Direct Immunofluorescence (IF)

Direct immunofluorescence (IF) can improve the diagnostic capability of conventional histopathology in the diagnosis of fungal diseases (18). The IF procedure, which can be performed on formalin-fixed paraffin-embedded tissue sections is helpful in confirming a presumptive histologic diagnosis, especially when fresh tissues are not available for culture or when atypical fungal forms are seen. The Division of Mycotic Diseases, Center for Disease Control, Atlanta (United States) and others have a broad battery of sensitive and specific reagents available for identifying the more common pathogenic fungi.

The immunofluorescence procedure has several advantages. Final identification of an unknown fungus is possible within hours after H&E and GMS stained sections are initially examined. The need for time consuming and costly cultures is often obviated by IF, since the special stains have been laboratories for confirmatory identification. Most service laboratories, embedded tissue and the shipment of specimens to distant reference embedded, does not appear to affect the antigenecity of fungi. this for time consuming and costly cultures is often obviated by iF, and common pathogenic fungi.

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References

Background

Myelodysplastic syndromes (MDS) are neoplastic clonal hematologic stem cell disorders which occur mainly in older adults (1). These are a heterogeneous group of diseases characterized hematologically by conspicuous cytopenias and possess a variable rate of progression to overt acute leukemia. (1, 2). Cyclic dysplasia, the morphologic hallmark of MDS, is defined in bone marrow aspirate samples with May-Grünwald Giemsa stain. The staining is so important in this field, that it formed the basis for the classification of MDS in 1982 by the French-American-British (FAB) group. Today, several additions to these cytologic findings – such as, refined morphology, risk evaluation, and cytogenetic data form the basis for the diagnosis of MDS. According to the current World Health Organization (WHO) classification, trephine biopsies are considered to be very important in the initial diagnosis of MDS (2, 3).

Histopathological studies of core biopsies yield complementary data to those provided by bone marrow aspirates and blood smears. They also provide complementary information with respect to the amount of hematopoietic cellularity and topographic alterations of the progenies. The use of appropriate special techniques permits grading bone marrow fibrotic content and defines the presence of fibrosis. Both cellularity and fibrosis constitute parameters of significant prognostic importance (4-8).

Histopathology Methods and Special Techniques

Hematolyn and eosin stain (H&E), Giemsa, Gomori’s silver impregnation technique and Perls’ stain in combination with immunostaining are some of the most common methods used to study MDS. Bone marrow cylinders that were optimally fixed, decalcified, embedded in paraffin and stained with H&E and other special stains were used for the quantitation of cellularity and the evaluation of histarchitectural displacement of progenies (Fig. 1).

In general, hyperplastic bone marrows predominate in MDS (Fig. 2), although in some patients hypoplastic marrows were seen, (8, 9). Histopathological findings of different progenies with H&E were used to differentiate MDS from secondary dysplastic changes produced by other neoplastic (lymphoma, metastasis) or reactive conditions (nutritional, toxic) (5). These changes consist of displacement of poorly formed erythroid groups to endosteal surface and, conversely, relocation of myeloid cells to centromedullary spaces (Fig. 2 and 3). In addition, dysmorphic megakaryocytes (hypo- or hyperlobated, atypical large cells) were frequently seen (Fig. 4) in MDS adopting a paratrabecular position.

Cytomorphological detail studied with Giemsa allowed us to discern fine nuclear details (such as the nuclear membrane, chromatin pattern, nucleoli) and also the presence of cytoplasmic granules, leading to a better definition of different cell populations (Fig. 5) (10,11). Giemsa stain was also helpful in the search for the presence of immature cell (blast) aggregates, formerly designed as ALIP (abnormal localization of immature precursors), which may represent an increase in blasts cells (Fig. 3). Based on this finding, one can make a differential diagnosis with other immature cells that present themselves in the marrow, as groups of megakaryotic erythroblasts, promyelocytes, and monocytic accumulates. However, in this case, in order to confirm the blastic nature of the cells, there was a need for additional immunostains, which included not only appropriate lineage markers such as glycophorin A, myeloperoxidase and CD68, respectively, but also CD34 and CD117 (12). Cases with more than 5% of CD34+ blasts in the marrow were included in the Refractory Anemia with Excess Blasts (RAEB) category, namely RAEB 1 (5-9% blasts cells) and RAEB 2 (10 to 19% blast cells) respectively (1) (Fig. 7). Bone marrows with CD34+ cells forming aggregates or clusters (i.e., three or more CD34+ cells) are currently described as having “multifocal accumulations of CD34+ progenitor cells” (2) (Fig. 8). These patients were defined as having both overall lower survival rates and leukemia free survival in a multivariable analysis (4).
Special Techniques Applied to Bone Marrow Biopsies in the Diagnosis of Myelodysplastic Syndromes

Figure 1. Normocellular MDS. Conspicuous dyserythropoiesis and increase in immature mononuclear cells (H&E, 250x). Arrow points to a fat cell.

Figure 2. Hypercellular MDS. Dyshematopoietic features include dyserythropoiesis, increase in immature cells (presumably of myeloid lineage) (arrows) and granular deposits of hemosiderin (iron-storage complex) (arrowheads) (H&E, 400x).

Figure 3. MDS. High power view showing aggregates of abnormally localized immature precursor (ALIP) cells (arrow). (H&E, 400x).

Figure 4. MDS. Grouped dysmorphic (hypolobated) megakaryocytes (arrow), immature cells with clear chromatin and finely granular cytoplasms (black arrowheads), and dark stained nuclei of erythroid cells (grey arrowhead) (Giemsa, 400x).
**Figure 5.** MDS Refractory anemia with excess blasts 2 (RAEB 2). There are numerous interstitial blasts cells (arrows) and isolated segmented granulocytes (arrowhead) (Giemsa, 400x).

**Figure 6.** Hypocellular MDS (RAEB 2). A cluster of immature myeloid cells is depicted (arrow) (Giemsa, 400x).

**Figure 7.** Hypocellular MDS (RAEB 2). Note the striking increase in CD34+ blast cells, which appear disperse (single cells) (arrowheads) and sometimes found as clusters (arrow). CD34+ immunostained, 100x.

**Figure 8.** Hypocellular MDS (RAEB 2). High power view of an aggregate of CD34+ progenitor cells (arrow). Also refer to Figure 7 (CD34 immunostain, 400x).
Special Techniques Applied to Bone Marrow Biopsies in the Diagnosis of Myelodysplastic Syndromes

In addition to Giemsa, the silver impregnation technique of Gomori (13) is also used in MDS in the detection of an abnormal increase of interstitial fibers or reticulin fibers. In fact, 60% of MDS marrows studied show some degree of an increase in reticulin fibers or myelofibrosis (MF) (4). According to the European Consensus guidelines, the silver impregnation technique allows one to semiquantitatively grade the fiber content into four grades (13,14) (a) Grade 0 (or normal) defined as the presence of only scattered linear reticulin; (b) Grade 1 (mild fibrosis) as a loose network of reticulin with many intersections (Fig. 9); (c) Grade 2 (moderate fibrosis) with a diffuse and dense increase in reticulin with extensive intersections and sometimes focal collections of collagen (10); and (d) Grade 3 (severe fibrosis) as having a diffuse and dense increase in reticulin fibers with extensive intersections and coarse bundles of collagen.

MDS is also frequently associated with anemia caused by ineffective erythropoiesis, and increased hemosiderin (iron-storage complex) deposits that can be readily detected in H&E-stained sections. However, for a detailed semiquantitative analysis (1 to 4+), a special stain for iron such as Perls’ (Prussian blue) is needed. With Perls’ stain, blue hemosiderin granules can be easily seen in the bone marrow interstitium, or inside macrophages. Sometimes, ringed sideroblasts can also be observed in thin sections.

Conclusion

Currently, the diagnosis of MDS is made with the integration of clinical data, cytomorphological data and genetics. The bone marrow biopsy is an essential tool in the diagnosis of MDS, for not only producing additional data on cellularity, but also in detecting the presence of topographical distortion and fibrosis, quantity of iron stores, an increase in blasts cells and/or aggregates, and other reactive or paraneoplastic conditions associated to cytopenias and dysplastic changes. To that end, the use of Giemsa, reticulin and Perls’ stain is strongly recommended in order to better appreciate cell morphology and interstitial changes associated to this group of diseases.
Special Techniques Applied to Bone Marrow Biopsies in the Diagnosis of Myelodysplastic Syndromes

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References


Chapter 29 | The Evolution of the Artisan™ Special Stains Instrument

Ron Zeheb, PhD and Steven A. Bogen, MD, PhD

“Special stains cannot be automated” was a common early objection to the Artisan™ project, when we started in the early 1990’s. The highly diverse nature of the special stains protocols, the instability of freshly mixed working reagents, and the unpredictability of the color development time were all factors weighing against the feasibility of special stains automation. Since there was no previously established market for special stains automation, companies in the field were not interested in financing such an effort. At the time, immunohistochemistry automation was still in its infancy and struggling to find a market foothold. Against this backdrop, creating a special stains instrument was a daunting challenge. As the inventors of the Artisan™, we considered that the story of the instrument and how it was developed might be of general interest.

Funding the Research and Development

The project was initiated by one of the authors (SAB) who, at the time, was in residency training at the Department of Pathology, Brigham & Women’s Hospital, Boston, MA. Early simple breadboard prototypes were fabricated using out-of-pocket funds. However, it soon became clear that professional engineering expertise was required. In addition, the project needed funding. Both problems were solved by teaming up with Mr. Herb Loeffler who, at the time, was Principal of Loeffler-MacConkey Design Inc., Arlington, MA. Together, we submitted a grant application to the National Institutes of Health, under the Small Business Innovative Research (SBIR) program. In total, we received approximately $550,000 to fund the R&D for the first prototypes. Later, in 1996, Richard Foemmel, PhD, joined the team and we formed CytoLogix Corporation. Dr. Foemmel, as President and CEO, raised venture capital to fund the transition from R&D to manufacturing. The Artisan™ product line was transferred to Dako in 2002.

The First Breadboard Prototype

For a variety of special stains (when performed manually), it is common practice to monitor color contrast development under the microscope. When the tissue section achieves the appropriate level of contrast, the histotechnologist stops the reaction by immersing the slide in a liquid. Unfortunately, this protocol is incompatible with automation. To solve the problem, it seemed likely that if it tightly controlled all of the reaction variables, such as time, temperature, and reagent concentration, then the chemical reaction should be reasonably predictable. If so, then visual inspection should not be necessary. As a first step, we needed to create a reaction chamber that could be applied to microscope slides, in order to contain the reagents that were added and removed. Without containment, the intensely colored stains and dyes might wreak havoc on the interior of the instrument.

Figure 1a and b show one of our first breadboards for testing this concept. Ten slide chambers were mounted on a clear plastic base. Each slide chamber was attached to a spring-loaded clamp, pressing the chamber down onto the glass microscope slide, thereby creating a seal. For early experiments, we added and removed reagents by pipette, simulating what an instrument would later do. The slide clip concept was kept throughout the Artisan™ design process and is incorporated into the present-day Artisan™ instrument (Fig. 1c).