

Specialized Testing in Hematopoietic Disorders Aids Diagnosis and Prognosis

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Introduction

The classification of hematopoietic, or bone marrow and lymph node, disorders (eg, leukemia, lymphoma, myelodysplastic syndrome, myeloproliferative syndrome) has changed significantly over the last 10 to 15 years. Historically, leukemias and lymphomas had been categorized largely by morphology (microscopic appearance). The resulting broad categories gave some prognostic and therapeutic guidance, but the heterogeneous nature of disease entities within each group limited the accuracy of the information.

The tide started turning in the early 1970s with the discovery of the so-called Philadelphia chromosome in patients with chronic myelogenous leukemia,¹ a blood disorder in which the bone marrow typically produces too many white blood cells, which have impaired function. The Philadelphia chromosome is a result of a chromosomal translocation that juxtaposes the gene *ABL* on chromosome 9 to the gene *BCR* on chromosome 22. This results in the production of an abnormal protein that causes the unregulated growth of bone marrow cells. This monumental discovery added focus to the genetic basis of many disorders, especially hematopoietic ones.

There are many methods in the clinical pathology laboratory

to examine chromosomes and their respective genes. Karyotyping involves microscopic examination of the chromosome structure itself. It offers an overview of all the chromosomes and can detect some abnormalities. This method remains very useful, despite being time-consuming and requiring cells to divide in culture, a potential technical challenge. If a known, specific genetic abnormality is being sought, fluorescence *in-situ* hybridization (FISH) can be used to detect translocations, gene deletions, monosomies (loss of an entire chromosome), trisomies (gain of an entire chromosome), and other abnormalities. The most sensitive method for detecting targeted chromosomal abnormalities is the polymerase chain reaction (PCR). However, this is used on a somewhat more limited basis due to the technically demanding nature of the test and the general requirement of an unfixed specimen in some circumstances, although recent advances in PCR automation are making its use more widespread.

While the science of cytogenetics has been evolving, another technology called flow cytometry has found a vital niche in the categorization of leukemias and lymphomas. The power of this technology lies in its ability to help classify these disorders based on the pattern of expression of certain cell surface molecules and to detect a very tiny population of abnormal cells among predominantly normal ones.

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All of these technological advances are used in the clinical pathology laboratory, and data from these tests, in conjunction with morphologic features, form the foundation of the most recent classification scheme for disorders of the hematopoietic system from the World Health Organization (WHO).² This scheme is widely accepted by health care professionals around the world because it is based largely on genetic characteristics that have direct impact on treatment and prognosis. Elucidation of mechanisms by which these genetic abnormalities produce disease has led to the discovery of targeted therapies with dramatic clinical success. One example is imatinib mesylate (Gleevec® from Novartis Pharmaceuticals), which has proved a great therapeutic success for patients with chronic myelogenous leukemia.

Although targeted therapies are not available for many hematopoietic disorders, the genetic and flow cytometric characteristics of a hematopoietic disease can play an enormous role in evaluating an individual's prognosis and choosing the most appropriate therapy. These methodologies will be explained in greater detail as they relate to the new classification scheme of hematopoietic disorders, and examples of how the technologies are used for diagnostic, therapeutic, and prognostic purposes will be given.

Karyotype

Karyotyping, the standardized arrangement and morphologic analysis of cell chromosomes, has long been used to diagnose congenital genetic abnormalities, and its significance in evaluating hematopoietic diseases is now well entrenched. Since karyotyping requires cells to divide, this technique is useful in the evaluation of primary bone marrow diseases such as myelodysplastic syndrome, myeloproliferative disease, and acute leukemia. Diseases producing more mature cells, such as many types of lymphoma, are difficult to study using this method because they do not divide readily in culture. Multiple, well-documented cytogenetic abnormalities have been described in patients with myelodysplastic syndrome (MDS). These include abnormalities involving chromosomes 5 and 7 and trisomy 8.² In conjunction with morphology, detection of these abnormalities is used to help make the diagnosis of MDS and to track the progression of disease. For example, as some patients with MDS progress toward acute leukemia, additional cytogenetic

abnormalities are acquired and serial karyotype analyses can detect this evolution. These changes help predict which patients will persist with a relatively indolent disease versus those who are at a greater risk of developing acute leukemia. Also, there is a particular type of MDS, known as 5q minus syndrome, in which a unique set of clinical and morphologic findings exist. The loss of the genetic material on the long arm of chromosome 5 confers a good prognosis with a very low risk of progression to acute leukemia.³

There are several acute leukemias that are now classified primarily based on cytogenetic findings that directly affect treatment and prognosis (Table 1). One example is acute promyelocytic leukemia (APML), which is characterized typically by a translocation of the *PML* gene on chromosome 15 next to the *RARα* gene on chromosome 17. The translocation results in the overproduction of the retinoic acid receptor, making retinoic acid an essential component of the therapy by inducing maturation of the abnormal promyelocytes.⁴ The cytogenetic

Table 1.
Examples of Genetic Findings Used for Prognosis

Disease based on WHO classification	Prognosis	Characteristic flow cytometric or morphologic findings
Myelodysplastic syndrome		
Multiple chromosomal abnormalities or complex karyotypes	Poor	
5q minus	Good	X
Acute myeloid leukemia		
Translocation (8;21)	Good	X
Inversion 16	Good	X
Translocation (15;17)	Good	X
Abnormalities of 11q23	Intermediate	
Acute lymphoid leukemia		
Hyperdiploid (>50 chromosomes)	Good	
Translocation (12;21)	Good	X
Translocation (9;22)	Poor	
Abnormalities of 11q23	Poor	X
Translocation (1;19)	Poor	
Hypodiploid	Poor	
Chronic lymphocytic leukemia/Small lymphocytic lymphoma		
Trisomy 12	Poor	
Deletion 13q14	Good	
Deletion 17p13	Poor	
Deletion 11q22-23	Poor	
Multiple myeloma		
Deletion 13q14	Poor	
Translocation (11;14)	Good	
Deletion 17p13	Poor	

finding is important to recognize due to this unique therapy. It also predicts a good prognosis, and bone marrow transplantation is often not considered as a treatment option. This is in contradistinction to many other types of acute leukemia for which it occasionally offers the only chance for extended remission.

Fluorescence *In-situ* Hybridization (FISH)

This technology offers similar information to a karyotype but generally detects abnormalities on a much more targeted part of the genome. However, FISH has the advantage of not requiring dividing cells and, hence, can be performed much more quickly and on a wider variety of specimens than a karyotype. Cells are incubated with fluorescently-labeled primers (manufactured segments of DNA) that bind, or hybridize, to a specific DNA sequence within the cell. The cells are then viewed under fluorescent microscopy and the fluorescent signals analyzed. The relatively rapid turnaround time is important in certain situations, such as in APL. If certain features present in an acute leukemia raise the suspicion of APL, FISH for the translocation can confirm the diagnosis and appropriate therapy can begin promptly. This is vital in this setting because conventional chemotherapy can actually be harmful for patients with APL. Another instance where FISH plays a role in rapid confirmation of a diagnosis is Burkitt lymphoma. This lymphoma grows very rapidly due to overreplication of the *c-MYC* gene on chromosome 8 that causes the cells to remain in a near constant state of division. The confirmation of Burkitt lymphoma is important because treatment typically begins very soon after diagnosis, and it is treated more aggressively than other types of lymphoma.⁵

FISH plays a crucial role in prognostication of diseases that have historically been difficult to characterize by karyotype because they do not divide readily in culture. Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and multiple myeloma are two notable examples. (See Table 1.) A panel of FISH studies is typically performed on these to arrive at a genetic profile of an individual's disease. This information is then integrated with clinical parameters to arrive at an overall prognosis that guides treatment options. Consequently, some patients are treated with a "watch and wait" approach because of a very low risk of significant progression, whereas others are treated very aggressively at initial diagnosis because of a significant risk of rapid progression. Prior to these genetic advances, the outcome of patients with CLL/SLL and multiple myeloma was quite variable, and there were only limited ways to predict how an individual's disease would behave.

Polymerase Chain Reaction (PCR)

Polymerase chain reaction is an exquisitely sensitive method of genetic investigation and has many applications. Relevant to this discussion, PCR is used to detect genetic abnormalities and, in some instances, to measure the quantity of the abnormality. Although advances in automation are currently available, PCR remains time consuming and requires relatively high technical

expertise due to the sensitivity of the method to contamination. The test can use an unfixed sample or, for some PCR primers, a fixed sample. A series of tightly controlled steps amplify, by making many copies, and then detect a genetic target. A practical application of PCR in evaluating lymphomas is the detection of clonality in B and T cell lymphomas. Detecting monoclonality can confirm malignancy, but it is generally not used as the sole determining factor. Furthermore, the PCR characteristics of an individual's lymphoma are often unique and can be used to determine if a subsequent tumor is a recurrence of the former lymphoma or a new primary. This distinction is often of prognostic and therapeutic importance.

Quantitative analysis precisely measures the amount of PCR product, and this can be of value in some settings. Quantitation of the gene fusion product resulting from translocation between chromosomes 9 and 22 that characterizes chronic myelogenous leukemia (CML) can be followed over time to assess the response to imatinib mesylate, the targeted therapy for CML. A negative or decreasing quantitative PCR test is reassurance that the current treatment regimen is controlling the disease, whereas an increasing amount of PCR product could trigger an increase in the dose of imatinib mesylate or consideration of other treatment, such as bone marrow transplant.

Flow Cytometry

Flow cytometry is a technology that detects the presence and quantity of certain molecules that exist on cell surfaces or in the cytoplasm. By examining the pattern of expression (presence) of these molecules, cells from peripheral blood, bone marrow, or a lymph node are grouped into populations of similar cells. Flow cytometry is used primarily as a diagnostic aide in the classification of lymphoma and leukemia, and, as mentioned, the diagnostic categories in the WHO classification² carry therapeutic and prognostic significance. One practical application of flow cytometry allows classification of acute leukemia into two major categories, myeloid and lymphoid. Lymphoid tumors can be further subcategorized into B and T lymphoblastic types. These categories of acute leukemias are treated differently and carry different prognoses, especially when correlated with genetic findings. (See Table 1.) Some acute leukemias express molecules that are not characteristic of a particular cell line, known as aberrant expression. These aberrant markers can be unique to an individual's disease and offer a useful way to detect minimal residual disease by easily separating the abnormal cell population from primarily normal cells.

The molecules that are detected by flow cytometry can serve as a surrogate marker for some of the genetic findings described earlier. (See Table 1.) For example, APL has a distinctive profile by flow cytometry in that it lacks expression of HLA-DR and CD34, two molecules that are very frequently present on other types of acute leukemia.⁶ Along with morphology, these findings prompt the pathologist to investigate for the characteristic translocation.

The Future

The methods of molecular and genetic evaluation discussed so far originated as research tools, and their utility in the clinical pathology laboratory has evolved quickly. Another type of test that might make this transition is gene microarray technology, sometimes called "gene chip." The results of this test, on a research basis, have been shown to be a very powerful tool to further evaluate how hematopoietic diseases relate to each other and, in some instances, offer an even clearer understanding of the mechanism of disease, prognosis, and optimal therapy.⁷ Researchers hope that the identification of specific gene expression in these diseases will lead to effective gene-targeted therapies. This assay entails extracting DNA from tissue and simultaneously analyzing for the overexpression or underexpression of thousands of genes to create a gene expression profile. At present, the gene microarray chips are generally too expensive for routine clinical testing, and the amount of data generated can take many hours to analyze using today's fastest computers. Moreover, storage of these massive amounts of data presents another challenge. Great advances in automation of this test have been made recently, and the cost has also decreased substantially in just a few years. As all of these technological and economic aspects improve, this test will very likely play some role in the evaluation of hematopoietic disorders and may subsequently alter the classification of these diseases.

Table 2.
Examples of Genetic Findings Used for Diagnosis

Genetic Abnormality	WHO Classification Diagnosis
Translocation involving 8q24	Burkitt lymphoma
Translocation (14;18)	Follicular lymphoma
Translocation (11;14)*	Mantle cell lymphoma
Translocation (11;18)	Extranodal marginal zone lymphoma
Translocation (2;5)	Anaplastic large cell lymphoma

* This translocation defines mantle cell lymphoma but has also been reported in some cases of multiple myeloma.² These diseases can be differentiated by morphology and flow cytometry.

Conclusion

Karyotype, FISH, PCR, and flow cytometry are being used currently in everyday practice to aid diagnosis (Table 2) and prognostication of hematopoietic disorders and to guide therapy. While each test can add an important level of understanding to a patient's disease, none of them should be used in isolation or without regard to other clinical information. The pathologist plays a critical role in this process by correlating the microscopic morphology with these data from specialized tests and making an overall assessment. Pathologists oversee the performance of these tests, interpret the results in light of the clinical context, and communicate this information to oncologists, radiation oncologists, surgeons, and other treating physicians. This invaluable information about an individual patient's disease has a direct, and often dramatic, impact on the type and duration of therapy and offers an indication of the individual patient's prognosis. **NCMJ**

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