Chapter 3 Ancillary Tests in Bone Marrow Diagnosis

ABSTRACT

Selection of the appropriate ancillary tests, often based on careful morphologic examination of peripheral blood and bone marrow and adequate patient data, are critical for accurate diagnosis. Sometimes a characteristic clinical picture or pathognomonic morphologic feature directly reflects the need for a specific panel applied simultaneously with the initial examination. Routine ancillary tests range from cytochemical and histochemical staining to more complex immunophenotyping, genetic, and molecular techniques. Sample processing and laboratory conditions affect the accuracy and reliability of these tests. The technical test procedure, interpretation, quality control measure, and source of error are essential considerations discussed for each test category in this chapter.

INTRODUCTION

Careful morphologic examination of the bone marrow will often suggest one or more ancillary tests to confirm the diagnosis, exclude differential diagnoses, or assess prognostic factors. A characteristic clinical picture or a previous diagnosis may indicate a simultaneous ancillary test application at the initial examination. These tests encompass technical procedures applied to specific sample preparation, including aspirate smears, biopsy touch imprints, clot sections, and paraffin-embedded cores.

Ancillary tests involve cytochemical and histochemical stains, immunophenotyping, and genetic and molecular tests. The proper selection of the test panel requires a deep knowledge of the disease pathology and diagnostic criteria and the professional skill for the appropriate application of the technique, its interpretation, recognition of its diagnostic specificity and sensitivity, and test limitations.

Some tests are integral within the bone marrow pathology laboratory, e.g., cytochemical and histochemical tests and IHC, while others require specialized units such as flow cytometry and molecular tests. In the latter case, careful attention to the type and quality of the sample is critical. This chapter will give an overview of the main ancillary test applied in bone marrow diagnosis:

- 1. The categories of ancillary tests
- 2. The rationale for test application, standard technique, limitations, and interpretation
- 3. The quality control and assurance measures applied to the test

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CATEGORIES AND APPLICATIONS OF ANCILLARY TESTS FOR BONE MARROW DIAGNOSIS

The ancillary test can identify cell type and differentiation stage, microorganisms, or abnormal deposition in the bone marrow cells or stroma.

They can be applied to bone marrow aspirate smear, anticoagulated sample, or bone marrow biopsy and are significantly affected by the initial sampling and processing protocols. It is, therefore, essential to determine all test requirements before commencing sampling.

Cytochemical and Histochemical Stains

Cytochemistry

Despite the infrequent use of many cytochemical stains, few are still of value in specific conditions, particularly in limited-resource locations. Bone marrow aspirate smears, particle crush smears, and biopsy touch imprints are the most suitable for their application. Most of the stains are commercially available, and the most common are: (Rice & Bain, 2012)

- i) The Perl's (Prussian blue) iron stain: is indispensable for demonstrating erythroblastic iron and counting pathological form, in addition to assessing iron stores
- ii) Myeloperoxidase (MPO) and Sudan Black Stains are two rapid and cheap stains for the rapid identification of myeloid differentiation of leukemic blasts, especially with the unavailability of flow cytometry.
- iii) Leukocyte alkaline phosphatase is helpful in rapidly differentiating reactive leucocytosis from suspected chronic myelogenous leukemia.
- iv) Tartrate-resistant acid phosphatase differentiates hairy cell leukemia from similar morphologic subtypes, though it is no longer a diagnostic criterion in the 2016 WHO classification

Histochemical Stains

Histochemical stains can be applied to core biopsy or clot sections. The most common stains in practice are:

- a) *Leucocyte Stains:* Only a few of the above cytochemical stains can be applied to bone marrow biopsy, such as Sudan black because tissue processing destroys most cellular enzymes. Chloroacetate esterase is one of the few enzymes that could stand processing. However, its use is somewhat limited except in the absence of other myeloid markers. Some enzymes now have an immunostaining substitute, e.g., myeloperoxidase and lysozyme.
- b) *Microorganisms Stains:* These are especially useful in patients with undiagnosed fever, particularly in immunocompromised cases, where detecting the organism from the peripheral blood is difficult. They include
 - a. **Gram stain:** bacteria appear as blue rods or cocci in the H&E section regardless of gram reaction. Gram staining of marrow sections has the same procedure as in the microbiology laboratory, except that neutral red is used instead of safranin. With the modified Brown and Hopps method, gram-positive organisms usually stain well, while gram-negative remain unstained due to the loss of bacterial lipid walls during tissue processing. (Taylor, 1966).

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