Fanconi anemia (FA) is an autosomal recessive condition associated with congenital abnormalities, progressive pancytopenia, and a predisposition to leukemia and solid tumors. We studied a retrospective cohort of North American patients with FA. We calculated relative risks of cancer compared to the general population and cause-specific hazards of the first major adverse outcomes of FA: bone marrow transplantation (BMT) for marrow complications, acute myeloid leukemia (AML), solid tumors, or death from bone marrow failure. We also estimated the cumulative incidence of each adverse event in the presence of the competing risks. Among 145 patients with FA, 9 developed leukemia and 14 developed a total of 18 solid tumors. The ratio of observed to expected cancers (O/E ratio) was 50 for all cancers, 48 for all solid tumors, and 785 for leukemia; these increased risks were statistically significant. The highest solid tumor O/E ratios were 4317 for vulvar cancer, 2362 for esophageal cancer, and 706 for head and neck cancer. Cause-specific hazards of both death and AML peaked at 1%/y in teenage years; the hazard of BMT peaked at 4%/y at age 7. In contrast, the hazard of a solid tumor approached 8%/y by age 40 years. The cumulative incidence to age 48 was 10% for leukemia, 11% for death from marrow failure, 29% for a solid tumor, and 43% for BMT. The risk of a solid tumor may become even higher as death from aplastic anemia is reduced and as patients survive longer after BMT. (Blood. 2003;101:822-826)

© 2003 by The American Society of Hematology

Cancer incidence in persons with Fanconi anemia

Philip S. Rosenberg, Mark H. Greene, and Blanche P. Alter

Fanconi anemia (FA) is an autosomal recessive condition associated with congenital abnormalities, progressive pancytopenia, and a predisposition to cancer. Although the most frequent malignancy in FA is acute myeloid leukemia (AML), an extensive series of case reports documents that persons with FA also develop specific solid tumors at remarkably young ages. Although the susceptibility to AML and solid tumors has now been recognized, the risk or penetrance of each adverse event by age has not been thoroughly quantified.

FA is a genomic instability syndrome. Cultured cells from patients with FA are characteristically sensitive to chromosome breakage by DNA cross-linking agents (for a review, see Young and Alter). Seven different genes have been cloned. In response to DNA damage, 5 of the FA gene products form a nuclear complex that promotes monoubiquitination of the FANCD2 protein, which in turn may interact with BRCA1 at nuclear foci. The precise functions of the FA complex and the etiology of malignancies in FA remain to be elucidated. Therefore, understanding patterns of cancer risk in FA may be informative about genetic mechanisms and gene/environment interactions that affect the risks of specific cancers, both in FA and in the general population. Refined estimates of cancer risk may also help patients with FA and their physicians make better-informed health decisions.

To quantify the cancer risk in FA, we assembled a retrospective cohort of persons with FA and collected data on the age at onset of cancers and hematologic consequences of the syndrome. We calculated both the relative risk of cancer in the FA cohort compared to the general population and the absolute risk of cancer as a function of age within the FA cohort.

Patients, materials, and methods

Study population

In January 2000, the Fanconi Anemia Research Fund mailed a survey to 284 members who had been proven to have FA by chromosome breakage analysis and who lived in the United States. Those who had not replied after 1 month were mailed monthly reminder cards for a total of 3 contacts. In August 2000, the survey was mailed to 34 members of Fanconi Canada who also had FA proven by chromosome breakage. Follow-up information was accepted through October of 2000, after which the cohort was closed.

The Human Subjects Committee of the Johns Hopkins Bloomberg School of Public Health approved the study, which was initiated while one of the authors (B.P.A.) was a graduate student at the school. The survey consisted of 4 pages of questions to be answered by the patients or their parents. Because proxy respondents (parents) were available, we analyzed the data as a retrospective cohort study. Questionnaire items included demographics, physical manifestations of FA, age at bone marrow transplantation (BMT), and age at onset of any cancers. Cancer diagnoses were confirmed using medical records, pathology reports, or death certificates.

Statistical analysis

For each cancer type, we compared the observed number of cancers to the expected number based on the experience of the Connecticut Tumor Registry. Expected incidence was calculated using age, sex, and birth/cohort-specific incidence rates for persons of white race/ethnicity. The ratio of observed to expected incidence measures the relative incidence of cancer in the FA cohort compared to a demographically matched cohort from the general population. We calculated 95% confidence intervals (CIs) for the incidence ratio using the standard assumption that the observed numbers of cases have a Poisson distribution. CIs were computed using an exact method that remains valid when the observed counts are sparse (eg, < 5).
The absolute risk of cancer observed in FA reflects the fact that individuals with FA are subject to multiple competing causes of morbidity and mortality. The characteristic bone marrow failure of FA may progress and require therapeutic BMT; a hematopoietic stem cell may become malignant, leading to leukemia (usually AML); or a somatic cell may become malignant, leading to a solid tumor. Therefore, we considered death, BMT, AML, and the development of a solid tumor to be competing risks, in the sense that the occurrence of any one of these conditions censors or alters the natural history of the other conditions. Because the relationship between myelodysplastic syndrome (MDS) and leukemia is not yet clearly established in FA, we considered MDS as nonmalignant and did not analyze it as a first event. Our analysis focused on the age-specific risk of each of the events in its role as the first major adverse outcome attributable to FA. Hence, to obtain estimates of absolute risk, we used competing risks survival methods.11

We obtained smoothed estimates of the absolute cause-specific hazard functions using a nonparametric procedure.12 The hazard functions specify the risk of each type of event by age in persons who are still susceptible, that is, who have not experienced any of the competing events. We modeled the hazards nonparametrically using spline functions. Because the joint likelihood function for the cause-specific hazards is equivalent to the product of the likelihood functions for each cause, with all other causes treated as censoring events,13 we were able to apply existing methods and software programs to estimate the hazard of each type of event. The number and location of the knots or join-points of each spline were chosen adaptively, and the variances of the hazard functions were estimated by bootstrap resampling.12

We estimated the cumulative incidence of each event in the presence of the competing risks using the nonparametric maximum likelihood estimator.11 We also obtained smoothed estimates of cumulative incidence by integration of the spline-smoothed cause-specific hazards according to the definition of cumulative incidence. These functions specify the cumulative proportion of subjects by age who experienced each adverse event as their initial failure. This estimator differs meaningfully from the more familiar statistical technique, the complement of the Kaplan-Meier product-limit estimate (the “1-KM” estimator).14 The 1-KM estimator (and the spline-smoothed version of it) estimates the hypothetical cumulative incidence that would be observed if the competing causes could be removed. In contrast, the maximum likelihood estimator describes the cumulative incidence that is actually observed in the presence of the competing risks.

We used the Cox proportional hazards model15 to study the effect of MDS on the hazard of developing AML, with the occurrence of MDS treated as a time-dependent covariate. Descriptively, we compared continuous distributions using the Wilcoxon rank sum test,16 tested for the independence of sex and outcomes using Stata7 software (Stata, College Station, TX). and Pearson χ², and compared the incidence rate of tumors with and without BMT using an exact binomial test.17

### Results

We received 145 questionnaires from 318 eligible individuals with FA in North America, a 45% response rate (Table 1). The median age of the subjects at the time of the survey was 12.7 years (range, 0.49 years). Sixteen percent (27 cancers in 23 individuals) had a history of cancer, including leukemia but excluding MDS. There were 9 cases of leukemia and 18 solid tumors in 14 individuals. The male-to-female ratio in all of the respondents was 1.1:1, whereas the proportion of male individuals was significantly elevated at 2.8:1 in those with MDS. The female excess among solid tumor cases reported in the literature was not observed in this study.2 The median age at diagnosis of FA in those with solid tumors, 7.6 years, was significantly older than in those without tumors, 4.6 years (P = .006), suggesting that those patients who went on to develop such neoplasms might have a milder FA phenotype (ie, fewer birth defects and less severe bone marrow involvement). The median age at onset of the leukemias, 11.3 years, was significantly lower than the median age at onset of the solid tumors, 28.9 years (P = .004), supporting the suggestion that solid tumors comprise the major neoplastic risk among the older patients with FA.

Nine cases of leukemia were observed, compared to 0.011 expected (observed-expected [O/E] = 785; 95% CI = 360-1490; Table 2). In addition, there was a statistically significant increase in all solid tumors combined (O/E = 48; 95% CI = 30-80). Among cancers that affect both men and women, there were large and statistically significant increases for liver (O/E = 386; 95% CI = 45-1395), head and neck (O/E = 706; 95% CI = 260-1540), and esophageal (O/E = 2362; 95% CI = 265-8530) cancers. Among gynecologic tumors, there were large and statistically significant increases for uterine cervix (O/E = 179; 95% CI = 20-645) and for vulvar (O/E = 4317; 95% CI = 870-12615) cancers. The overall excess also includes nonsignificant increases in osteosarcoma, soft-tissue sarcoma, and brain cancer.

Not included in the above analyses were 4 cancers in 3 individuals among 44 who underwent BMT and who had follow-up after BMT. (One individual who underwent BMT was censored at the BMT date.) The indications for BMT were aplastic anemia in 37 subjects and MDS in 8. The specific malignancies occurring after BMT were a gastrointestinal lymphoma in a patient receiving cyclosporin A, one patient with oral cavity carcinoma, and one patient with both tongue and vulvar carcinomas. The crude rate for solid tumors prior to BMT, AML, or death was 0.7% per year, whereas it was 1.99% after BMT. This rate ratio was 2.79, with a trend toward significance (P = .073); however, the small number of events and person-years in the BMT group limits the accuracy of this observation.

In the competing risks model whichever outcome is the first event precludes analysis of any later event in that individual in its role as first failure. The cause-specific hazard function for each adverse event (instantaneous risk of each event type, by age, among persons still susceptible) is shown in Figure 1A. The hazard of

<table>
<thead>
<tr>
<th>Table 1. Demographics of North American respondents with FA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
</tr>
<tr>
<td>Male to female ratio‡</td>
</tr>
<tr>
<td>Median age, y (range) at FA diagnosis</td>
</tr>
<tr>
<td>Median age, y (range) at outcome</td>
</tr>
</tbody>
</table>

NA indicates data not applicable.‡MDS was not considered to be a cancer. This column includes 4 subjects who had MDS prior to leukemia, and 1 who had MDS prior to a solid tumor. Persons with MDS are included in the solid tumors, leukemia, and no cancer columns.

†Fourteen individuals had 18 tumors: 6 head and neck cancers, 2 each of esophageal, liver, and cervix cancers, 3 vulvar cancers, and 1 each of osteosarcoma, soft-tissue sarcoma, and brain cancer. None of the respondents reported having developed either breast or ovary cancer.

‡Male-to-female ratio after exclusion of 2 women with only gynecologic cancers.
Table 2. Observed cancers, ratio of observed to expected cancers, and 95% CIs among North American respondents with FA

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>No. of observed cancers</th>
<th>O/E†</th>
<th>95% CI‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia (AML)</td>
<td>9</td>
<td>785§</td>
<td>360-1490</td>
</tr>
<tr>
<td>Head and neck</td>
<td>6</td>
<td>705§</td>
<td>260-1540</td>
</tr>
<tr>
<td>Esophagus</td>
<td>2</td>
<td>2362§</td>
<td>265-8530</td>
</tr>
<tr>
<td>Liver</td>
<td>2</td>
<td>386§</td>
<td>45-1395</td>
</tr>
<tr>
<td>Vulva</td>
<td>3</td>
<td>4317§</td>
<td>870-12 615</td>
</tr>
<tr>
<td>Cervix</td>
<td>2</td>
<td>179§</td>
<td>20-645</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>1</td>
<td>79</td>
<td>1-440</td>
</tr>
<tr>
<td>Soft-tissue sarcoma</td>
<td>1</td>
<td>49</td>
<td>0.6-270</td>
</tr>
<tr>
<td>Brain</td>
<td>1</td>
<td>17</td>
<td>0.2-95</td>
</tr>
<tr>
<td>Total cancers</td>
<td>27</td>
<td>50§</td>
<td>35-80</td>
</tr>
<tr>
<td>Total solid tumors</td>
<td>18</td>
<td>48§</td>
<td>30-80</td>
</tr>
</tbody>
</table>

*A total of 27 cancers observed in 23 patients (Table 1). Two patients had 2 solid tumors (cervix and vulva, and vulva and esophagus) and 1 patient had 3 solid tumors (esophagus, liver, and cervix).

†Expected cancer incidence rates calculated from the Connecticut Tumor Registry.

‡Limits of the 95% CIs rounded to the nearest 5 for values greater than or equal to 10.

§P < .05 that true O/E ratio equals 1.0 (exact 2-sided tests).

BMT (for aplastic anemia or nonmalignant but clinically significant MDS) increased during childhood to a peak of around 4%/y by age 7 years, and then declined to approximately 1.5%/y by age 20 years (Figure 1A). The hazards of death and AML increased during childhood, peaked at around 1%/y during teenage years, and were stable or decreasing in adulthood.

The leveling of the hazard for AML reflects the experience of the entire cohort, including 23 patients who developed MDS at a median age of 12.3 years (Table 1). After the onset of MDS, the relative hazard of developing AML was 24 (95% CI = 5-111, P < .0001). The crude hazard rate for AML was 0.3%/y prior to MDS and 9.4%/y subsequent to MDS (P = .00003).

In contrast to the patterns seen for AML, death, or BMT, the hazard of a solid tumor was relatively low (but not zero) during childhood, and then increased at a greater than linear rate. The hazard of a solid tumor was approximately 1%/y by age 17 years, 2%/y by age 24 years, and 4%/y by age 30 years. The hazard may be close to 8%/y by age 40 years, although this estimate is uncertain because relatively few subjects remained at risk by the time this age was reached, and consequently the confidence limits are broad.

The cumulative incidence function for each adverse event (cumulative probability by age of each event type as the initial cause of failure) is shown in Figure 1B. The cumulative incidence of BMT increased during childhood. By age 13 years, 33% had undergone transplantation as their initial failure (Figure 1B). The cumulative incidence increased more slowly after this age, a consequence of the declining hazard of BMT and the stable or increasing hazards for the competing adverse events.

By age 17 years, 12% of patients with FA had died from hematologic complications prior to undergoing BMT or developing either leukemia or a solid tumor. The cumulative incidence of death as the primary (nonmalignant) outcome was constant thereafter. Similarly, by age 24 years, 10% had developed AML as their initial failure, and the cumulative incidence of AML was constant thereafter. These observed plateaus in the cumulative incidence of death and AML have 2 possible explanations. First, the risk of death from aplastic anemia, or of development of AML, was maximal during childhood and adolescence and then declined. Second, the risk of BMT or of solid tumors as first adverse events was now relatively higher than the risk of death or leukemia.

The cumulative incidence of a solid tumor was relatively low through the middle twenties. It increased to 9% by age 28 years, 22% by age 34 years, and 29% by age 48 years. By age 48 years, almost all of the subjects (93%) had either died, received a transplant, or developed leukemia or a solid tumor.

Figure 2 contrasts the cumulative incidence that was observed for each event to the hypothetical curve that would be expected if the competing risks could be removed. (These latter hypothetical curves were estimated by the complement of the Kaplan-Meier curves.) In this scenario, the cumulative incidence of death, leukemia, and BMT would increase only modestly, reflecting that these adverse events tend to occur early in life. In contrast, the cumulative incidence of a solid tumor would increase more slowly after age 17 years, a consequence of the declining hazard of BMT and the stable or increasing hazards for the competing adverse events.
increase substantially: from 25% to 55% by age 40 and from 30% to 75% by age 48. This large increase reflects the fact that solid tumors occur relatively later than the competing risks: if the latter could be removed, a much larger number of subjects would live long enough to be at risk of a solid tumor.

Discussion

In addition to their well-known risk of AML, persons with FA have an extraordinary risk of developing a solid tumor. Compared to the general population, the risk is approximately 50-fold higher for all solid tumors combined, and several hundred- to several thousand-fold higher for cancers of the head and neck, esophagus, liver, vulva, and cervix. Among persons with FA, the absolute annual hazard rate of development of a solid tumor is also notably high and increases rapidly with age. The hazard is 2%/y at age 24 years, 4%/y at age 30 years, and close to 8%/y at age 40 years. Despite the presence of the competing risks, almost 1 in 3 persons with FA are expected to develop a solid tumor by the age of 48 years, compared to the 1 in 10 persons with FA who are expected to develop AML by the same age. These high cancer incidence rates are not an artifact of any increase in cancer risk resulting from BMT conditioning, because this analysis considered only cancers that arose prior to BMT. BMT appears to further increase the risk of cancer,18 but our data are limited by the small number of such observations in the current cohort.

The cause-specific hazards show a striking pattern. The risk of each hematologic event—death (most of the early deaths were due to complications of bone marrow failure), BMT, or AML—peaks by the age of 20 years and is stable or decreasing thereafter. In contrast, the risk of a solid tumor is relatively low prior to the age of 20 years and then increases progressively after that time. This pattern is reminiscent of the latent period distribution of second malignancies following chemotherapy or radiation treatment. Cases of leukemia first occur about 2 years after the initial therapy, and the risk peaks at 10 to 12 years after treatment and then disappears. Solid tumors begin to appear after 5 years and show a more than linear rise, reaching 5% cumulative incidence at 25 years since diagnosis of the first malignancy, without a plateau.19

Our estimates of the cumulative incidence of FA-related solid tumors are likely underestimate because many persons with FA do not yet live long enough to develop a tumor. This may not always be so because management of aplastic anemia, MDS, or leukemia may improve, leading to longer survival times. In addition, increasing numbers of patients with FA will be cured of their hematologic disease with successful hematopoietic stem cell transplants from bone marrow, peripheral blood, or cord blood. These types of interventions result in an individual who, genetically, is a somatic mosaic. The genome in the bone marrow has non-FA genes (and thus would no longer be at increased risk of malignant transformation), whereas the genome of all other somatic cell lineages retains defective FA genes with their attendant susceptibility to carcinogenesis. Current data, though sparse, suggest that FA transplantation survivors may have a risk of oral cancer, which is even higher than that determined by the natural history of patients with FA not receiving a transplant, perhaps related to immunosuppression or graft-versus-host disease or both.18 Thus, improved management of the early adverse hematologic outcomes, that is, aplastic anemia and leukemia, whether by supportive, pharmacologic, or transplantation methods, will increase the number of FA patients who are at risk of developing solid tumors later in their course.

Assuming that patients with FA do not die from bone marrow failure, our “1-KM” curves show the hypothetical penetrance of solid tumors expected in persons with FA who are “cured” of their anemia. In this scenario, our data suggest that the cumulative incidence of solid tumors would increase to 55% by age 40 years and 75% by age 48 years. This hypothetical increase reflects the decreased risk of adverse events attributable to defective hematopoi-esis, rather than any underlying increase in the risk of solid tumors. In fact, our estimates may be a lower bound because BMT appears to increase the risk of oral cancers. Clearly, current and future generations of patients with FA will require rigorous and lifelong screening for solid tumors, regardless of the severity of their anemia or the origin of their bone marrow.

The mechanisms by which defects in the FA complex increase susceptibility to specific solid tumors are not completely elucidated. However, recent work suggests that these mechanisms may involve BRCA1 and BRCA2. The early age at onset of these tumors in FA is similar to what is observed in other cancer predisposition syndromes, such as hereditary breast cancer, hereditary colon cancer, Li-Fraumeni syndrome, xeroderma pigmentosum, ataxia telangiectasia, Bloom syndrome, and so forth.20 The susceptibility to solid tumors in FA may reflect a “2-hit” process in which the FA defect renders the cell susceptible to genomic instability caused by subsequent somatic events occurring relatively early in life. The specific solid tumors that occur excessively in patients with FA suggest that environmental exposures may play a role. There are known or assumed associations between oral androgens and liver tumors,21 certain human papillomaviruses (HPVs) and gynecologic cancers,22 and HPVs and head and neck cancers.23 Additional studies of these and other oncogenic agents are clearly indicated to determine whether they play a role in the development of these cancers in patients with FA.

Limitations of our study must be noted. First, the absolute number of subjects in our analysis was relatively small. As a consequence, our point estimates of risk are uncertain. Second and most important, our results may be influenced by selection bias, because fewer than half of the eligible subjects responded. If respondents were more likely to have a family history of cancer than nonrespondents, then our estimates may be too high. However, if individuals with FA died of cancer before the diagnosis of FA was even made, our estimates may be too low. Our hazard models stipulate that the risks of each event are statistically independent, a standard but untestable assumption.

Although these limitations remain a concern, our results are qualitatively consistent with the much larger number of case reports in the literature;2,3 and in cross-section at least, our cohort appears to be representative of the FA population.24 Although there are no published large cohort data on the risk of solid tumors in FA, the International Fanconi Anemia Registry (IFAR) has published quantitative estimates of leukemia risk.24,25 Our estimates of the risk of leukemia are substantially lower than in the IFAR reports. In the first report from IFAR, the proportion of FA patients with leukemia, 9%, was compared to an annual incidence of leukemia in the general population. This comparison of a proportion to an annual incidence rate results in a marked overestimation of the risk in FA.25 In its second report, the IFAR considered AML and MDS as a single entity for analytical purposes. Standard Kaplan-Meier survivals were calculated, without the use of competing risks methods. In fact, both their data and ours give an actuarial risk of 50% for the combination of AML and MDS by age 40.24 (and our data, not shown).

However, we believe that AML and MDS in FA each has a distinct natural history and prognosis and should, therefore, be
analyzed separately. In our cohort MDS was a significant risk factor for AML. The hazard of developing AML subsequent to MDS was 9.4%/y, suggesting that in FA, not all clones will necessarily progress. In vitro models provide plausible mechanisms for conversion of MDS to AML in some subsets of patients, but the cytogenetic and clinical heterogeneity of MDS in FA suggests that the natural history may well differ in other subsets. This supports “splitting” rather than “lumping” this disorder.

Despite the uncertainties inherent in the small size of our cohort and the limited number of cancer events available for analysis, our estimates reflect the extraordinary cancer experience reported among individuals affected by this rare autosomal recessive disorder. Our estimates of absolute and relative cancer risk may help to inform the counseling and management of individuals with FA. The biologic basis for the specific cancer excesses that we have noted certainly merit further study. To follow up on these observations and to investigate the etiology of cancer in FA, we have launched a prospective cohort study of persons with FA and other inherited bone marrow failure syndromes.

Acknowledgments

We are extremely grateful to the Fanconi Anemia Research Fund and Fanconi Canada for mailing out the questionnaires and to all of the families who completed them. We thank Drs Gloria Petersen, Marie Diener-West, and Katherine Helzlsouer at the Johns Hopkins Bloomberg School of Public Health for their helpful comments during the development and preliminary analyses of the study; Jeremy Miller and David Castenson at Information Management Systems (Silver Spring, MD); and Liliana Preiss at Research Triangle Institute (Rockville, MD) for assistance with data management and analysis.

References