



SCIENCE LETTER

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Toward Standards for FA Diagnosis

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Diagnostic Guidelines

A small group of medical clinicians met in Portland, OR in May 1998, to develop FA guidelines. A diagnostic algorithm was developed. Suspicion of FA should lead to referral for testing by a laboratory with expertise in FA. Whether the test of blood lymphocytes is DEB, MMC, or flow cytometry is left to the discretion of the laboratory. If the test is positive, the patient

should be managed by or in consultation with a hematologist with FA experience. If the test is negative, but FA is highly suspect, the patient should be referred to a hematologist with experience with FA. The test can be repeated by methods not used initially. Skin fibroblasts can be tested. Analysis of complementation or mutation can be considered.

FA is *highly suspect* in children with aplastic anemia and characteristic birth defects, as well as in cases of aplastic anemia <21 years old, children with characteristic birth defects and red cell macrocytosis for age who

are otherwise hematologically normal, patients in whom karyotype analysis reveals spontaneous chromosome breaks, children with an otherwise unexplained myelodysplastic syndrome (MDS), and patients treated for malignancy who are unusually sensitive to chemotherapy. *Intermediate suspicion* of FA is associated with macrocytosis for age, androgen-responsive aplastic anemia, birth defects in the major FA categories even without hematologic problems, non-immune thrombocytopenia without another cause, children with

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FA Complementation Analysis Update

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Research summarized in previous editions of the Newsletter has established eight complementation groups for FA (FA-A to FA-H), which were identified as a result of completing the analysis of 24 European FA patients. There is good reason to assume that each group is connected with a separate FA gene. With three genes identified to date (FA-A, FA-C, and very recently, FA-G) cloning the remaining

genes continues to be a major challenge.

Since the proteins encoded by the FA genes identified so far have no similarities to any other known protein there is as yet no clue for a molecular function. Antibodies raised against the FA proteins will be essential tools for unraveling the process defective in FA patients. Also, the identification of

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Genetic Testing

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Chromosomes (Cytogenetics)

Analysis of chromosomal response to clastogens, chemicals which cause DNA damage, is the first step in confirming the clinical diagnosis of Fanconi anemia (FA). Normal and carrier individuals are able to repair the damage caused by clastogens, such as mitomycin C (MMC) and diepoxybutane (DEB), at the concentrations used in the laboratory. However, presumably due to the underlying inherited genetic mutation in FA, FA patients' cells are unable to eliminate the damaged DNA, resulting in multiple breaks, gaps and radial forms in the chromosomes. Although most FA diagnoses are confirmed through this standard test on stimulated blood lymphocytes, some patients with classic features receive negative or ambiguous results. It is appropriate to request repeat testing, particularly in light of new laboratory technologies, advances in scientific understanding of the disease and variation in approach of different laboratories.

Acquired changes in chromosomes and underlying genes arise as a new event. These occur after conception

and are generally not passed on to offspring. Aberrations related to myelodysplasia (MDS) and leukemias can be observed by traditional cytogenetics (i.e. banded metaphase chromosome analysis) and by molecular cytogenetics (i.e. use of fluorescently tagged DNA probes in the process of fluorescent in situ hybridization or "FISH" on interphase cells in which no chromosomes can be seen because they are not dividing). Use of fluorescently tagged probes (pieces of DNA with known chromosomal locations) and the process of fluorescent in situ hybridization (FISH) allows detection of clonal chromosome abnormalities that may go undetected in the standard karyotype analysis.

DNA

With the cloning of the FA-A and FA-C genes, it is now possible to establish the specific mutation in families of complementation groups A and C. The search for the mutation generally follows confirmation of the diagnosis by breakage testing and complementation group assignment. Knowledge of the specific mutation allows carrier testing

in at-risk family members and more specific prenatal diagnosis.

Prenatal Diagnosis

Families are urged to seek genetic counseling in conjunction with prenatal diagnosis of FA. Such an interaction allows discussion of 1) procedure options with their risks and benefits, 2) experience of the center where a procedure is being done, 3) information regarding potential test results, their interpretation and timeliness of reporting, and 4) other genetic risks of which the couple should be aware. Current procedures for retrieval of fetal cells for breakage and mutation testing include chorionic villus sampling (CVS) at 10-12 weeks gestational age (GA), early amniocentesis (11-13.9 weeks GA), routine amniocentesis (greater than or equal to 14 weeks GA) and percutaneous umbilical blood sampling (PUBS) at 18-20 weeks GA. Ultrasonography accompanies all of these procedures and has the potential of revealing fetal structural abnormalities, particularly at the timing of amniocentesis and PUBS.

A new method of diagnosis which utilizes in vitro fertilization (IVF) and sampling of a single cell from the resulting 6-8 day embryo (blastocyst biopsy) prior to implantation is now being explored at a limited number of medical centers. Preimplantation diagnosis (PGD) is feasible for families in which a mutation is known. Mutation analysis can be done on a single cell. Only unaffected embryos are transferred to the uterus. Arrangements with the testing laboratory must be made many months in advance of the IVF in order to develop the test for the specific family mutation. Testing may also be available for HLA and chromosome status. Follow-up CVS or amniocentesis is strongly recommended to confirm the preimplantation diagnosis.



Cesar and Irma Lucero (Argentina) consult with Dr. Susan Olson (Oregon).

Complementation Studies in FA

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acute myelogenous leukemias (especially if super-sensitive to chemotherapy), patients with cancer at an atypically early age (e.g., head/neck/esophagus <40 years old, anal/vulvar <30 years old), or aplastic anemia <35 years old. A *low but real level of suspicion* of FA should occur for individuals with skin pigmentation (either hyperpigmented or areas of hypopigmentation) or café au lait spots, anyone with increased Hb F without a hemoglobinopathy, males with infertility, individuals with short stature and aplastic anemia or MDS, and aplastic anemia at any age.

Many patients are seen by orthopedic, hand, or plastic surgeons for their hand deformities, geneticists for various anomalies, gastroenterologists or endocrinologists for failure to grow, cardiologists for congenital heart defects, etc., before they develop hematologic manifestations. It is important to remember that perhaps 25% of patients in fact do not have birth defects, and will be missed if those are the only criteria.

FA patients develop characteristic hematologic and solid malignancies at an earlier age than in the general population, with an unexplained excess in females compared to males. The cancers include those of the head and neck, gastrointestinal, and gynecological areas. Most of the cancers are squamous cell. Some may have a viral association, such as head and neck, and anal-vulvar-cervical. Patients with these tumors may not have been known to have FA prior to development of cancer, but the diagnosis of FA is critical because it may guide management with regard to radiation or chemotherapy which might be toxic to the FA patient. FA patients also develop liver tumors, but these are usually in patients receiving androgen therapy. Leukemia in FA

patients is usually myeloid, unlike the typical childhood acute lymphoid leukemia.

Malignancies

Although surveillance does not guarantee early detection of a cancer, it does increase the possibility of detection at a stage which might require less aggressive management than in advanced disease.

To Monitor for Indications of Myelodysplastic Syndrome (MDS) or Leukemia: *Complete blood count* every 4 months, unless more frequent because of hematologic abnormality. *Bone marrow* examination annually, including aspirate for morphology, biopsy for cellularity and MDS, and cytogenetics for clonality. If available, testing procedures may include special stains and flow cytometry for markers of MDS or leukemia. Evaluation should be done at a center with experience in MDS and leukemia.

Gynecologic Cancer: Vaginal, anal, and cervical cancers occur earlier than in normal women, and are probably associated with human papillomavirus (HPV). *Gynecologic exam and Papanicolaou smear* are recommended every year after age 16 or menarche, whichever is first. *HPV testing* can be done on vaginal or cervical scrapings. The Pap smear and HPV tests may only need to be done in sexually active FA females. *Colposcopy* can be reserved for patients with abnormal Pap smears. *Breast* self-exam should be done monthly, and by physician annually.

Head, Neck, and Upper Esophagus Cancer: *Medical history* of throat pain, soreness, otalgia (earache), odynophagia (pain on swallowing), dysphagia (difficulty swallowing), hoarseness. *Physical examination* every 4 months, with careful attention to mouth, mucous membranes, throat, neck, lymph nodes.

Gastrointestinal Cancer: *Medical history* of weight loss, dietary changes, vomiting, blood in stools.

Liver Tumors: *Physical examination* for liver size, tenderness, masses. *Liver function tests*, including enzymes, bilirubin, alpha-fetoprotein. Do all annually, and enzymes and bilirubin every 3-4 months in patients on androgen therapy. *Liver ultrasound* every 6 to 12 months in patients on androgen therapy.

Gynecology and Pregnancy Guidelines

Menarche: Menarche is often delayed, and menses are irregular and may be anovulatory (without ovulating). However, more than 2 dozen FA women have had children, and thus some menses are clearly functional.

Menopause: Menopause is uniformly early in FA, mostly before age 40 years. FA women are thus at risk to develop estrogen insufficiency prematurely, with the risks of osteoporosis and heart disease. *Estrogen replacement* is indicated, with the caveat that estrogens may be bone marrow suppressive.

Pregnancy: In most of the more than 2 dozen FA pregnancies, the mother's hematologic status worsened, often requiring red blood cell and platelet *transfusions*. Preeclampsia or eclampsia was increased, as was the need for *Cesarean sections* for the former or for failure of labor to progress. FA pregnancies are high risk, and should be managed by experts in maternal-fetal medicine. Consideration should be given to cryopreservation of the baby's placental blood, as a potential source of stem cells for the mother.

Gynecologic Cancer: See above.

Leukemia, Myelodysplastic Syndrome and FA

J. Martin Johnston, MD

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Each cell in the human body arises from a "parent" cell through a process of division. Many millions of such divisions occur daily, each requiring the duplication of about 3 billion "bases" of DNA. In spite of remarkable biological safeguards, this feat of duplication is not error-free. Random alterations of the DNA, "mutations", are inevitable. Thankfully, most mutations are either inconsequential (i.e., they don't affect the cell) or detrimental (i.e., they make it more likely that the cell will simply die). Rarely, a mutation will give the cell a growth advantage. Such a mutation will be passed to the cell's "daughters," which will accumulate. Eventually, a second random event in one daughter may yield a doubly mutated cell. Over time, the stepwise acquisition of more mutations can lead to a cell with uncontrolled

growth: a cancerous cell. The type of cancer (e.g., leukemia) reflects the particular cell in which the mutations have occurred (e.g., a developing blood cell).

By definition, then, leukemic cells bear mutated DNA. Most of these mutations are so subtle as to be invisible, even under a microscope. Sometimes, however, a mutation will disrupt the DNA structure in a "gross" way: whole chromosomes (each of which is simply a long ribbon of DNA) may be lost or duplicated; portions of chromosomes may be deleted or rearranged. These changes are visible under the microscope and their presence defines a "clonal (meaning genetically related) abnormality." However, not all clonally abnormal cells are cancerous: even a "gross" mutation may not alter the cell's growth to such a dramatic and

irreversible degree. Thus, a clonal abnormality may warn (in a patient not yet diagnosed with leukemia) of an impending cancer. "Myelodysplastic syndrome" (MDS) is the term used to describe such premalignant conditions in the bone marrow.

In patients with FA, we suspect that the processes of mutation recognition and repair are defective. Thus, FA patients are at increased risk of acquiring the mutations that can lead to cancer. A particularly common cancer in FA patients is acute myelogenous leukemia (AML), an otherwise relatively rare form of leukemia. Furthermore, FA patients have difficulty tolerating standard cancer therapies, since chemotherapy and radiation therapy cause damage to the DNA of the FA patient's fragile, but otherwise healthy cells. Thus, the development of leukemia in an FA patient is ominous.

On the other hand, FA patients can demonstrate clonal abnormalities in their bone marrow without having leukemia. They may have other findings to support a diagnosis of MDS or the marrow may appear normal (aside from the chromosomal alteration). Interestingly, clonal abnormalities in FA patients will sometimes disappear; thus, leukemia is not an inevitable consequence of clonality. A better understanding of the biology of FA will allow a better understanding of all these phenomena.



Dr. Martin Johnston (Wisconsin) visits with Tom Massino (FA parent, Arizona).

Bone Marrow Transplant in Fanconi Anemia: An Update

John E. Wagner, MD

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At this time, hematopoietic cell transplant (from a healthy related or unrelated donor) is the only treatment with curative potential. In the late 1970s, Gluckman et al (Paris) proposed the use of low dose cytoxan and limited field irradiation in the treatment of FA patients in an attempt to reduce toxicity and improve survival. We now know that these modifications in chemotherapy and radiation (as compared to therapy given to non-FA patients) have indeed improved outcome particularly in FA patients with HLA matched sibling donors.

Today, hematopoietic cell transplant (HCT) from HLA matched sibling donors is generally associated with an excellent outcome if performed early in life (0-20 years) and prior to development of myelodysplastic syndrome (MDS) or leukemia (in FA patients, usually AML). Gluckman et al observed a low incidence of regimen-related toxicity and overall probability of survival of 75.6% (n=45, group includes all ages). Kohli-Kuma et al observed an overall probability of survival in patients <10 years of age with HLA matched sibling donors of >85% (n=17). Therefore, the optimum time of sibling donor HCT is when the patient is <10 years of age, has had few if any transfusions, prior to initiation of androgens or growth factors, and prior to development of MDS/AML. Patients should receive low dose cytoxan (20 mg/kg) and limited field irradiation, i.e., total abdominal irradiation (TAI), total nodal irradiation (TNI) or total lymphoid irradiation (TLI).

Unfortunately, the majority of FA patients do not have an HLA matched sibling donor; therefore, it is necessary to search for a closely matched relative (parent, cousin, etc.) or unrelated donor (cord blood or marrow).

Because of the higher risk of treatment-related mortality (treatment side effects, infections, graft rejection, graft versus host disease), non-matched donor transplants have not been recommended prior to treatment with other modalities (in contrast to the recommendation for those with HLA matched sibling donors). While patients with FA should undergo transplant prior to the development of leukemia or transfusion-requirement, early use of androgens and/or hematopoietic growth factors (G-CSF, GM-CSF, Il-11, EPO) at the onset of marrow failure with close follow-up is recommended.

The results of unrelated donor BMT/umbilical cord blood are poor compared to results with a matched sibling donor. The overall probability of survival is approximately 30-35%. The major obstacles to success are: 1) graft rejection and 2) infection (viral and fungal). We now know that patients with HLA matched unrelated donors do better than those with HLA-1 antigen mismatched donors. While we are evaluating the effects of age, transfusion number, radiation dose, presence of clonal cytogenetic

abnormalities, DEB sensitivity, mosaicism, etc. on various transplant outcomes (i.e., engraftment, GVHD, toxicity, survival), there are no clear-cut predictors of any outcome. Therefore at the present time, we recommend:

1. Conventional transplant, i.e., TBI 450 cGy and cytoxan 40 mg/kg for FA patients with an HLA matched unrelated donor or 1 antigen mismatched related donor;
2. Novel approach for other patients (not necessarily in this order):
 - A. Non-myeloablative therapy;
 - B. Megadose stem cell transplant without chemotherapy/radiation
 - C. Chemotherapy/TBI followed by transplantation of T-cell depleted marrow plus lymphocytes containing a "suicide" gene;
 - D. Genetic corrections of the stem cell;
 - E. *Isolation of the normal stem cell in FA patients with mosaicism (*to be proven).

Each of these novel approaches is being explored.



Parents seek advice from Dr. Nasrollah Shahidi.

Gene Therapy for Fanconi Anemia Group A Patients

Christopher Walsh, MD, PhD, UNC Gene Therapy Center, Chapel Hill, NC

We have developed several recombinant viral vectors carrying the Fanconi anemia group A gene for delivery to hematopoietic stem/progenitor cells. Currently retroviral vectors appear best suited for the transfer of genetic material to hematopoietic cells. We have produced a retroviral vector which transduces CD 34+ cells isolated from FA-A patients. In the laboratory, the transduced cells grow as well as normal cells after exposure to agents such as mitomycin C. Using a variety of strategies to enhance the transfer of the virus to CD 34+ cells we are now able to correct cells at higher levels. These results form the basis of a clinical gene therapy trial which has been submitted to the FDA.

The criteria for entry into the study require that patients be documented to carry mutations for FA-A, that they not be candidates for allogeneic bone marrow transplantation and in general that they have no other severe organ (lung, liver, kidney) dysfunction. In this trial patients will be

required to take injections of two cytokines which should allow for an enrichment of circulating peripheral blood CD 34+ cells. Recent experiments suggest that not only are more CD 34 cells obtained by this procedure, but that they may be more easily infected by the retroviral vector. CD 34 cells will then be collected following plasmapheresis. This procedure requires that the patients be attached through a venous catheter to a machine which captures the population of circulating peripheral blood CD 34 cells. These cells are further purified using an antibody affinity column and the CD 34 cells are then mixed with the retrovirus carrying the normal FA-A gene. The transduced cells are returned to the patient. The level of gene transfer can be easily monitored by assaying the growth of CD 34 cells. This procedure can be repeated an additional two times if necessary.

The protocol is designed as an outpatient procedure. However, patients

will stay overnight in the clinical research ward for observation following infusion of corrected cells. Patients and their physicians will be required to send blood and bone marrow samples to monitor the degree of gene transfer and to assess changes in blood counts and bone marrow. Based on developments in the laboratory, the cells, vectors and procedures used to enhance gene transfer will be subject to change. Thus we feel that this protocol is a first step towards the ultimate goal of restoring the hematologic defects in FA to normal.

The trial has been funded for 10 patients. We anticipate that costs to participants will be limited to travel expenses and limited lab tests. We anticipate beginning the study in the fall of 1998. Patients, family members and family physicians who wish more information may contact Dr. Christopher Walsh at (919) 966-9116 or e-mail: cwalsh@med.unc.edu.

Parents seeking answers about gene therapy. Lorne Shelson and Annette Waxberg (Canada).



Information for Dentists Caring for Patients with Fanconi Anemia

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Fanconi anemia (FA) is a familial disorder inherited in an autosomal recessive pattern. At the molecular level, FA is marked by increased chromosomal breakage. The molecular defect manifests itself in bone marrow failure (not just anemia as the name implies), birth defects that vary from patient to patient and an increased risk of cancer and leukemia. Dentists treating patients with FA should become familiar with the problems common to all FA patients and should ask that the patient's primary physician or hematologist provide a summary of the medical problems specific to the patient he or she is treating. The following is a general guideline for evaluating and treating FA patients, which must be adapted to the individual patient.

Problems common to all patients with FA:

1. Patients are at an increased risk of cancers of the gastrointestinal tract, including oral mucosal and tongue carcinoma. These problems are usually seen after the first decade of life, but baseline head and neck examination and oral cancer screening should be started at the first visit and continued on a semi-annual basis. Suspicious lesions, including ulcerations, persistently swollen tissue and leukoplakia should be subjected to biopsy. If a biopsy is contemplated, contact the patient's hematologist to see if special processing is required.
2. Patients are at an increased risk of leukemia. Persistent gingival swelling, oral bleeding or loose teeth without apparent cause may be symptoms of leukemia and should be reported to the patient's hematologist.

3. Patients may have low platelet counts from an early age. A simple examination and uncomplicated prophylaxis might be done when the patient has mild thrombocytopenia, but the same patient may need a platelet transfusion before an extraction, a biopsy, a procedure that requires a mandibular block anesthetic, or other procedures with a significant risk of bleeding. Therefore, the patient's hematologist should be consulted to check the status of the blood counts and review possible treatment plans several days before each visit.
4. FA patients often develop a low white blood cell count, making them susceptible to bacterial infections. Therefore, aggressive preventive care is important. Bottle weaning should be encouraged at one year of age and dental check ups should begin by the age of 18 months and continue semi-annually.

Specific problems seen in some FA patients:

1. Some patients will have an indwelling venous access device or a cardiac defect. Such patients should be given prophylaxis against sub-acute bacterial endocarditis (SBE) according to American Heart Association guidelines.
2. Patients may have upper extremity deformities that interfere with the maintenance of daily oral hygiene. In such cases, the parents should be given the responsibility for daily brushing and flossing. An electric toothbrush may be an aid to maintenance of oral hygiene in such patients.

FA Complementation Analysis Update

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new FA genes may provide new functional clues. There is good evidence indicating that FA proteins engage in complex formation that is essential for their functional activity.

Mosaicism Update

For an introduction to the topic of mosaicism in FA, the reader may consult earlier editions of the Newsletter. Some 25% of FA patients appear to have a proportion of their blood lymphocytes that have lost their FA character and apparently have reverted to normal (non-FA), that is, they are no longer sensitive, but are resistant to crosslinking agents (as normal cells are). The proportion of reverted lymphocytes may be small (e.g. less than 10%) or high (up to 100%).

In some patients a high proportion of reverted lymphocytes is associated with mild or no hematological symptoms; in others the symptoms may still be severe. This is thought to be related to the type of hematopoietic progenitor cell that has undergone the reversion. If a lymphocyte progenitor has reverted, only the lymphocytes will have the reverted character. In such a case the mosaicism is expected to be transient, since lymphocyte progenitors are not able to renew themselves indefinitely. If, on the other hand, a true pluripotent stem cell has undergone the reversion, this is likely to improve the formation of blood cells in *all* lineages. For most mosaic patients, it is currently not possible to determine the type of progenitor that has undergone reversion. The implications of mosaicism for treatment are currently difficult to delineate and should be considered in each individual FA patient, depending on the therapeutic choices that are being considered.



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