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Immunophenotyping in Hematopathology: Applications in Leukemia Diagnosis

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Description

Acute Lymphoblastic Leukaemia (ALL) is a lymphoid precursor illness that is the most prevalent malignancy identified in children, with about 3000 new cases diagnosed each year (SEER 2004-2008 data). The current WHO classification of ALL includes three main diagnoses: B-lymphoblastic Leukemia/lymphoma, not otherwise specified, B-ALL (B-cell Acute Lymphoblastic Leukemia) with recurring cytogenetic abnormalities, and T-ALL. The evaluation of morphology in conjunction with immunophenotyping is sufficient for the diagnosis of ALL and distinguishing between B-ALL and T-ALL. More cytogenetic study is needed to determine whether a specific instance of B-ALL may be further subcategorized based on specific chromosomal abnormalities.

Cells of small to medium size with limited cytoplasm, a high nuclear-to-cytoplasmic ratio, round to oval nuclei with smeared or coarsely reticular chromatin and inconspicuous to variably prominent nucleoli are found in peripheral blood and bone marrow aspirates. Cases with larger cells with more cytoplasm and more varied nuclear contour and chromatin are less prevalent. The cytoplasm is basophilic in nature and may contain vacuoles or granules.

Tissue taken from a bone marrow biopsy is often significantly hypercellular, while it may be normocellular or even transiently hypocellular in unusual circumstances. At the time of presentation, bone marrow involvement is normally diffuse and homogenous, but it can be localized upon relapse. Homogenous lymphoblasts with limited cytoplasm displace typical hematopoietic components, revealing just a few megakaryocytes and modest groups of erythroid precursors. Lymphoblast nuclei can be spherical or convoluted, with chromatin that is often described as equally scattered or stippled but can be relatively condensed. Nucleoli are inconspicuous yet can be noticeable. Mitotic activity is virtually always brisk.

Sometimes there is partial or severe necrosis. A fully necrotic marrow provides a diagnostic problem, and a second biopsy and aspirate from the contralateral location is strongly advised and frequently beneficial. Reticulin fibrosis can occur, resulting in a dry tap. Inability to aspirate is also caused by "packed" marrow. When an aspirate is not accessible, immunohistochemistry can be used to undertake immunophenotypic characterization. When an aspirate cannot be collected, a second core is sent to the cytogenetic laboratory, and cytogenetic analysis is done on material recovered from the core biopsy after disaggregation.

The preceding information applies to the vast majority of cases, but unusual manifestations do arise. Azurophilic granules are found in 5% to 7% of ALL cases, with the granules being coarser and bigger than those found in Acute Myeloid Leukaemia (AML). These situations are more common in children with Down syndrome and in those who have a translocation between chromosomes 9 and 22 [t(9;22)]. Immunophenotyping and the absence of myeloperoxidase staining distinguish them from AML.

Because of the presence of significant vacuolation, some ALL cases may resemble Burkitt's lymphoma, although the profoundly basophilic cytoplasmic feature of Burkitt's lymphoma is absent. Hypereosinophilia is seen in some cases, most notably in B-ALL with t(5;14) and T-ALL with an 8p11.2 anomaly. Finally, some patients may present with an aplastic state that is followed by a partial recovery and then overt leukaemia.

The outcomes of ALL patients have improved considerably over the years and continue to improve; with current Event-Free Survival (EFS) rates reaching 85%. Better risk classifications of patients has been a key component of therapeutic approach success. The immunophenotype of the leukemic blasts and the classification of a patient as having B-ALL or T-ALL are used for initial stratification.

White blood cell count at diagnosis was one of the first recognized prognostic variables for individuals with B-ALL and remains relevant as part of the National Cancer Institute risk classification scheme. Patients with B-ALL who are >365 days and 10 years old at diagnosis and have WBC counts of 50 109/L are considered standard risk, whereas those who are 10 years old at diagnosis or have WBC counts of 50 109/L regardless of age are considered high risk. These parameters govern initial induction therapy, with standard-risk patients receiving a three-drug induction of vincristine, l-asparaginase, and glucocorticoids and high-risk patients receiving a four-drug induction that includes an anthracycline. Patients with T-ALL are classified as HR and are given a four-drug induction regimen.

Survival in ALL has improved substantially over the years, owing partly to risk classification and the capacity to adjust therapy correctly; continuing advances in risk stratification would potentially enhance outcomes even further. The inclusion of cytogenetic information for B-ALL in the 2008 WHO classification of precursor lymphoid neoplasms reflected the importance of chromosomal abnormalities in determining the biology and response to therapy in ALL patients. Progress in ALL biomedical research will eventually necessitate updating of these criteria to incorporate more subclasses based on specific genetic anomalies, especially those with prognostic value and those that can be exploited for targeted therapeutics. The biggest advantage of categorizing cases as having t(9;22) such cases can be targeted with imatinib.