagnosis of triphenotipic acute leukemias also an extremely rare diagnosis and requires strong signs of three lineages in blastic cells.
Coexpression of B- and T-lineage associated antigens or antigens of all three lineages is exceedingly rare, accounting for <5% of MLLs.

Material and method. Results. Current diagnosis in our department is done based on ELN panels, and immunophenotyping on a FACS Calibur cytometer. We present a difficult case of 31 years male who was diagnosed with ambiguous lineage acute leukemia in 2014, February.

Clinical presentation was typically for acute leukemia with anemia, thrombocytopenia and leukocytosis with 49% blasts in peripheral blood and 95% in bone marrow, with medium size, fine chromatin, rare nucleoli, small amount basophilic cytoplasm, positive at myeloperoxidase (MPO) 30% and periodic acid Schiff (PAS) 9%. Immunophenotyping by flow cytometry on bone marrow aspirate identified 85% blasts with CD45 medium/low, SSC medium with expression of lineage markers cCD3+ (60%) cMPO- cCD79a+ (64%) TdT-/+ and stem cell markers CD34+ CD38+; other surface markers were found for myeloid lineage CD33+/- CD13+ CD117+ CD15- CD36- CD11b- CD14- CD64-, B cell lineage CD19- CD10+/-, T cell lineage CD2+ CD3- CD7+ CD5- CD1a- and NK lineage CD56- CD16-. Immunohistochemical staining on bone marrow biopsy described the same blasts with expression of CD34, MPO, CD33, cCD3, negative for cCD79a and CD68, suggestive for MPAL T/myeloid.
Based on scoring system for MPAL, we considered the diagnosis for trilineage ambiguous acute leukemia, T/B/myeloid.

Cytogenetic and molecular analysis detected only a few monosomias and no specific fusion gene.

Treatment choice was difficult but regimen for ALL, **UKALL XII/ECOG 2993,** was done, with good response at interimar analysis after first month of induction therapy. Further treatment will include consolidation with allogeneic SCT.

Conclusion. Ambiguous lineage acute leukemias are rare cases, associated with cytogenetic high risk changes. Powerfull diagnostic tools by multiparametric immunophenotyping are extremely important in diagnosis and aplying scoring system developed by EGIL could be a chalenge. Most MPAL are biphenotypic, but MPAL with three lineages could be found and treament choice is difficult, but ALL regimen have been more efficient.