Hematologic Abnormalities in Fanconi Anemia: An International Fanconi Anemia Registry Study

By Anna Butturini, Robert Peter Gale, Peter C. Verlander, Barbara Adler-Brecher, Alfred P. Gillio, and Arleen D. Auerbach

We analyzed data from 388 subjects with Fanconi anemia reported to the International Fanconi Anemia Registry (IFAR). Of those, 332 developed hematologic abnormalities at a median age of 7 years (range, birth to 31 years). Actuarial risk of developing hematopoietic abnormalities was 98% (95% confidence interval, 93% to 99%) by 40 years of age. Common hematologic abnormalities were thrombocytopenia and pancytopenia. These were often associated with decreased bone marrow (BM) cellularity (75% of cases studied). Clonal cytogenetic abnormalities developed in 23 of 68 persons with BM failure who had adequate studies. Actuarial risk of clonal cytogenetic abnormalities during BM failure was 67% (47% to 87%) by 30 years of age. Fifty-nine subjects developed myelodysplastic syndrome (MDS) or acute myelogenous leukemia (AML). Actuarial risk of MDS or AML was 52% (37% to 67%) by 40 years of age. Risk was higher in persons with than in those without a prior clonal cytogenetic abnormality (3% [0% to 9%] v 35% [0% to 79%]; P = .006). One hundred twenty persons died of hematologic causes including BM failure, MDS or AML and treatment related complications. Actuarial risk of death from hematologic causes was 81% (67% to 90%) by 40 years of age.

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FANCONI ANEMIA is an autosomal recessive disease characterized by aplastic anemia and congenital abnormalities.1 Hypersensitivity to DNA cross-linking agents, like diepoxybutane (DEB), is often used as a diagnostic test.2 DEB testing allows diagnosis of Fanconi anemia in persons with diverse clinical features including those without clinically detectable congenital abnormalities.3

Complementation studies suggest that the genetic bases of Fanconi anemia are heterogeneous.4,5 Abnormalities in at least four genes, defined by complementation groups A, B, C, and D, result in increased DNA cross-linking sensitivity6 that characterizes Fanconi anemia. Group C complementing gene (FACC) was recently cloned.6 Mutation analysis suggests a correlation between genetic and clinical features in persons with abnormalities in this gene.7

Although hematologic abnormalities are common in persons with Fanconi anemia,8,9 their natural history is uncertain. Most studies are of small numbers of subjects with brief follow-up. We analyzed hematologic abnormalities in 388 persons with Fanconi anemia reported to the International Fanconi Anemia Registry (IFAR).

MATERIALS AND METHODS

Registry characteristics. The IFAR was established in 1982 to study clinical features of persons with Fanconi anemia.2,10 In almost all cases, diagnosis was confirmed by DEB testing performed at The Rockefeller University. Registration into the IFAR was usually at the time of DEB testing and included information on congenital and hematologic abnormalities. In persons with hematologic abnormalities, an attempt was made to obtain results of prior laboratory studies. The frequency and completeness of hematologic studies were not prospectively defined. Follow-up data were collected by different modalities. In about 30% of cases, physicians sent reports of some or all hematologic studies. In about 10% of cases, hematologic studies were performed on one or more occasions at The Rockefeller University Hospital or affiliated institutions. Comprehensive attempts to obtain follow-up data were made in 1989 and in 1993 by sending physicians of all living subjects detailed follow-up forms. Response rates were about 75% in 1989 and 50% in 1993.

Definition of abnormalities. Hematologic abnormalities were defined as follows: thrombocytopenia, platelets less than 100 × 10^9 L, anemia, hemoglobin (Hb) less than 10 g/dL and neutropenia, neutrophils less than 1 × 10^9/L. Pancytopenia was defined as an abnormality in two or three blood cell lineages. Less severe decreases in blood cells and alterations in red blood cell mean corpuscular volume (MCV) and fetal Hb (HbF) were noted, but not scored as abnormalities. Decreases in blood cell levels from concomitant nonhematologic diseases were also not scored as abnormalities.

Myelodysplastic syndrome (MDS) is a known complication of Fanconi anemia.6 Diagnosis of MDS usually does not require increased bone marrow (BM) myeloid blasts. However, because of interobserver variability in the diagnosis of MDS and the lack of central review of BM, we defined as MDS only those cases with 5% to 30% myeloid blasts in the BM or 5% to 20% myeloid blasts in the blood. BMs with myelodysplastic features but with less than 5% myeloid blasts were noted but not scored as MDS. Acute myelogenous leukemia (AML) was defined as greater than 30% myeloid blasts in the BM or greater than 20% myeloid blasts in the blood. MDS and AML were considered the initial hematologic abnormality when they were diagnosed within 6 months from first detection of thrombocytopenia or pancytopenia.

In this analysis, unless otherwise specified, we use the term BM failure to indicate hematologic abnormalities other than MDS or AML regardless of BM cellularity or whether these were diagnosed by BM studies or not.

Cytogenetic abnormalities were termed clonal when ≥2 metaphases had the same or a related abnormality. Cytogenetic studies showing only normal metaphases were considered adequate unless reported as inadequate by the referring laboratory or physician.

Statistical analysis. Actuarial risk of hematologic events and death was calculated by life-table analysis.11 Univariate analysis was performed by the Mantel-Cox test.12 Multivariate statistical models...
HEMATOLOGIC ABNORMALITIES IN FANCONI ANEMIA

Table 1. Actuarial Risk (95% confidence interval) of Hematologic Abnormalities in Fanconi Anemia

<table>
<thead>
<tr>
<th>From detection of hematologic abnormality</th>
<th>N*</th>
<th>10 yrs</th>
<th>20 yrs</th>
<th>30 yrs</th>
<th>40 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonal cytogenetic abnormalities</td>
<td>68</td>
<td>15% (6-25)</td>
<td>37% (21-56)</td>
<td>67% (47-87)</td>
<td>—</td>
</tr>
<tr>
<td>MDS/AML</td>
<td>398</td>
<td>7% (4-10)</td>
<td>27% (20-35)</td>
<td>43% (30-44)</td>
<td>52% (37-67)</td>
</tr>
<tr>
<td>Death†</td>
<td>332</td>
<td>16% (13-21)</td>
<td>45% (38-54)</td>
<td>65% (54-75)</td>
<td>81% (67-90)</td>
</tr>
</tbody>
</table>

From detection of hematologic abnormality

<table>
<thead>
<tr>
<th>From detection of hematologic abnormality</th>
<th>N*</th>
<th>10 yrs</th>
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<th>30 yrs</th>
<th>40 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonal cytogenetic abnormalities</td>
<td>68</td>
<td>42% (25-60)</td>
<td>81% (63-96)</td>
<td>91% (75-100)</td>
<td>—</td>
</tr>
<tr>
<td>MDS/AML</td>
<td>311</td>
<td>22% (14-31)</td>
<td>46% (35-60)</td>
<td>70% (62-79)</td>
<td>—</td>
</tr>
<tr>
<td>Death†</td>
<td>332</td>
<td>45% (30-53)</td>
<td>80% (59-92)</td>
<td>86% (74-95)</td>
<td>—</td>
</tr>
</tbody>
</table>

* Number at risk.
† Death caused by hematologic abnormality.

were obtained by the stepwise step-up Cox proportional hazard technique using the SMDP program.\(^1\) Variables considered were sex and age at DEB testing.

The relationship between clonal cytogenetic abnormalities during BM failure and successive development of MDS or AML was analyzed by Fisher exact test and by life-table analysis.\(^1\) Life-table analysis was performed by entering subjects at the time of the first cytogenetic study except as follows. Subjects in whom first detection of a clonal cytogenetic abnormality and diagnosis of MDS or AML were coincident were excluded. Persons with ≥2 cytogenetic studies in whom ≥1 studies showed a clonal abnormality detected before developing MDS or AML were entered into the life-table analysis at the time of the clonal abnormality.

Statistical analyses were performed with and without censoring persons who received BM or cord blood cell transplants at the time of transplant. Results were similar in each case. Consequently, data shown are the results of analyses performed without censoring transplant recipients at time of transplant unless otherwise specified.

RESULTS

Subject characteristics. 388 subjects were registered between 1982 and 1992. Of those, 205 were male and 183 female. Median age at DEB testing and registration was 6 years (range, birth to 36 years). In 328 persons, diagnosis of Fanconi anemia was suspected because of congenital and/or hematologic abnormalities. The remaining 60 persons were siblings of probands. Additional details are published.\(^1\)

Median follow-up was 5 years (0 to 10 years) from DEB testing and 6 years (0 to 36 years) from first detection of a hematologic abnormality. Median age of subjects at the last observation or death was 11 years (1 month to 41 years). Median number of blood studies per subject per year of known hematologic abnormality was 0.7 (0.1 to 11). Median number of BM studies per subject per year of known hematologic abnormality was 0.1 (0 to 3).

Initial hematologic abnormalities. Hematologic abnormalities were detected in 332 persons. In 274 persons, these were present at the time of DEB testing. In 58 others, these were detected a median of 3 years (1 to 13 years) after DEB testing. Median age at detection of a hematologic abnormality was 7 years (birth to 31 years). Actuarial risk of developing a hematologic abnormality by 40 years of age was 98% (95% confidence interval, 93% to 99%) (Table 1, Fig 1).

Initial hematologic findings were diverse; 128 persons (38%) presented with thrombocytopenia, 176 (53%) with pancytopenia, 13 with MDS, 7 with AML, 6 with anemia, 1 with acute lymphoblastic leukemia (ALL) and 1 with neutropenia. Platelets were higher (50 [20 to 98] v 35 [2 to 65] × 10^9 L; \(P < .001\)) and age at diagnosis younger (5 years [birth to 17 years] v 8 years [birth to 31 years]; \(P = .009\)) in persons presenting with thrombocytopenia than in those presenting with pancytopenia. MCV was increased in 189 of 191 evaluable persons and HbF in all 79 studied persons.

BM studies at first detection of a hematologic abnormality were reported in 168 persons; 125 (75%) showed reduced BM cellularity, 22 (13%) normal or increased cellularity, 20 (12%) MDS or AML, and 1 ALL.

Disease evolution. Of 128 persons presenting with thrombocytopenia, 62 progressed to pancytopenia after a median of 3 years (3 months to 15 years). Actuarial risk of progression to pancytopenia was 84% (72% to 92%) 20 years after thrombocytopenia was first detected.

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Of the 311 persons with BM failure, 39 developed MDS or AML after a median of 9 years (1 to 17 years) from detection of a hematologic abnormality. Actuarial risk of developing MDS or AML was 48% (35% to 60%) 20 years after BM failure was detected.

Persons with BM failure received diverse treatments including androgens, corticosteroids, hematopoietic growth factors and transfusions. Fifty-eight persons received BM or umbilical cord blood cell transplants from HLA identical siblings or alternative donors. Because treatment was not given in a prescribed way and treatment modalities evolved over several years, no attempt was made to analyze the impact of treatment on disease evolution or survival.

**MDS and AML.** 34 persons developed MDS and 25 AML, including 10 with prior MDS. Median age at detection of MDS or AML was 13 years (1 month to 32 years). Actuarial risk of developing MDS or AML by 40 years of age was 52% (37% to 67%) (Table 1, Fig 2).

In 20 persons, MDS or AML was the first hematologic abnormality detected. In the other 39 cases, MDS or AML developed after BM failure. Age at diagnosis of leukemia was significantly younger in persons without than those with prior BM failure (7 years [1 month to 20 years] vs 17 years [6 to 32 years]; P < .0001) (Fig 2). Age at detection of first hematologic abnormality did not differ significantly between these groups (7 years [1 month to 20 years] vs 9 years [1 month to 30 years]).

Eight of 34 persons with MDS received a BM transplant, and therefore, were unevaluable for disease evolution. Of the remaining 26 persons, 10 progressed to AML after a median of 2.5 years (0.5 to 7 years), 9 of the remaining 16 died of hematologic complications after a median of 2 years (0.5 to 6 years), and 7 are alive at a median of 1 year (1 month to 10 years) after diagnosis of MDS.

Five of 35 persons with AML (10 with prior MDS) received transplants, and therefore, were unevaluable for disease evolution. Of the remaining 30 persons, 26 died within 3 months from diagnosis of AML with BM aplasia or resistant leukemia, and 1 has been followed-up less than 3 months. In 3 persons, treatment decreased BM myeloblasts to less than 5%; 2 had persistent myelodysplastic BM and clonal cytogenetic abnormalities and died of infection after 1 and 2 years. The third subject had hypoplastic BM with only normal metaphases for 9 years until dying of hepatocellular carcinoma.

**Survival.** 135 of the 388 persons with Fanconi anemia died at a median age of 13 years (1 month to 40 years). In the 332 persons with hematologic abnormalities, there were 129 deaths; 120 deaths were directly or indirectly caused by hematologic abnormalities and 9 were from other causes, including 6 from cancers other than leukemia.

Of 120 deaths from hematologic abnormalities, 49 were from BM failure, 37 from treatment-related complications after transplant and 34 from MDS or AML. Actuarial risk of death from a hematologic cause by 40 years of age was 81% (67% to 90%) (Table 1 and Fig 3). Median interval from onset of a hematologic abnormality to death was 7 years (1 month to 35 years). Actuarial risk of death after 20 years from first detection of hematologic abnormalities was 80% (59% to 92%). Censoring the 71 persons who received transplants at the time of transplant did not significantly affect these results.

**BM cytogenetics.** Cytogenetic studies were performed one or more times on BM samples from 105 persons. Some of these data are published. Eighty-eight persons were studied during BM failure. Cytogenetic studies were performed a median of 5 years (0 to 20 years) after detection of a hematologic abnormality. In 85 persons there was no report of myelodysplasia in a concurrent histologic study; 3 were reported to have myelodysplasia with less than 5% myeloblasts.

Adequate cytogenetic studies were obtained in 68 persons: 45 (66%) had only normal metaphases and 23 (34%), clonal...
cytogenetic abnormalities on ≥1 study. Results of cytogenetic analyses are summarized in Fig 4. Studies showing only normal metaphases were performed in younger persons (median age 9 years [2 to 27 years] v 16 years [3 to 32 years]; P < .0001) and earlier after detection of hematologic abnormalities (2 years [0 to 24 years] v 7 years [0 to 20 years]; P = .004) than those showing clonal cytogenetic abnormalities. Actuarial risk of developing clonal abnormalities was 67% (47% to 87%) by 30 years of age and 81% (62% to 95%) by 20 years from detection of a hematologic abnormality (Table 1).

Of 68 persons with adequate cytogenetic studies, 7 developed AML or MDS including 1 of 45 (2%) with only normal metaphases and 6 of 23 (26%) with clonal cytogenetic abnormalities (P = .005). Actuarial risk of developing MDS or AML within 3 years from cytogenetic analysis in persons with BM failure and only normal metaphases was 3% (0% to 9%) versus 35% (0% to 79%) in persons with BM failure and clonal cytogenetic abnormalities (P = .006).

Clonal cytogenetic abnormalities were often detected in close temporal proximity to the diagnosis of MDS or AML. Of 3 persons who had only normal metaphases in the initial study, two had prior clonal cytogenetic abnormalities (intervals 12 and 15 months) and one did not (interval 6 months). These cases are too few for further analysis.

Evolution of clonal cytogenetic abnormalities was analyzed in persons who had BM cytogenetic studies on two or more occasions. Eight persons had ≥2 studies during BM failure. Two with only normal metaphases in the initial study subsequently developed clonal cytogenetic abnormalities. Six others with clonal cytogenetic abnormalities in the initial study had the same abnormality on retesting.

Five other subjects were studied during BM failure and after developing MDS or AML (Table 2). Three who had only normal metaphases during BM failure developed clonal abnormalities after progressing to leukemia. The other two had the same or related clonal abnormalities during BM failure and after developing leukemia.

Twenty-three persons were studied after developing MDS or AML. All had at least one study showing clonal cytogenetic abnormalities. Loss of chromosome 7 or rearrangement or loss of 7q and rearrangements of 1q36 and 1q24-34 were similarly detected in persons with BM and MDS or AML; rearrangements of 11q22-25 were more frequent in persons with MDS or AML (Fig 4).

Sibling. We analyzed hematologic abnormalities and outcome in 106 members of 46 affected sibships. Hematologic abnormalities were detected in all 46 probands and 40 siblings. Median age at detection of hematologic abnormalities in probands was 8 years (0.5 to 29).

<table>
<thead>
<tr>
<th>UPN</th>
<th>Age (yrs)</th>
<th>Status</th>
<th>Cytogenetics</th>
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<tbody>
<tr>
<td>412-1</td>
<td>14</td>
<td>BMF</td>
<td>46XY</td>
</tr>
<tr>
<td>15</td>
<td>BMF</td>
<td>46XY</td>
<td></td>
</tr>
<tr>
<td>378-1</td>
<td>8</td>
<td>BMF</td>
<td>46XX</td>
</tr>
<tr>
<td>15</td>
<td>AML</td>
<td>46XX, -21, t(3;11)(q27;q21) [31/46XX, t(3;11;21) q27;q21;pl1]</td>
<td></td>
</tr>
<tr>
<td>187-1</td>
<td>6</td>
<td>BMF</td>
<td>46XY</td>
</tr>
<tr>
<td>8</td>
<td>MDS</td>
<td>45XY, -7</td>
<td></td>
</tr>
<tr>
<td>390-1</td>
<td>30</td>
<td>BMF</td>
<td>46XX, del(7q)(q22);q36</td>
</tr>
<tr>
<td>31</td>
<td>MDS</td>
<td>46XY, del(7q)(q22);q36 [13]46XY, del(6)(p23), del(7q)(q22);q36 [5]</td>
<td></td>
</tr>
<tr>
<td>44-2</td>
<td>8</td>
<td>BMF</td>
<td>46XX</td>
</tr>
<tr>
<td>9</td>
<td>BMF</td>
<td>46XX, [8]46XX, t(1;8)(p36;q21) [11]</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>MDS</td>
<td>46XX, t(1;8)(p36;q21) [8]46XX, t(1;8)(q21;p21) [10]</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: UPN, unique patient number; BMF, bone marrow failure.

Table 2. BM Cytogenetic Analyses in Persons Studied During BM Failure and After Development of MDS or AML
Actuarial risk of developing a hematologic abnormality by 40 years of age was 100%. Median age at detection of hematologic abnormalities in siblings was 9 years (0.6 to 27 years). Actuarial risk of developing a hematologic abnormality by 40 years of age was 83% (71% to 92%). Age at detection of hematologic abnormalities in probands and siblings was correlated (R, 0.72; P = .0006). However, there was no concordance for the type of first hematologic abnormality within affected sibships. Also, development of leukemia or death in one member of a sibship did not increase the risk of leukemia or death in the other siblings.

**DISCUSSION**

We used an observational database of 388 subjects with Fanconi anemia most of whose diagnosis was confirmed by DEB testing. Although large numbers of subjects and long follow-up make this database unique in analyzing hematologic abnormalities of Fanconi anemia, there are potential limitations. One is possible selective reporting. Another is that there was no prospectively defined study design; frequency and extent of hematologic testing were determined by the participating physicians even after subjects were registered. A third consideration is completeness and accuracy of data reporting; no audits of reporting centers were performed. Certainty of our conclusions could be increased by a prospective study. However, this would require substantial numbers of subjects and a long observation period.

Our study indicates that most persons with Fanconi anemia develop a hematologic abnormality; actuarial risk was 98% (93% to 99%) at 40 years of age. Whether the few older persons currently without hematologic abnormalities will eventually develop them is unknown. A 50-year-old Fanconi anemia-dying without ever developing hematologic abnormalities is reported (E. Gordon-Smith, personal communication, September 1993).

Our study indicates an actuarial risk of developing MDS and/or AML of 52% (37% to 67%) at 40 years of age. This exceeds the 15% rate cited in most studies. This difference is not surprising because prior estimates were based on small sample sizes and were not actuarial. It should be emphasized that our study may underestimate risk of developing MDS and/or AML because of the relatively strict definitions we used and because of nonprospective testing and ascertainment of hematologic data.

An interesting problem in Fanconi anemia is the relationship between the different hematologic features. Data we review suggest that thrombocytopenia and pancytopenia are phases of a single process. For example, persons presenting with thrombocytopenia were younger than those presenting with pancytopenia. Also, most persons with thrombocytopenia had multilineage abnormalities, as shown by elevated HbF and macrocytosis. The actuarial probability of progression to pancytopenia was 84% (72% to 92%) after 20 years.

A similar problem is the relationship between BM failure and leukemia (Fig 5). In our study, the most striking data suggesting that BM failure is a phase of leukemia development is the 67% (47% to 87%) actuarial risk of developing clonal cytogenetic abnormality during BM failure by 30 years of age. Most of these clonal cytogenetic abnormalities were similar to those reported in MDS and therapy-linked AML in persons without Fanconi anemia, developed late in the course of hematologic disease and persisted when leukemia developed. Also, in two subjects, the same clonal cytogenetic abnormality was detected during BM failure and leukemia. When we analyzed the association between detection of clonal cytogenetic abnormalities during BM failure and subsequent risk of developing MDS or AML, we found an increase in leukemia risk in persons with these abnormalities compared with those with only normal metaphases. Because our strategy of analysis excluded cases where clonal cytogenetic abnormalities and leukemia diagnosis were concurrent, our results may underestimate the risk of leukemia in persons with these abnormalities.

Controversy regarding the relationship between BM failure and leukemia in Fanconi anemia is similar to that regarding the relationship between aplastic anemia and leukemia in otherwise normal persons. Although cytogenetic abnormalities are rare, some data suggest a substantial incidence of clonal hematopoiesis in persons with aplastic anemia. Also, some studies suggest an increased risk of leukemia in persons who recover from aplastic anemia. What is still uncertain is whether detection of clonal hematopoiesis during aplastic anemia increases leukemia risk. Experimental data link development of clonal hematopoiesis in aplastic anemia to decreased stem cells rather than leukemia.

Fanconi anemia is an interesting model of leukemia development. Because many persons with Fanconi anemia will develop MDS and/or AML if they survive long enough, it is reasonable to regard the Fanconi anemia genotype as "preleukemia." The subsequent development of hematologic abnormalities might occur as a direct consequence of absence of the Fanconi gene product or because of an interaction of this abnormality with other intrinsic or extrinsic potentially leukemogenic factors.
ACKNOWLEDGMENT

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REFERENCES

Hematologic abnormalities in Fanconi anemia: an International Fanconi Anemia Registry study [see comments]

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