Chapter 2: Laboratory Diagnostics

Introduction

Good to Know

United States: The Clinical Laboratory Improvement Amendments (CLIA) and the College of American Pathologists (CAP) provide laboratory certification and accreditation. The American College of Medical Genetics (ACMG) provides detailed guidelines for genetic testing.

Canada: The Ontario Laboratory Accreditation and the Canadian College of Medical Genetics (CCMG) provide laboratory oversight and guidelines, respectively.

Europe: Recognized accreditation bodies include the Belgian Accreditation Council (BELAC), the French Accreditation Committee (COFRAC), the German Accreditation Council (DAkkS), the Swiss Accreditation Service (SAS), and the United Kingdom Accreditation Service (UKAS).

Any physician who suspects that a patient may have Fanconi anemia (FA) should refer the patient to a hematologist and/or geneticist, who can arrange for diagnostic testing. The laboratory should be accredited and certified to perform FA testing for clinical care, and should have evaluated many patients with and without FA. Evaluating a large number of patients enables a laboratory to validate its FA testing procedures, and to establish ranges for normal and abnormal test results. The recommended testing procedures are outlined in the flow chart in Figure 1.

This chapter will describe three types of analyses that are commonly used in the diagnosis of FA:

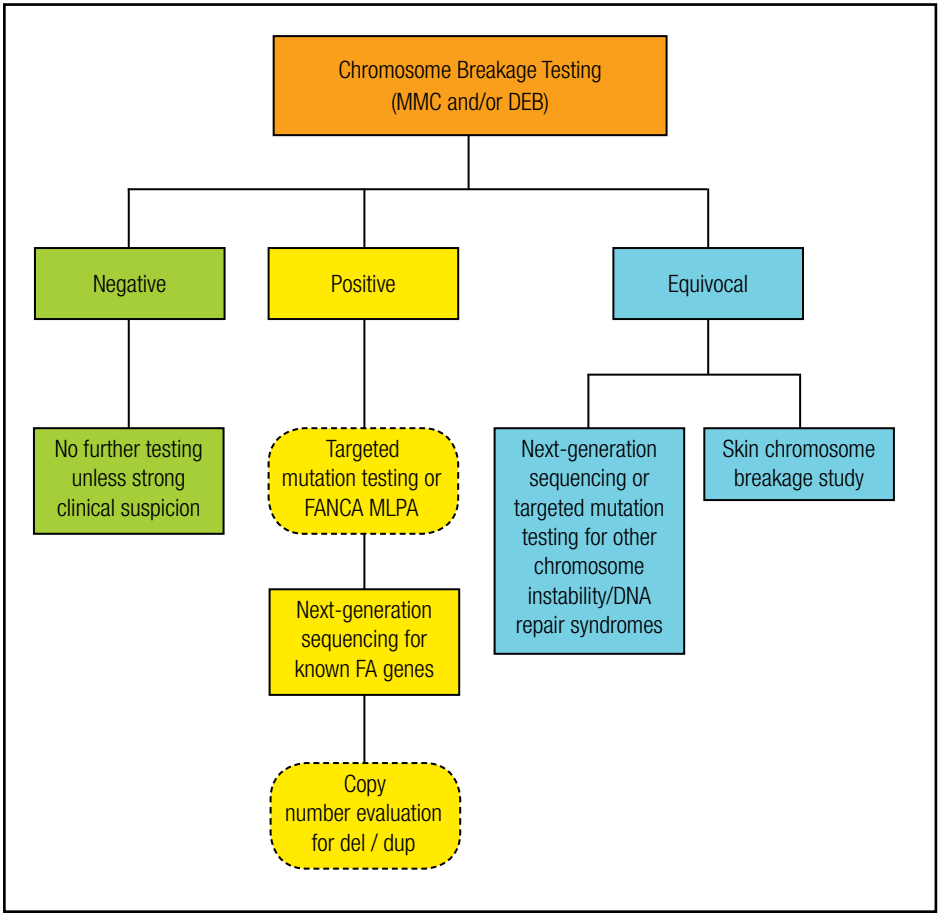
- *Chromosome breakage tests*
- *Mutation analyses*
- *Bone marrow chromosome analyses*

Test 1: Chromosome Breakage in Peripheral Blood Lymphocytes

Chromosome breakage test

The first test that should be used to diagnose FA is the chromosome breakage test, which is performed on a sample of the patient's blood in a clinical cytogenetics laboratory. The initial step involves culturing a sample of the patient's blood with a chemical substance known as a T-cell mitogen, which stimulates lymphocytes (a type of white blood cell) to divide. Next, the culture is treated with chemicals known as DNA cross-linking agents, such as mitomycin C (MMC) and/or diepoxybutane (DEB). Finally, the types and rates of breakages and rearrangements found in the chromosomes of cells are

Figure 1. Flow chart for FA-related laboratory tests.



evaluated [1, 2]. Normal cells can correct most of the chromosomal damage caused by the DNA cross-linking agents, whereas cells from patients with FA typically show multiple chromosomal breaks and rearrangements per cell, including complex rearrangements such as radial figures. As detailed by the American College of Medical Genetics guidelines for cytogenetic laboratories, the test results report should include the breakage and rearrangement rates, as well as the distribution of chromosomal breakage among cells or the average number of aberrations per cell with and without radial figures. Further, all tests should include at least two independent cultures (e.g., samples treated with different concentrations of MMC, or one sample treated with MMC and the second with DEB, or another relevant combination) to show that the results are reliable. Because some patients' specimens will have very low white blood cell counts, it may not be possible to set up two cultures for a given test. In such cases, a second specimen should be obtained from the patient, if possible, to confirm the findings obtained from the first culture.

The laboratory should also obtain measures of baseline chromosome breakage by evaluating cells that have not been treated with MMC and/or DEB. These findings may help to guide the follow-up molecular testing, because the measurements of baseline breakage can vary markedly among the various complementation groups. For example, patients with mutations in the *FANCD1* gene have very high levels of baseline breakage and unusual constellations of abnormalities compared with other groups of patients with FA [3]. The baseline breakage may also aid the differential diagnosis of other chromosome instability disorders that display specific types of chromosomal abnormalities, such as rearrangements of chromosomes 7 and/or 14, which commonly occur in ataxia-telangiectasia and Nijmegen breakage syndrome; telomeric rearrangements, which often occur in dyskeratosis congenita; and railroad figures and premature centromere separation, both of which are characteristic of Roberts syndrome [4, 5].

Cell cycle analysis in peripheral blood lymphocytes

MMC- and/or DEB-induced chromosome breakage analysis is the most common first-line test for the diagnosis of FA. However, a few laboratories diagnose FA by measuring cell cycle kinetics, rather than chromosome breakage, in peripheral blood lymphocytes treated with mitogen and DNA cross-linking agents [6, 7]. Normal lymphocytes that do not have any DNA damage will progress through all the normal phases of the cell cycle [the phases are Gap1 (G1)-> DNA Synthesis (S)-> Gap 2 (G2)-> Mitosis (M)]

without significant delay. However, cells that have DNA damage will stop at the G2 phase of the cycle to repair the damage before they progress to M. Because FA cells have more unrepaired damage after treatment with DNA crosslinking agents, a higher percentage of cells (generally 40% or more) from FA patients will be arrested during the G2 phase when compared to cells from individuals without FA. A flow cytometer instrument is used to measure the progression of the cells through the cell cycle and provide the percentage of cells arrested at G2. Some laboratories may use cell cycle analysis in conjunction with a chromosome breakage test. The principles and flow chart delineated for the chromosome breakage test should be applied to cell cycle analysis. Positive, negative, and equivocal results should be followed up as described for the chromosome breakage test results delineated in Figure 1.

Interpreting chromosome breakage test results

Positive: A patient is considered to have a positive test for FA if the lymphocytes display markedly increased chromosomal breakage and rearrangement after treatment with MMC and/or DEB. Typically, more than 90% of the lymphocytes in the culture of blood derived from a patient with FA will show increased breakage, and the rates and types of breakage observed will fall within the abnormal range. In the event of a positive test result, the patient and his or her family should be referred to a genetic counselor, who can help coordinate the necessary follow-up testing and explain the test results to the family after the testing is completed (see *Chapter 17*). Importantly, follow-up testing should be performed to identify the patient's disease-causing genetic mutation(s) using the molecular methods described under "*Test 2: Mutation Analysis*." All of the patient's siblings should be tested for FA either by chromosome breakage analysis or, if his or her sibling's disease-causing gene mutation(s) have been identified, by mutation analysis [(2)].

Negative: A test result is considered to be negative if the patient's lymphocytes do not show increased chromosomal breakage or rearrangement in response to MMC and/or DEB, and the types and rates of breakage are within the normal range. If the chromosome breakage test is negative and the clinical evidence that the patient may have FA is weak, no further studies are needed. By contrast, if the chromosome breakage test is negative but there is *strong* clinical evidence that the patient may have FA, then skin fibroblast testing should be performed to rule out the possibility of mosaicism as described below in the discussion of equivocal results. In addition, many disorders have some clinical features in common with FA and are associated with some form

of chromosome instability (Table 1). Therefore, patients who have a negative chromosome breakage test but have some of the clinical features of FA should undergo DNA sequencing that includes the genes implicated in FA as well as genes relevant to the conditions described in Table 1.

Table 1. Disorders that may share clinical features with FA and manifest with chromosome instability.

Disorder	Putative Genes Involved
Ataxia-telangiectasia	*ATM*
Ataxia-telangiectasia-like disorder	*MRE11*
Bloom syndrome	*BLM*
DNA ligase 4 syndrome	*LIG4*
Dubowitz syndrome	
Dyskeratosis congenita	*DKC1, TERT, TERC, WRAP53, NOP10, NHP2, TINF2, RTEL1, CTC1*
Nijmegen breakage syndrome	*NBN*
Nijmegen breakage syndrome-like disorder	*RAD50*
Roberts syndrome	*ESCO2*
Rothmund-Thomson syndrome	*RECQL4*
Seckel syndrome 1	*ATR*
Severe combined immunodeficiency	*NHEJ1*
Warsaw breakage syndrome	*DDX11*

***Equivocal*:** Test results are considered equivocal, or inconclusive, if the percentage of cells that display chromosomal breakage patterns characteristic of FA is much lower than the laboratory typically sees for FA or if there is increased breakage but the pattern is not characteristic of FA. In general, there are two underlying causes of inconclusive test results:

- ***Possibility #1:*** *There is mosaicism in the patient's peripheral blood.*

  Mosaicism is characterized by two distinct populations of lymphocytes in the blood. One population has normal sensitivity to DNA cross-linking agents due to a spontaneous correction of an FA mutation, while the other population is hypersensitive to DNA cross-linking agents due to the presence of FA mutations. Mosaicism can be diagnosed by sending a sample of the patient's skin, obtained via a skin biopsy, to a certified clinical cytogenetics laboratory, which can perform the chromosome

breakage test on fibroblast cells present in the skin sample. The diagnosis of FA can be confirmed by a chromosome breakage test that reveals increased breakage in the fibroblasts, with the types of breaks and rearrangements characteristic of FA. Mosaicism testing should be performed if the clinical evidence that the patient may have FA is strong, but the blood chromosome breakage test results were reported as negative or equivocal.

Approximately 10-20% of patients with FA have a form of mosaicism in which the fibroblast cultures show increased breakage, while the lymphocytes do not. The percentage of normal cells in the blood of these patients may range from less than 50% to 100%. Over time, a patient with a low percentage of normal cells may develop a high percentage of normal cells, and this process may be associated with spontaneous improvement in the patient's blood cell counts. However, the mosaicism measured in peripheral blood lymphocytes may not reflect mosaicism in the bone marrow cells. This means that a patient with a high percentage of normal cells in the tested lympohcytes may have no (or a very low percentage of) normal cells in his or her bone marrow. As the bone marrow cells are involved in the development of leukemia, their status should not be generalized from the lymphocyte results. It is not possible to directly test the bone marrow cells using the same chromosome breakage tests used for lymphocytes. Thus, it remains unclear whether the clinical course of the disease will be altered in patients who have normal cells in the peripheral blood. Importantly, the presence of mosaicism—either in the blood or bone marrow—does not protect the individual from the development of clonal chromosome abnormalities within the population of cells that retain their FA mutations. This, in turn, can lead to the development of hematologic malignancies and solid tumors.

- ***Possibility #2:*** *The patient has a condition other than FA that manifests with increased chromosomal breakage.*

  Depending on the pattern of breakage and the clinical findings, the patient may have a condition other than FA that is associated with chromosome instability, such as Nijmegen breakage syndrome, ataxia-telangiectasia, ataxia-telangiectasia-like disorder, DNA ligase 4 syndrome, Seckel syndrome 1, Bloom syndrome, dyskeratosis congenita, Roberts syndrome, Warsaw breakage syndrome, Cornelia de Lange syndrome, or FAN1 deficiency.

Because most of the gene mutations that cause these conditions have been identified, molecular testing can be performed to establish the diagnosis.

Test 2: Mutation Analysis

If the results from the chromosome breakage test are positive, then mutation analysis should be performed to identify the specific genetic mutation that has caused the patient to develop FA. Identifying the mutation is valuable for the following reasons:

- It enables mutation-specific testing of family members, and permits the accurate diagnosis of individuals who have only one mutated copy of a FA gene (e.g., the parents of FA patients) and who do not have the clinical findings of FA, as well as the diagnosis of individuals who have two mutated copies of a FA gene (e.g., the patients) and manifest, or will be expected to manifest, the clinical findings of FA. This information allows for appropriate medical management and focused genetic counseling.
- It can be used for premarital screening, prenatal diagnosis, and preimplantation genetic diagnosis.
- It aids the accurate genotyping of potential bone marrow donors, such as siblings who do not appear to have FA, so that any individuals who have undiagnosed FA will not be used as donors.
- It enables patients who are clinically well to be monitored closely for the potential development of aplastic anemia, myelodysplastic syndrome, leukemia, or solid tumors.
- It provides information that determines a patient's future prospects for pharmacologic or gene therapies.

Genetic mutations in patients with FA

Researchers are just beginning to identify the associations between certain FA gene mutations and the physical abnormalities and bone marrow disease that they cause (see *Chapter 1*, Table 3). The most severe physical defects, which sometimes include features of VACTERL-H syndrome, are most frequently reported in patients with mutations in the following genes: *FANCC* (specifically, the IVS4+4 A > T mutation), *FANCD1/BRCA2, FANCD2, FANCG, FANCI*, and *FANCN/PALB2*. An early onset of aplastic anemia has been reported for *FANCC* (specifically the IVS4 mutation in Ashkenazi Jewish patients) and *FANCG* has been associated with more severe aplastic anemia and increased incidence of leukemia. Patients with mutations in *FANCD1/BRCA2*

and *FANCN/PALB2* tend to develop leukemia, or solid tumors (particularly medulloblastoma and Wilms' tumors) by age 5 [21]. In general, null mutations, which result in the complete loss of a gene's normal function, are thought to be more severe than hypomorphic mutations, which result in a partial loss of a gene's function [8, 9]. However, it was recently shown that this is not the case for *FANCA* mutations [22]. Researchers have concluded that other genetic and environmental factors influence the genotype-phenotype relationship. Two examples that illustrate this point are the observed variability in disease severity between siblings with the same *FANC* mutations, and the much more severe disease that occurs in patients of Ashkenazi heritage who harbor the IVS4 mutation, compared with patients of Japanese heritage with the same mutation [23].

Worldwide, the majority of patients with FA have mutations in the *FANCA* gene; several hundred different *FANCA* mutations have been documented. However, a limited number of specific mutations tend to be common in certain populations of people that have descended from a small group of founders (see Table 1 in *Chapter 17*). For patients and their families that belong to such populations, and for individuals with clinical findings and/or a family history of cancer associated with a particular mutation, analysis may begin with targeted tests for the specific suspected mutations. However, for most new diagnoses of FA, there will likely be no specific mutation that is suspected. Several strategies have been adapted by different laboratories to ensure that the testing maximizes the possibility of identifying the patient's mutations while at the same time minimizing costs and decreasing the amount of time it takes to get the test results. Gene sequencing is a critical component of these strategies.

Gene sequencing approaches

Until recently, a genetic test known as complementation analysis, which involves somatic cell-based methods such as retroviral gene transfer, was the primary method available for determining which *FANC* genes were mutated in a given patient. However, such complementation analysis is labor-intensive, expensive, and time-consuming. In the last few years, the development of next generation sequencing (NGS) methodology, also referred to as massively parallel sequencing, has transformed the field of genetic testing because it enables detailed analysis of thousands of genes simultaneously (i.e., in parallel). Such analyses would be too time-consuming and costly to attempt using classic DNA sequencing methodologies, such as Sanger sequencing, that analyze a single gene at a time. Many laboratories have developed targeted

panels of genes to be assessed by NGS to search for mutations among a group of genes that have been previously documented or have been suggested to be important in a particular disease. Such panels may include anywhere from a few genes to greater than 500. The number of genes examined varies from laboratory to laboratory depending on the testing platform and algorithm being used. An NGS approach that is more comprehensive than the sequencing of targeted panels of genes is known as whole-exome sequencing, which involves sequencing of all of the exons (segments of DNA that contain information needed to make proteins) of all known genes, representing approximately 2-3% of the human genome. An even more comprehensive NGS application is whole-genome sequencing, which involves analyzing the entire human genome. At the time of this writing, whole-genome sequencing is primarily limited to research studies. The high cost of such testing prohibits this from being used as a frontline testing tool at this time. However, sequencing technologies are rapidly evolving, and it is likely that by the time of publication of this chapter, there will be new methods and instrumentation being evaluated that not only improve sensitivity for detection of different types of mutations, but also increase efficiency and decrease cost. Multiple laboratories are now offering, or developing, NGS-based applications for FA testing and have targeted panels that include all 16 of the known FA genes [10]. Some panels also include genes that are known to be associated with other bone marrow failure or chromosome instability disorders. Targeted panels can identify novel mutations within known FA genes, but only tests such as whole exome sequencing, which screen regions of the genome that do not contain one of the targeted genes, can identify novel FA genes [11]. Further, other whole genome screening methods, such as genomic microarray testing, are being successfully implemented to detect FA gene mutations (specifically large deletions) that cannot be identified by NGS [24]. Complementation testing and functional studies can be used to validate and confirm the clinical significance of novel mutations identified using these methods.

Limitations of next generation sequencing and complementary molecular tests

Fanconi anemia gene function can be affected by numerous types of deleterious mutations, such as base pair substitutions, small deletions of only one or a couple of base pairs, large deletions involving hundreds of thousands of base pairs of DNA, and insertions. These various types of mutations have implications for FA testing. NGS is very effective for certain types of

mutations, such as single base pair substitutions and small deletions, but is problematic for other mutation types such as large deletions and duplications [12]. Large deletions represent the most common type of mutation encountered in the *FANCA* gene [13, 14]. Ameziane et al. (2012) developed a statistical method for detecting large deletions by NGS. Other laboratories currently use techniques other than sequencing to detect these larger abnormalities. One such technique is known as multiplex ligation-dependent probe amplification (MLPA), a very efficient and sensitive method for identifying large deletions as part of an FA testing algorithm. MLPA is used as a first-line test to rule out large deletions in *FANCA*, followed by either conventional Sanger sequencing for a small number of FA genes [15] or NGS for a targeted FA gene panel.

Genomic microarray is another technique that can be used as a first-line strategy to search for deletions and duplications among FA and related genes. Several different types of microarrays are available, two of which are commonly referred to as array comparative genomic hybridization (aCGH) and SNP arrays. Similar to NGS, these microarrays can be targeted for detection of deletions and duplications (collectively referred to as copy number variants (CNV) or copy number aberrations (CNA)) within a set of known genes, or can be designed to detect these CNA anywhere in the genome. Most laboratories use a hybrid model, where the microarray is enriched (i.e., has extra coverage) for certain genes of interest, while also having some coverage of the remaining genome. As with all of the testing methods described in this chapter, the laboratory performing the microarray analysis should be certified and have well-established guidelines to distinguish a clinically significant result from a technical artifact or normal benign variation. The choice of testing methods, and the order in which they are performed to identify a given patient's mutation, depends in part on the patient's clinical features and ancestry and the expertise and experience of the laboratory. As there is no single test method that is equally able to detect all types of mutations, and there is more than one technique that can detect a particular type of mutation, the combination and priority of testing applied varies between laboratories.

The ability of a DNA sequencing assay to detect and characterize a mutation can be influenced by a number of variables, including the statistical methods used to analyze the findings. Both the technical and statistical methods for NGS are rapidly evolving. It is critical that a clinically certified laboratory perform the test to ensure adherence to rigorous standards for quality control and quality assurance. Moreover, it is strongly recommended that a genetic counselor or other genetics professional help guide the testing. Close communication

between the laboratory director and the genetics professional is critical. Prior to the initiation of testing, the genetic counselor should confer with the laboratory director about the limitations of the testing methodology and analysis being used. Specifically, the genetic counselor and laboratory director should discuss the types of mutations that can and cannot be detected, and the number of FA genes and other relevant genes that will be included in the testing. This information should be summarized by the genetic counselor and communicated to the patient and the patient's family. The laboratory should also share its methods for validating positive test results.

Test 3: Bone Marrow Chromosome Analysis

G-banding analysis

Following the diagnosis of FA, the chromosomes of the patient's bone marrow cells should be analyzed using Giemsa banding (G-banding; a cytogenetics technique which marks the chromosomes with colored bands and is used to elicit the unique and characteristic staining pattern of each chromosome) to determine whether a clone with acquired chromosome abnormalities is present, and if so, to characterize the abnormalities observed. Specifically, G-banding analysis can detect clonal chromosome abnormalities acquired by a subset of bone marrow cells.

Good to Know

A **clone** is a population of cells.

Clonal abnormalities are changes in the structure or number of chromosomes in certain cells (in FA, generally cells of the bone marrow).

Clonal evolution is a process by which cells acquire new abnormalities.

Clonal expansion is an increase in the percentage of cells with identical abnormalities.

Myelodysplastic syndrome (MDS), leukemia, and other hematologic malignancies are associated with clonal abnormalities that arise in the malignant cells; therefore, the observation of a clonal abnormality may herald the emergence of cancer or of a precancerous condition. Some clonal abnormalities in patients with FA may persist for a long time without causing adverse consequences; others have been recognized as being associated with more rapid progression or more aggressive disease. In either case, clonal evolution and clonal expansion are frequently associated with disease

progression. If no clonal abnormalities are observed in the patient's bone marrow, then the G-banding analysis should be repeated annually. If a clonal abnormality is observed, then follow-up analyses should be performed more than once per year to monitor the behavior of the clone. To fully interpret the results of the bone marrow chromosome analysis, a hematopathologist should also analyze a sample of the patient's bone marrow using additional techniques to enable correlation between the hematopathology and cytogenetics findings.

The guidelines for chromosome analysis for acquired abnormalities are specified in the 2009 (revised January 2010) edition of the *Standards and Guidelines for Clinical Genetics Laboratories* by the American College of Medical Genetics (available at: www.acmg.net/ACMG/Publications/Laboratory_Standards___Guidlines/ACMG/Publications/Laboratory_Standards___Guidelines.aspx?hkey=8d2a38c5-97f9-4c3e-9f41-38ee683bcc84). Specifically, the guidelines state that:

- At least 20 different cells in the metaphase stage of the cell cycle should be analyzed using G-banding, with follow-up and screening of additional cells as necessary.
- The chromosomes from normal and abnormal cells should be documented with karyograms (digital images or photographs of the chromosomes, with each pair of the chromosomes aligned in numerical order from 1 – 22, XX or XY).
- The results should be summarized using the standard nomenclature found in the most recent version of the International Standards for Cytogenetic Nomenclature (ISCN).

Recurring clonal chromosome abnormalities may be found in patients with MDS, acute myelogenous leukemia (AML), and other cancers. Certain chromosomal abnormalities occur more frequently in patients with FA, including a gain of material from the long arm of chromosome 1 (1qG), gain of material from the long arm of chromosome 3 (3qG), and loss of chromosome 7 (7L). These abnormalities can occur alone or in combination with each other, or with other abnormalities involving other chromosomes [16-20]. One study found that 1qG, 3qG, and/or 7L accounted for 75% of the clonal abnormalities observed in patients with FA [16].

FISH analysis

The clinical laboratory performing the chromosome analysis should have expertise in cancer cytogenetics and be familiar with FA and the types of

abnormalities associated with the disorder. Because the cells of patients with FA are genetically unstable, it is likely that some cells will develop random, non-clonal abnormalities; therefore, it is important for the laboratory to distinguish *non-clonal* abnormalities, which are limited to single cells and do not represent an emerging malignant process, from *clonal* abnormalities, which can herald the development of a premalignant or malignant condition. Clonal chromosome abnormalities can involve the loss or gain of a whole chromosome, the loss or gain of parts of chromosomes, or the structural rearrangement of parts of different chromosomes. Some clones have cells with only one clonal abnormality, while other so-called complex clones have cells with multiple numerical and structural abnormalities. G-banding is sometimes insufficient to accurately characterize these abnormalities. The same is true for the reverse of G-banding, known as R-banding.

Accurate characterization is important because some abnormalities (such as 3q gain) are associated with higher risk for transformation of disease than other abnormalities. In such cases, a technique known as fluorescence in situ hybridization (FISH), which employs fluorescently labeled chromosome region-specific probes, can be a highly informative tool. For example, the gain of a 3q (3qG) abnormality can be challenging to identify by G-banding, because it often involves the translocation of only a small portion of chromosome 3 to another chromosome. To definitively determine whether the translocated material originated from 3q, it might be necessary to perform FISH analysis. Other subtle abnormalities may be completely overlooked without the use of FISH. While G-banding examines all chromosomes for abnormalities, FISH analysis typically examines cells for a small set of pre-specified abnormalities. Furthermore, G-banding is limited to the dividing cells and is rather labor intensive, which limits the overall number of cells analyzed. FISH analysis, on the other hand, can be used to quickly examine more than 100 cells. Thus, the two techniques of G-banding and FISH complement each other. Because the gain of 1q (1qG) and/or 3q (3qG), and loss of 7 (7L) comprise the majority of the clonal abnormalities seen in cells from patients with FA, it is recommended that, in addition to the G-band analysis of 20 metaphase cells, FISH analysis of 100 to 200 interphase cells be performed to detect low-level presence of a clone harboring one of these three abnormalities. Some laboratories use FISH analysis for a larger number of regions involved in MDS and AML (e.g., 5q, 20q) in both FA and non-FA patients. Such FISH panels can be applied to either unstimulated peripheral blood or to bone marrow. The concordance between FISH results on blood and bone marrow in

patients with FA has not yet been clearly established; however, some physicians and laboratories have started to perform FISH analyses on peripheral blood samples that are collected at time points in between the annual scheduled bone marrow testing. This intervening blood FISH study is being tested as a noninvasive means of monitoring, on a more frequent basis, for the emergence of an abnormal clone with 1qG, 3qG or 7L.

Genomic microarray testing

Genomic microarray testing is a relatively new technique that has become a major tool for cytogenetics and/or molecular laboratories. Microarray techniques such as array comparative hybridization and/or SNP analysis can identify regions of chromosomal loss and/or gain that may be too small, too ambiguous in banding pattern, or too complex to be identified by G- (or R-) banding. Sometimes there are so many abnormalities in a single cell, that a specific abnormality is essentially hidden. Microarray techniques are highly sensitive for detecting and identifying the origin of regions of chromosome loss and gain. For example, microarray techniques can rapidly detect and characterize the presence of a 3qG abnormality and provide specific information about the boundaries of the region that is gained. However, one limitation of this technique is that the clonal abnormality must be present in a sufficiently high percentage of cells (generally higher than 10%) to be detected. Unlike FISH and conventional G-banding analyses, microarray analysis does not provide information about individual cells, but rather provides results based on the total population of cells sampled.

As noted above for the G-banding analyses, all cytogenetic findings should be interpreted within the context of the patient's complete hematological profile and other clinical features to obtain a comprehensive assessment of the patient's status. Communication between the cytogenetics laboratory director, other laboratory directors (e.g., molecular genetics and hematopathology directors), physicians, and the genetic counselor is critical for optimal patient care.

Chapter Committee

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