Incidence of Aplastic Anemia: The Relevance of Diagnostic Criteria

By the International Agranulocytosis and Aplastic Anemia Study

The incidence of aplastic anemia was estimated in a 4-year study conducted in Israel and seven areas in Europe. Strict definition criteria were used, and all data and bone marrow specimens were reviewed by a panel of experienced hematologists. Complete ascertainment of cases was attempted by establishing a telephone network including all relevant hospitals in the study areas. The incidence of aplastic anemia was estimated to be less than three per million per year, a figure lower than previously reported. The most likely explanation for the differences among studies is variation in diagnostic criteria, which might lead to an overestimation of the incidence in some previous studies.

A PLASTIC ANEMIA is a rare but serious blood dyscrasia that may be induced by environmental factors such as drugs or viral infections. There are only limited data on its incidence, which appears to vary according to the period of observation and geographic area.1,6 It is possible that the variation is due to genetic differences and to varying prevalences of exposure to potentially myelotoxic agents. However, methodological problems such as the completeness of case ascertainment or stringency of diagnostic criteria may also be responsible for at least some of the differences observed. In this paper we report incidence data obtained in a multicenter study of agranulocytosis and aplastic anemia that was carried out from 1980 to 1984 in Israel and seven regions of Europe. The aim of the study was to quantify associations among drugs, especially antiinflammatory and analgesic agents, and two blood dyscrasias, agranulocytosis and aplastic anemia.

METHODS

The methods of the study have been described.7 Here the description is limited to aspects of particular relevance to estimating the incidence of aplastic anemia. The study base included the entire population of eight defined geographic regions: Ulm, FRG; Berlin; Barcelona, Spain; Milan, Italy; Budapest; Sofia, Bulgaria; Stockholm/Uppsala; and Israel. The total population was 20.5 million, as determined from census data obtained from official national sources. Case ascertainment began in July 1980 and ended in June 1984 (not all regions participated for the full 4-year period).

Efforts were made to identify all cases of aplastic anemia occurring in the regions. A study center in each region obtained information on potential cases from hospitals within a defined area; these hospitals included all clinical institutions known to treat diseases of the severity of aplastic anemia. The study centers maintained regular liaison with the cooperating hospitals at intervals of no more than 2 weeks. In each hospital, an appropriate individual, usually a hematologist or technician, was contacted by telephone to ascertain all cases of aplastic anemia. Once notification of a potential case was received, a specially trained nurse or physician monitor was sent to collect more detailed clinical information and histopathologic specimens. A consulting hematologist reviewed the information in each of the eight regional centers. If the case clearly did not comply with the definition of aplastic anemia as required by the protocol, it was excluded locally. All remaining potential cases were reviewed subsequently by the Hematology Review Committee (HRC, see the Appendix) using clinical summaries, hematologic data, and microscopic examination of bone marrow and peripheral blood specimens. This panel had no information on previous exposures to drugs or chemicals.

For confirmation of diagnosis, the following conditions were obligatory: peripheral blood (at least two of the following three criteria): (a) hemoglobin, ≤100 g/L, or hematocrit, ≤30%; (b) platelets, ≤50 × 10^9/L; and (c) leukocytes ≤3.5 × 10^9/L, or granulocytes, ≤1.5 × 10^9/L. For bone marrow to confirm the diagnosis, there had to be an adequate bone marrow biopsy specimen or histology specimen obtained at autopsy showing the following: (a) a decrease in cellularity with the absence or depletion of all hematopoietic cells or normal cellularity due to focal erythroid hyperplasia with depletion of granulopoietic cells and megakaryocytes and (b) the absence of significant fibrosis or neoplastic infiltration. Cases with pancytopenia due to cytostatic drugs or irradiation were excluded.

If there were equivocal data that did not permit a definite diagnosis, the following additional information was sought from the local hematologist for second review: follow-up blood counts including reticulocyte counts, subsequent bone marrow specimens, and autopsy reports. If one of the two peripheral blood criteria to be fulfilled was hemoglobin, ≤100 g/L, or hematocrit, ≤30%; a reticulocyte count of less than 30 × 10^9/L was required.

All patients were observed until death, recovery, or bone marrow transplantation, or for a period of at least 2 years.

RESULTS

There were 315 cases initially identified as aplastic anemia and four cases originally identified as agranulocytosis but subsequently confirmed as aplastic anemia. Of the 319 potential cases, 34 were clearly misdiagnosed and were therefore ruled out by the consulting hematologists at the local centers (Fig 1). Among the 285 potential cases reviewed by the HRC, 168 were confirmed as definite aplastic anemia; nine cases were included even though a bone marrow biopsy specimen was not available because at least two bone marrow aspirations were typical and all clinical data over a prolonged period of observation were compatible with the diagnosis. Among the potential cases excluded by the HRC were 40 patients in whom a definite diagnosis could not be made due to insufficient documentation, in the majority due to the absence or inadequate technical quality of the biopsy specimen. Seven cases of moderate pancytopen-
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There were 70 potential cases identified as having pancytopenia due to another disease. The largest group (17 cases) consisted of pancytopenia with normal or hypercellular bone marrow that could not be further classified from the data available to the HRC; ten of these were recognized as transient pancytopenia on further follow-up. In 13 patients, a definite diagnosis of leukemia could not be made, but increased cellularity of the bone marrow and morphological aberrations of myelopoietic precursors permitted their identification as myelodysplasias that were potentially preleukemia. Nine cases were identified as aleukemic myeloid or myelomonocytic leukemia, usually of an oligoblastic or smouldering type. There were seven cases with severe neutropenia and additional anemia or thrombocytopenia; review of the bone marrow biopsy specimens, details of clinical presentation, and subsequent patterns of blood counts revealed that the anemia or thrombocytopenia was due to secondary events such as bleeding or infection and that the correct diagnosis was agranulocytosis or chronic neutropenia. Non-Hodgkin’s malignant lymphoma involving the bone marrow (including two cases of aleukemic hairy cell leukemia) was identified in six cases. Five cases were determined to have hypersplenism or liver disease, and the remaining 13 cases included diagnoses such as megaloblastic anemia, chronic renal disease, and multiple myeloma, none of which accounted for more than three individuals.

The overall incidence of aplastic anemia, based on the total of 166 complete and confirmed cases, was estimated to be 2.2 cases per million per year (Table 1). (Data from Stockholm/Uppsala are not included in the overall incidence rates because clot sections from aspirated bone marrow spicules are often taken instead of a core biopsy, thereby resulting in a larger proportion of potential cases in that region being excluded due to insufficient documentation in terms of the study criteria.) The region-specific incidence estimates ranged from 0.6 per million in Budapest (based on only three cases) to 3.1 per million in Milan. A test for heterogeneity in the region-specific rates among the five centers contributing at least 18 cases was not statistically significant.

Table 1. Annual Incidence of Aplastic Anemia According to Region, July 1980 to June 1984

<table>
<thead>
<tr>
<th>Region</th>
<th>Population (10^6)</th>
<th>Interval (mo)</th>
<th>Incidence (per 10^6/yr)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulm</td>
<td>5.3†‡</td>
<td>48</td>
<td>56</td>
</tr>
<tr>
<td>Berlin</td>
<td>1.8</td>
<td>48</td>
<td>18</td>
</tr>
<tr>
<td>Barcelona</td>
<td>4.1</td>
<td>48</td>
<td>36</td>
</tr>
<tr>
<td>Israel</td>
<td>3.9</td>
<td>48</td>
<td>21</td>
</tr>
<tr>
<td>Milan</td>
<td>2.3</td>
<td>45†</td>
<td>27</td>
</tr>
<tr>
<td>Budapest</td>
<td>2.0</td>
<td>31‡</td>
<td>3</td>
</tr>
<tr>
<td>Sofia</td>
<td>1.1</td>
<td>23§</td>
<td>5</td>
</tr>
<tr>
<td>Stockholm/Uppsala</td>
<td>1.8</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>20.5#</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Standardized to the age and sex distribution of the study population.
†October 1980 to June 1984.
§August 1982 to June 1984.
††The incidence was not calculated for Stockholm/Uppsala for methodological reasons (see the text).
#The total population does not include Sweden.
**Calculated as a population- and time-weighted average of the age- and sex-standardized region-specific rates.
††Including a large region surrounding the city.

The incidence according to age and sex is given in Table 2. The sex ratio for all regions combined was close to unity. Among females, the incidence of aplastic anemia increased steadily with age to a peak of 6.3 per million above the age of 60. Among males, the highest incidence was in the 15- to 24-year-old age group. However, nine of the 22 cases were from one military brigade in Barcelona; this result will be reported more fully elsewhere. If the latter age group is ignored, the incidence in males was highest (3.2 per million) over the age of 60 years. No attempt was made to compare age or sex-specific incidence rates between the study regions due to the limited number of cases. The fatality rate 2 years after diagnosis was 49%.

DISCUSSION

The overall incidence of aplastic anemia as estimated in this study is considerably lower than both incidence and mortality estimates obtained from other studies. Aoki et al1 and Hayakawa and Kurihara3 used death certificate statistics to compare mortality rates in different regions.

Table 2. Annual Incidence of Aplastic Anemia According to Age and Sex

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Male</th>
<th>Incidence (per 10^6/yr)*</th>
<th>Female</th>
<th>Incidence (per 10^6/yr)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;15</td>
<td>12</td>
<td>1.7</td>
<td>5</td>
<td>0.7</td>
</tr>
<tr>
<td>15-24</td>
<td>22†</td>
<td>3.5</td>
<td>4</td>
<td>0.9</td>
</tr>
<tr>
<td>25-44</td>
<td>12</td>
<td>1.1</td>
<td>18</td>
<td>1.8</td>
</tr>
<tr>
<td>45-59</td>
<td>11</td>
<td>1.8</td>
<td>15</td>
<td>2.0</td>
</tr>
<tr>
<td>≥60</td>
<td>16</td>
<td>3.2</td>
<td>51</td>
<td>6.3</td>
</tr>
<tr>
<td>Total</td>
<td>73</td>
<td>2.1</td>
<td>93</td>
<td>2.4</td>
</tr>
</tbody>
</table>

*Standardized to the regional distribution of the study population.
†Includes nine cases from Barcelona.
High rates for aplastic anemia were reported from Japan, compared with most European countries and the United States. It is unknown what proportion of deaths attributed to aplastic anemia in these reports were actually aplastic anemia. In the studies of Wallerstein et al.6 and Szkelo et al., outpatients and hospital charts, autopsy records, or laboratory material was used to confirm the diagnosis in cases selected by a review of death certificates. In no more than 60 of 290 and 60 of 111 cases, respectively, could aplastic anemia be confirmed, although it is possible that a proportion of those who failed to satisfy study criteria lacked sufficient documentation. Even larger discrepancies may exist in countries with less adequate diagnostic facilities than in the United States. Uncontrolled mortality statistics, therefore, give at best only a very rough estimate of the true incidence of the disease.

Even the highest regional incidence in the present study (3.1 per million in Milan) is lower than previous estimates, which range from 4 to 25 cases per million per year (Table 3). The latter figures are based on data derived from both mortality and morbidity statistics collected in different regions and at different times. Potential methodological problems and other possible explanations must be considered in interpreting the apparent differences.

The estimate of Wallerstein et al.6 was obtained in the course of quantifying the risk of aplastic anemia in relation to exposure to chloramphenicol; incidence estimates were derived from mortality data, assuming a 50% disease-specific death rate. The limitations of death certificate data have already been mentioned. In addition, it is not clear that the assumption of a 50% death rate is correct. We observed a similar death rate in our series after only 2 years of follow-up, and it is probable that the rate would have been higher with a longer follow-up. Thus, the total incidence could have been overestimated by Wallerstein et al.

It is important to consider whether the lower incidence of aplastic anemia observed in our study, and based on recent data, reflects a true decrease over the last 20 years (for example, because of decreased use of potentially harmful drugs such as chloramphenicol). This explanation seems unlikely because no clear trend has been observed in mortality statistics from different areas between 1950 and 1980 or between 1970 and 1979 in a study by Szkelo et al using morbidity data.5

It also does not seem likely that differences in age between the study populations can completely account for the observed differences in incidence. Presumably, the age distributions in the study regions have not changed materially over time; if anything, the populations have aged, thus increasing rather than decreasing the incidence of a disease with the age dependency of aplastic anemia. Evidence against differences in age distributions was suggested by a comparison of the study population of Szkelo et al.6 with our data: the age and sex characteristics of the population of metropolitan Baltimore were virtually identical to those in our study.

There is no evidence that the lower incidence in our study is due to lower ascertainment than in previous studies. Utmost care was taken to obtain regular information from all cooperating hospitals in the telephone network. Although it is possible that some patients with aplastic anemia from the defined geographic areas had been referred to hospitals outside of these areas, this is also true for the other studies listed in Table 3. There were 47 cases excluded because of insufficient documentation or not fulfilling our study criteria; if the assumption is made that all of those excluded were actually cases of aplastic anemia, the incidence rate would only have increased from 2.2 to 2.8 per million per year.

We suggest that the major reason for the differences observed between the present study and those listed in Table 3 is differences in the stringency of diagnostic criteria among studies. Quantitative estimation of bone marrow cellularity is of paramount importance for the diagnosis of aplastic anemia, as against other blood dyscrasias resulting in pancytopenia. This is particularly relevant for the differential diagnosis between aplastic anemia and various myelodysplastic syndromes. An adequate bone marrow biopsy specimen is therefore regarded as mandatory for a definitive diagnosis of aplastic anemia.5,9,10 and is a requirement for inclusion in therapeutic trials.13,14 In this study, the results of the review by the HRC of the cases originally diagnosed as aplastic anemia, including an examination of blood films and bone marrow specimens, demonstrated that it is not sufficient to rely on the diagnosis as derived from hospital reports. The primary reason for the exclusion of potential cases was a bone marrow specimen of insufficient quantity or quality or an inadequate histopathologic evaluation. Only 5% of the cases were accepted without a bone marrow biopsy. Biopsy was not required in the studies of Wallerstein et al.,6 Böttiger and Westerholm,10 or Szkelo et al.; and in the study of Modan et al there was no bone marrow examination at all. Thus, it appears that pancytopenias other than aplastic anemia may have been included as true aplastic anemia in previous studies and that the incidence estimates were erroneously high.

<table>
<thead>
<tr>
<th>Author</th>
<th>Study Period</th>
<th>Area</th>
<th>Population (× 10⁶)</th>
<th>n</th>
<th>Incidence (per 10⁶/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modan et al.6</td>
<td>1961-1965</td>
<td>Israel</td>
<td>2.4</td>
<td>93</td>
<td>7.8</td>
</tr>
<tr>
<td>Wallerstein et al.6</td>
<td>1963-1964</td>
<td>California</td>
<td>17.5</td>
<td>60</td>
<td>4.8</td>
</tr>
<tr>
<td>Böttiger et al.10</td>
<td>1964-1968</td>
<td>Sweden</td>
<td>1.2</td>
<td>80</td>
<td>13</td>
</tr>
<tr>
<td>Szkelo et al.6</td>
<td>1970-1978</td>
<td>Baltimore</td>
<td>1.6*</td>
<td>62</td>
<td>4.1*</td>
</tr>
<tr>
<td>Böttiger et al.11</td>
<td>1973-1977</td>
<td>Sweden</td>
<td>1.3</td>
<td>157</td>
<td>25</td>
</tr>
<tr>
<td>Present series</td>
<td>1980-1984</td>
<td>Europe/Israel</td>
<td>20.5</td>
<td>166</td>
<td>2.2</td>
</tr>
</tbody>
</table>

*White population only.
Our criteria confined the definition of aplastic anemia to severe and moderate cases; borderline cases, of somewhat less concern due to good prognosis and low mortality, were not included. Such cases may have been included in other series, but this also cannot explain completely the differences shown in Table 3. Blood counts were described as “pancytopenia” or “severe pancytopenia” in the reports of Böttiger et al.10,11 and Wallerstein et al.8 respectively. Upper thresholds for hematocrit and neutrophil values as indicated by Szklo et al.4 and Modan et al.4 were similar to ours.

The most striking difference exists between our data and the incidence estimates of Böttiger et al.10,11 According to the detailed information in their more recent paper,11 they have included cases of bone marrow failure other than aplastic anemia such as hematopoietic neoplasia, myelofibrosis, or pancytopenia due to cytostatics. Exclusion of these cases would reduce the incidence rate from 25 to 12.3 cases per million per year. Because only 10% of the cases of “true” aplastic anemia are attributed to drugs other than cytostatics,11 differences in diagnostic criteria rather than different drug exposure rates may explain the discrepancy between Böttiger’s data and those reported here and from the Baltimore area.5

The possibility of misclassification of a substantial fraction of cases may have important implications for the validity of the data concerning the drug etiology of aplastic anemia (as well as the effects of other chemicals) that are derived from many previous reports. Most hematologists would agree that in practice, knowledge of exposure to potentially myelotoxic drugs can often influence the physician in making a diagnosis of aplastic anemia when a patient has pancytopenia. This could result in bias due to selective reporting of exposure-positive pancytopenia as drug-induced aplastic anemia irrespective of whether the diagnosis is correct.

In conclusion, we suggest that the lower incidence reported in our study is closer to the actual incidence than the estimates obtained from previous studies. Revisions of the commonly accepted assumptions of risk due to environmental factors may therefore be necessary.

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APPENDIX

Participants are as follows: Chairman, M. Levy, Jerusalem; Coordination and Data Analysis (Slone Epidemiology Unit), D. Slone,1 S. Shapiro, D.W. Kaufman, T. Anderson, L.J. Blair, P.T. Cook, J.J. Farrell, L.F. Gaetano, R. Matis, J.P. Kelly, C.L. Wells, Boston; Investigators, J.R. Laporte, Barcelona, Spain; H. Kewitz, Berlin; G. Retagi, S. Hollan, Budapest; M. Levy, Z. Stern, Jerusalem; G. Tognoni, Milan, Italy; V. Vlahov, Sofia, Bulgaria; B.E. Wiholm, Stockholm; H. Heimpel, W. Heit, Ulm, FRG; Data Collection, X. Carne, L. Ibanez, Barcelona, Spain; M. Klein, C. Scott, Berlin; E. Czink, A. Laszlo, Budapest; M. Beiski, F. Simon, Z. Toledano, Jerusalem; C. LaVecchia, P. Liati, M.G. Zurlo, Milan, Italy; N. Bacracheva, Sofia, Bulgaria; K. Jacobsen, M. Sandberg, Stockholm; I. Bayer, R. Kreuzer, Ulm, FRG; Hematology Review Committee, H. Heimpel, W. Heit, Ulm, FRG; G. Lambertenghi-Delliliers, Milan, Italy; A. Polliack, Jerusalem. The study is supervised by an Honorary International Advisory Board whose members are Sir Richard Doll, FRCP, FRSc, Oxford, Chairman; Per Knut M. Lunde, MD, Oslo; and Sven Moeschlin, MD Solothurn, Switzerland.

1Deceased.

REFERENCES


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