Fanconi anemia: Current management

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Abstract
Fanconi anemia (FA) is an autosomal recessive chromosomal instability disorder, characterized by congenital anomalies, defective hematopoiesis and a high risk of developing acute myeloid leukemia and certain solid tumors. All racial and ethnic groups are at risk, and at least 11 complementation groups have been identified and the genes defective in eight of these have been identified (FANCA, C, D2, E, F, G, L and BRCA2). FA-A is the most common complementation group, accounting for approximately 65% of all affected individuals. The gold-standard screening test for FA is based on the characteristic hypersensitivity of FA cells to the crosslinking agents, such as mitomycin C or diepoxybutane. Recent progress has been made in identifying the genes bearing pathogenetically relevant mutations, but slower progress has been made in defining the precise functions of the proteins in normal cells, in part because that the proteins are multifunctional. Molecular studies have established that a common pathway exist, both between the FA proteins and other proteins involved in DNA repair such as NBS1, ATM, BRCA1 and BRCA2. Stem cell transplantation (SCT) is the only option for establishing normal hematopoiesis. To reduce undue toxicities due to inherent hypersensitivity, nonmyeloablative conditioning for transplants has been advocated. This review summarizes the general clinical and hematologic features and the current management of FA.

Clinical features
The most frequent characteristic birth defects in FA include skin hyperpigmentation and/or café au lait spots (55%), short stature (51%), abnormal thumbs and radii (43%), abnormal head (26%), eyes (23%), kidneys (21%) and ears (21%). Low birth weight (≤2,500 g) and developmental disability are found in 11% of patients, respectively. However, 25% or more of known FA patients have few or none of these features.

The most important clinical features of FA are hematological and these are responsible for the greatest morbidity and mortality in homozygotes. At birth, the blood count is usually normal and macrocytosis is often the first detected abnormalities. This is followed by thrombocytopenia and anemia, and pancytopenia typically presents between the ages of 5 and 10 years, with the median age of onset being 7 years. Moreover, patients with FA may even present with myelodysplastic syndrome (MDS), or acute myeloid leukemia (AML). The relative risk of AML is 800-fold, and the median age in reported cases is 14 years.

Also, patients with FA are at a particularly high risk of developing specific solid tumors at unusually young ages, including head, neck, esophagus, and gynecological squamous cell carcinomas, as well as liver tumors. The relative risk of developing total cancers is 48 in FA patients in comparison with general population, with the highest relative risk of 4,300 for vulva and/or anus tumor.

Therefore, FA should be considered in any young adult with any of the following subtle but characteristic physical anomalies, hematologic cytopenias, unexplained macrocytosis, MDS/AML, or squamous cell cancer even in the absence of severe pancytopenia or a positive family history.

FA diagnosis
In the proper clinical context the gold-standard screening test for FA is based on the characteristic
hypersensitivity of FA cells to the crosslinking agents (mitomycin C [MMC], diepoxybutane [DEB], cisplatin). Culture of replicative cells (usually phytohemagglutinin [PHA]-stimulated peripheral blood lymphocytes or skin fibroblasts) in the presence of low doses of either MMC, or DEB followed by examination of metaphase spreads for evidence of chromosomal breaks and radial chromosomes can establish the diagnosis of FA. Data are reported as aberration per cell, as well as percentage of cells with aberrations, usually for 20 to 100 cells. The percentage of cells with aberration may be useful, because patients with hematopoietic somatic mosaicism may have only a few cells with breaks.

Flow cytometry can detect the proportion of cells that are arrested at G2/M cell cycle after culture with a clastogen such as nitrogen mustard. It has advantage of examining thousands of cells and is less labor-intensive and subjective, but it is usually done in a specialized laboratory.

Complementation analysis requires patient lymphocytes, EBV-lymphoblasts, or fibroblasts to culture with cells or retroviruses which introduce known normal FANC genes into the patient’s cells. This test is limited to the availability of cells or cloned DNA from known FA genotypes, and is performed in a very limited number of research laboratories. Mutated genes can also be identified by denaturing high performance liquid chromatography (DHCLP) heteroduplex analysis.

**Genetics and molecular pathogenesis**

At least eleven complementation groups are known to date. Genes for 8 groups have been characterized (FANCA, C, D2, E, F, G, L and BRCA2). FANCA is the most common complementation group, in which more than 200 mutations have been documented. Identification of the 8 subtypes facilitated the cloning of the FA genes. The first gene, FANCC on chromosome 9q22.3, was discovered in 1992. The breast cancer susceptibility gene, BRCA2, has been identified as a FA gene (formerly known as FANCD1); biallelic mutations in BRCA2 have been observed in FA subtype B and D1 cells, suggesting that BRCA2 is the FANC gene corresponding to both of these complementation groups.

Knock-out mouse models of FA provide insight into the role of individual mutations. Knock-out of FANCA and FANCG and two different knock-outs of FANCC have been generated in mice. The phenotype of these mutant mice is identical and consists of cellular sensitivity to DNA cross-linking agents, abnormal G2–M progression of the cell cycle similar to Fanconi patients, and hypoplasia of gonads.

Cells and cell lines from Fanconi patients are phenotypically similar, regardless of the complementation group that they represent. The hypothesis that Fanconi proteins A, C, D2, E, F, and G function in a common cellular pathway was substantiated by data showing that proteins A, C, E, F, and G form a constitutive nuclear protein complex. Activation of this protein complex by DNA damage or cell cycle progression results in the conversion of the downstream FANCD2 protein from an unubiquitinated isoform for from to a monoubiquitinated isoform. Monoubiquitination does not occur if the protein complex A to G is not intact, and therefore Fanconi cells from A, C, E, F, and G patients do not show monoubiquitinated FANCD2. In normal cells after monoubiquitination, FANCD2 localizes to nuclear foci where it co-localizes with other DNA-repair proteins such as BRCA1. Although BRCA2 may also be genetically linked to the Fanconi pathway, its exact role is unclear.

A genotype-phenotype study examined the consequences of mutations of FANCC in patients. Kaplan-Meier analysis showed that IVS4 or exon 14 mutations define poor-risk subgroups clinically, they are associated with earlier onset of hematologic abnormalities and poorer survival compared with patients with other exon I mutation and with the non-FANCC population. This was confirmed in a 20-year follow-up perspective by the International Fanconi Anemia Registry (IFAR). Another report describing the association of complementation group and mutation type with clinical outcome showed that Fanconi patients with mutations in the FANCG gene and those homozygous for null mutations in FANCA are also high risk groups with a poor hematologic outcome.

**Management**

The projected median survival of patients with FA is approximately 30 years but survival is extraordinary variable. The most life-threatening early event is bone marrow failure. Matched sibling donor (MSD) SCT is now accepted as the best therapy available to cure the FA patient of marrow aplasia and to prevent progression to myelodysplasia or leukemia. An otherwise healthy patient with FA and significant pancytopenia (ANC <1,000/mm³, hemoglobin ≤8 g/dL or a platelet count ≤40–50,000/mm³) with an available HLA-MSD is an excellent candidate for hematopoietic SCT. Initial efforts to transplant FA patients using standard preparative regimens and graft-versus-host disease (GVHD) prophylaxis were plagued by two serious and often lethal problems: severe toxicity from chemotherapy and exaggerated GVHD. Bone marrow transplantation (BMT) protocols were subsequently modified for FA, and the outcomes improved substantially.

The data on HLA-identical MSD SCT performed on 151 FA patients from 42 institutions were summarized in 1995 with the 2-year survival rate of 66%.
Using cyclophosphamid (total dose, 20 mg/kg) and thoracoabdominal irradiation (5 Gy) for conditioning and cyclosporine A for GVHD prophylaxis, 50 FA patients transplanted form MSD had a 5-year disease-free survival of about 75%. Another protocol for 16 FA patients with MSD used cyclophosphamid alone (total dose 100 mg/kg). The actuarial survival rate at 37 months was 88%. Fludarabine, a purine antimetabolite with potent immunosuppressive properties, was successfully incorporated into a conditioning protocol without radiation. The University of Minnesota is currently using T-cell depleted bone marrow (with Isolex CD34 positive selection) or cord blood as a donor source. Engraftment and survival with cord blood is better than another. At this time, most centers have reserved the use of umbilical cord blood for those patients who cannot identify a suitable marrow donor.

For patients who are not candidates for transplantation, androgen therapy sometimes induces meaningful responses in pancytopenic patients. This treatment is known to affect liver function adversely. Thus, several transplant centers recommend androgens not be given to any FA patients unless no suitable donor is available. Use of cytokines such as G-CSF or erythropoetin has been beneficial in some patients, provided the marrow shows no evidence of a clone or dysplasia.

Knowledge of the complementation group or mutation may permit the potential use of gene therapy and pre-implantation genetic diagnosis and embryo selection both to rule out FA and rule in an HLA match.

For patients with stable disease, annual surveillance exams and bone marrow aspiration (with cytogentic studies) and biopsy are suggested. For patients with complex cytogenetic abnormalities or MDS, closer follow-up is warranted.

Suggested Readings