

Leukaemia Diagnosis 2004: The Integrated Final Report

Wendy N Erber

Haematology, The Western Australian Centre for Pathology and Medical Research

The World Health Organisation (WHO) 2001 publication “Tumours of Haematopoietic and Lymphoid Tissues” was proposed as a clinically useful classification, incorporating morphology, cell phenotype, genetics and clinical features to establish the precise diagnosis. Distinct entities were defined based, where possible, on cell lineage and stage of differentiation. The classification has been widely adopted thereby standardising diagnoses internationally and is being used to predict clinical behaviour. The proper use of this classification necessitates the aggregation of results from a number of diagnostic tests to diagnose specific WHO defined entities. For leukaemia diagnosis it is the responsibility of the Haematologist to provide this information in an integrated form for treating clinicians.

An “integrated report” incorporates results of all diagnostic laboratory investigations on samples from a patient with leukaemia into one report giving a final WHO diagnosis, together with any additional clinically useful information. The requirements for an integrated leukaemia report include:

1. Patient and sample details;
2. Morphology of the blood and bone marrow (aspirate and / or trephine biopsy);
3. Results of ancillary tests performed including cytochemistry, phenotyping, cytogenetics, and molecular genetic results;
4. Other information of diagnostic and / or clinical significance (eg. prognosis; recommended method for monitoring residual disease);
5. FINAL DIAGNOSIS using the WHO Classification; and,
6. Name of the reporting Haematologist

and date completed.

Relevant clinical information, including patient demographics, clinical history, blood count, specimen type and date of collection, should be included in the integrated final report. For some leukaemias the current and past patient history may be essential to the diagnosis, for example:

1. Constitutional symptoms and organomegaly;
2. Previous haematopoietic malignancy (e.g. myelodysplasia), cytotoxic drugs (e.g. alkylating agents) or radiotherapy;
3. Constitutional karyotypic abnormalities that may predispose to leukaemia (e.g. trisomy 21); and,
4. Other relevant pathology results (e.g. LDH) or viral serology (e.g. EBV; HTLV-1).

The morphology of the blood and bone marrow are the essential first steps (“screening tests”) in the diagnostic repertoire for leukaemias and other haematopoietic malignancies, and, in many cases is diagnostic. Morphology should be used to determine which ancillary tests are required to confirm or validate the diagnosis and classify the malignancy by WHO criteria. Initial blood film and bone marrow morphology reports must be issued with the morphological features and provisional diagnosis. This report should state which ancillary tests will be performed on the sample. These will vary by the leukaemia type, as determined by morphological assessment. For example:

- a) Peripheral blood lymphocytosis with classical morphologic features of chronic lymphocytic leukaemia requires cell

phenotyping to confirm the phenotype and detect prognostic antigens (e.g. CD38; ZAP70), FISH for prognosis (e.g. 17p, 6q) and a bone marrow trephine to determine the pattern and extent of disease.

- b) Peripheral blood neutrophilia with left shift and basophilia, associated with bone marrow morphology consistent with chronic myeloid leukaemia, requires cytogenetics (full karyotype) for the Ph chromosome and/or other abnormalities, FISH for t(9;22) and/or deletion of der(9), and molecular genetics (for BCR/ABL). Only if the blast count is significant would phenotyping be required.
- c) Bone marrow aspirate with blast cell count and morphology of acute leukaemia requires immunophenotyping for cell lineage, differentiation stage and to detect antigens that may be useful for monitoring residual disease, and cytogenetics (full karyotype). The results of morphology and cell phenotype will determine which genetic abnormalities should be investigated by FISH and molecular techniques.

The final integrated report must include morphology results of both the bone marrow aspirate and trephine biopsy as these two samples provide complementary information. The bone marrow aspirate provides quantitative and qualitative cytological assessment, whilst the trephine yields information regarding cellularity, pattern, extent of disease and fibrosis which may be important for management and prognosis.

Cell phenotyping is an essential component in the laboratory work-up for the diagnosis and classification of many leukaemias, particularly acute leukaemias and chronic lymphoid malignancies. Phenotyping, which establishes the cell lineage, stage of cell differentiation and the presence or absence of specific antigens, may be diagnostic (e.g. CD10+/CD19+/TdT+ precursor B-lymphoblastic leukaemia) or of value in confirming a diagnosis (e.g. CD13+/CD33+/CD117+ expression by

blast cells with myeloid morphology). Phenotyping can detect unique antigenic characteristics for diagnosis of specific entities (e.g. CD11c+/CD19+/CD103+ hairy cell leukaemia), add prognostic information (e.g. CD38 or ZAP70 in B-chronic lymphocytic leukaemia), assist in monitoring residual disease (e.g. dual CD4+/CD8+ T-prolymphocytic leukaemia) and detect antigens which can be targeted with immunotherapy (e.g. CD20). It is important that these results, together with their interpretation, be incorporated into the integrated final report.

Cytogenetics has become a critical component in the testing repertoire of leukaemias with both conventional karyotyping with G-banding and FISH having roles. Karyotyping, assessing all chromosomes, is essential in the diagnosis and classification of acute leukaemias, chronic myeloid leukaemia and myelodysplasia. FISH, by targeting specific genetic lesions, is valuable when distinct abnormalities are diagnostic of a particular entity (e.g. PML/RAR α of t(15;17) in acute promyelocytic leukaemia) and provides prognostic information (e.g. 11q23 abnormalities in MLL leukaemias). Results of karyotyping and FISH, both positive and negative, should be included in the integrated final report.

Molecular genetic analysis is becoming increasingly important in the analysis of haematological malignancies. In general, such testing has a longer turn-around-time than the above-mentioned tests, and, as a result the information gained is usually confirmatory validating the diagnosis established by other techniques. In certain settings molecular genetic testing is required to confirm clonality (e.g. T cell malignancies) or for the detection and monitoring of specific disease-associated translocations (e.g. BCR/ABL in chronic myeloid leukaemia). It is important that these molecular genetic features be determined on the diagnostic sample, even though they may not be essential for diagnosis and classification, as they may be critical for monitoring residual disease and relapse following therapy.

The final integrated leukaemia report should include the results of all investigations performed on the diagnostic sample to give a total specimen interpretation and final diagnosis using the WHO classification. This report should be able to be generated automatically by extracting individual test results from the data repository of the laboratory information system (LIS). Repeat data entry to generate the integrated report should be avoided due to the labour required and the potential for introducing errors. Other information of clinical significance, such as likely prognosis, most useful investigations for monitoring residual disease and photographic images, should also be able to be added to the report. All integrated reports should be included into an institutional leukaemia database. This is useful, not only for the individual patient, but also for teaching and research purposes. A large searchable database will enable new disease entities to be discovered and epidemiological and statistical data to be extracted.

The integrated leukaemia report may require initial information technology expertise to develop but is feasible, improves data quality and will be of benefit to patients and clinicians. As different test types differ in their turn-around-times the final integrated report will not be immediately available and individual test reports (e.g. morphology; phenotyping) must still be issued. However the final leukaemia diagnosis and WHO classification may only be possible when all tests are completed. An example of an integrated report is provided.

Example of an integrated leukaemia report for a case of precursor B-lymphoblastic leukaemia.

Patient Details:			
Name:	John AKIMURA	Ref Number:	123456A
Date of Birth:	2/9/73	Gender:	M
Hospital:	St Elsewhere's	Clinician:	Brown
Clinical History:	Persistent sore throat and fever.		

Sample Details			
Date of Collection:	16/1/04	Accession Number:	678901Z

Specimen Type:	Blood: <input type="checkbox"/>	Bone Marrow Aspirate: <input checked="" type="checkbox"/>	Bone Marrow Trephine: <input checked="" type="checkbox"/>
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Blood Count and Film:			
Hb: 125 g/L	WCC: 10.1	Platelets: 85	Blasts: 3%
Blood Film Comment:	Intermediate sized atypical mononuclear cells with fine chromatin, a single nucleolus and basophilic cytoplasm.		

Bone Marrow Aspirate: (Romanowsky stain): Hypercellular marrow with 90% blast cells of intermediate size with round nucleus, fine chromatin, scanty basophilic cytoplasm and prominent vacuolation. No granules or Auer rods.
CONCLUSION: Morphological appearances of Burkitt's Leukaemia / Lymphoma

Bone Marrow Trephine: (H&E stain): The marrow is markedly hypercellular with a diffuse infiltrate of blast cells of intermediate size with round nuclei, fine chromatin and one or more nucleoli. There is a high mitotic rate. The blast cells were CD79a and TdT positive.
CONCLUSION: Precursor B Lymphoblastic Leukaemia / Lymphoma

Immunophenotyping: (Flow cytometry): The blast cells in the bone marrow (weak CD45 expression and low side scatter) made up 83% of cells in the sample. These expressed B-lymphoid antigens CD10, CD19 and CD79a and were positive for TdT, CD34 and HLA-DR. There was no surface IgM or cytoplasmic μ chain and no expression of κ or λ light chains.
COMMENT: B-lineage blast cells. Phenotype of Precursor B-lymphoblastic Leukaemia.

Cytogenetics: Karyotype: 46, XY, -6q,+8,-11.
 FISH: t(8;14) NOT detected with the c-myc breakapart probe.

Molecular Genetics: c-MYC/IgH rearrangement NOT detected.

Prognosis: No specific information regarding prognosis.

Final Diagnosis (WHO Classification): **Precursor B-Lymphoblastic Leukaemia**
 Comments: Although the morphology of the marrow aspirate was consistent with Burkitt's Leukaemia the blast cell phenotype (TdT+, IgM-), cytogenetics and molecular genetics (no c-MYC rearrangement) do not support this. The overall interpretation is therefore Precursor B-Lymphoblastic Leukaemia.

Reported By: Dr Wendy Erber, Haematologist	Date: 5/02/2004
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