Population-Based Drug-Induced Agranulocytosis

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Background: Since the publication of a major international case-control study on the risk of agranulocytosis associated with the use of medicines in the 1980s, many new drugs have been introduced in therapeutics.

Methods: Seventeen units of hematology contribute to the case-control surveillance of agranulocytosis and aplastic anemia in Barcelona, Spain. After a follow-up of 78.73 million person-years, 177 community cases of agranulocytosis were compared with 586 sex-, age, and hospital-matched control subjects with regard to previous use of medicines.

Results: The annual incidence of community-acquired agranulocytosis was 3.46:1 million, and it increased with age. The fatality rate was 7.0%, and the mortality rate was 0.24:1 million. The drug most strongly associated with a risk of agranulocytosis was ticlopidine hydrochloride with an odds ratio (OR) of 103.23 (95% confidence interval [CI], 12.73-837.44), followed by calcium dobesilate (OR, 77.84 [95% CI, 4.50-1346.20]), antithyroid drugs (OR, 52.75 [95% CI, 5.82-478.03]), dipyrone (metamizole sodium and metamizole magnesium) (OR, 25.76 [95% CI, 8.39-179.12]), and spironolactone (OR, 19.97 [95% CI, 2.27-175.89]). Other drugs associated with a significant risk were pyrithyldione, cinepazide, aprindine hydrochloride, carbamazepine, sulfonamides, phenytoin and phenytoin sodium, β-lactam antibiotics, erythromycin stearate and erythromycin ethylsuccinate, and diclofenac sodium. Individual attributable incidences for all these drugs, which collectively accounted for 68.6% of cases, were less than 1:1 million per year.

Conclusions: Agranulocytosis is rare but serious. A few drugs account for two thirds of the cases. Our results also provide reassurance regarding the risk associated with a number of newly marketed drugs.

Arch Intern Med. 2005;165:869-874
With the aim of identifying all cases of agranulocytosis occurring in the study population, our center maintains regular contact with all hospitals in the area, through weekly or bi-weekly telephone calls to a designated contact person in the hematology laboratory. Potential cases were patients with a granulocyte count of less than 500/mm$^3$ or a total white blood cell count series of less than 3000/µL in 2 consecutive counts, with a hemoglobin level of at least 10 g/dL and a platelet count of at least 100 $\times$ 10$^9$/L. A bone marrow aspirate or a biopsy sample was generally required, but it was not mandatory if all other diagnostic criteria were met and if the neutrophil count was within the reference range within 30 days. Primary exclusion criteria were applied to patients receiving chemotherapy for cancer, radiation therapy, or immunosuppressive drugs; those with hypersplenism, lupus erythematosus, leukemia, lymphoma, megaloblastic anemia, or AIDS; asymptomatic cases discovered coincidently by complete blood cell counts performed for other reasons (eg, routine presurgical blood cell count); and those younger than 2 years (among whom a significant proportion of cases of neutropenia are of viral origin).

Secondary exclusion criteria were applied to patients who could not be interviewed during the first 28 days of hospital stay (to avoid memory bias); those with psychiatric conditions, blindness, or deafness (because the interview would not be reliable); and those living in a nursing home (because they rarely know the names of the drugs they take). Because of the difficulty of establishing acceptable criteria for the selection of adequate controls for in-hospital cases of agranulocytosis without incurring selection bias, in-hospital cases were excluded from the case-control analysis.

Initially, for each case of agranulocytosis or aplastic anemia, we selected no more than 4 controls matched by sex, age, and hospital in the 3 months after admission of the corresponding case, among those of a list of admission diagnoses judged independent of the reasons for use of most of the drugs, such as non–alcohol-related trauma, asymptomatic conditions needing surgical treatment, or acute infection.

The protocol was approved by the ethics committee of the coordinating hospital. After obtaining informed consent, cases and controls were interviewed by trained personnel, with a structured questionnaire, during their hospital stay. Detailed information was obtained on the use of drugs in the 6 months before admission, by means of an open question on the use of medicines, a list of frequent symptoms often prompting use of medicines, and a list of the top-selling drugs. Since 1998, this list has been shown to patients with color pictures of the boxes or flasks of each product. Cases and their corresponding controls were interviewed by the same monitor. Clinical and laboratory data were also recorded. On the basis of the examination of the clinical and laboratory information, a hematologist confirmed the diagnosis and established the index day, ie, the day when the first symptom attributable to agranulocytosis occurred; this review was performed blindly with respect to previous drug exposures. From 1980 to 1986, cases were blindly reviewed by an IAAAS international hematologic committee.

Odds ratios (ORs) were calculated after controlling for confounding by applying a multiple logistic regression model, including potential known confounders and drug terms. The terms finally included in the model were aspirin, dipyrone (metamizole [sodium and magnesium]), propyphenazon, acetaminophen, diclofenac sodium, indomethacin, other NSAIDs (nonsteroidal anti-inflammatory drugs), ticlopidine, calcium dobesilate, spironolactone, antithyroid drugs, phenytoin, carbamazepine, sulfonamides (including the combination of trimethoprim and sulfamethoxazole, and sulfasalazine), β-lactam antibiotics, erythromycin (stearate and ethylsuccinate), and a group of drugs with relatively low levels of consumption that are known to cause agranulocytosis (ie, aprindine [hydrochloride], cimapride, clopidogrel, clozapine, calcium carbimide [cyamamide], gold salts, mianserin [hydrochloride], and pyrityldione). The pharmacological terms included in the model were chosen because they were well-established potential causes of agranulocytosis, or because they showed a higher prevalence of use among the cases compared with the controls.

The primary analysis was performed with a conditional multiple logistic regression model. However, with the aim of increasing statistical power for estimation of the risk associated with drugs having a low prevalence of use, a second analysis was done with all the cases for which information on drug exposures was available, and all the controls of both agranulocytosis and aplastic anemia, with an unconditional model that included sex, age, and interviewer as additional terms. Drug exposures were considered in different ways. The main analysis refers to the week before the index day; this definition of exposure was decided after taking into account that for most of the cases of agranulocytosis, the time elapsed from injury of the bone marrow or of peripheral neutrophils to the appearance of the initial symptoms of infection is usually less than 7 days. The following 2 additional confirmatory analyses were performed: one, with the aim of exploring possible information bias (regarding each drug that was used at least 3 days in the preceding week), and the other, to evaluate protopathic bias (regarding each drug that was used between 9 and 3 days before the index day) (described in detail at http://www.icf.uab.es/farmavigila/protocols/agranulodrugs.htm).

For drugs showing ORs significantly higher than 1, population-attributable risks were calculated from the OR, with the formula AR=[Pc×(OR–1)]/OR, where AR is the attributable risk and Pc is the proportion of exposed cases. We calculated 95% confidence intervals (CIs) as described by Greenland. We calculated the overall population-attributable risk by combining all exposures to individual drugs that showed a significant association as a single term.

For estimation of incidence, the source population was older than 2 years. Incidence rates were calculated by age and sex, and the attributable incidence (number of cases per million and per year) was calculated by multiplying the attributable risk by the incidence of the disease. All patients were followed up to 4 weeks or to hospital discharge, and case-fatality rates were estimated for the 4 weeks after the diagnosis.

RESULTS

Up to June 30, 2001, total follow-up was 78.73 million person-years. Four hundred fifty-four potential cases were identified for hematologic review. Fifty-eight patients were excluded at this stage, leaving 396 confirmed cases of agranulocytosis (210 women [53%]). Details on the ascertainment of potential cases, and exclusions can be found at http://www.icf.uab.es/farmavigila/protocols/agranulodrugs.htm. Of the 396 confirmed cases, agranulocytosis developed in 123 during hospital admission, and 273 had been admitted to a hospital because of agranulocytosis (community cases). The annual incidence of the disease was quite stable during the 22-year follow-up (described in detail at http://www.icf.uab.es/farmavigila/protocols/agranulodrugs.htm).
The overall incidence was 5.02:1 million and per year (3.46 for community cases). Among the 396 cases, there were 36 deaths, and the case-fatality rate was thus 9.1% (for community cases, the case-fatality rate was 7.0% [19 deaths among 273 individuals, with a mortality rate of 0.24 per million]). Incidence increased with age, as 55% of community cases were 65 years or older (Figure). The case-fatality rate was 3.57% among those aged 25 to 44 years, 3.48% among those aged 45 to 64 years, and 10.86% among those older than 64 years.

Ninety-six community cases (35%) were excluded (described in detail at http://www.icf.uab.es/farmavigila/protocols/agranulodrugs.htm), thus leaving 177 cases for the case-control analysis.

Five hundred eighty-six controls were interviewed and included in the analysis. Three hundred seventy-nine had trauma, 170 had medical emergencies, and 47 had been admitted for elective surgery. The rates of use of analgesics, nonsteroidal anti-inflammatory drugs, antibiotics, and other drugs of interest did not differ across the various categories of controls (described in detail at http://www.icf.uab.es/farmavigila/protocols/agranulodrugs.htm). The overall incidence was 5.02:1 million inhabitants per year, and the case-fatality rate was 3.46:1 million, and the case-fatality rate was 7%. With such low incidence rates, any moderate increase in the risk of agranulocytosis associated with a particular drug translates into a low number of attributable cases. More than half the cases were 65 years or older. The largest relative increases in risk were seen with pyrithyldione, cinepazide, ticlopidine, calcium dobesilate, and antithyroid drugs. Even for these, however, the estimated attributable incidences were small, of less than 1:1 million. In our milieu, a few drugs—dipyrone, ß-lactam antibiotics, ticlopidine, antithyroid drugs, pyrithyldione, aprindine, cinepazide, sulfonamides, calcium dobesilate, dicoledon, spironolactone, and carbamazepine—accounted for nearly 70% of cases. Our results also provide reassurance about a wide range of drugs in common use.

Three drugs with poor evidence of clinical efficacy, ie, cinepazide, pyrithyldione, and calcium dobesilate, accounted for a significant proportion of cases. This led to regulatory action, with the first two withdrawn from the market and the indications of the last one restricted. Our data indicate that ticlopidine, consumption of which increased sharply in the 1990s, is associated with a high relative risk of agranulocytosis, and that a substantial proportion of cases are attributable to this drug. The risk of agranulocytosis associated with ticlopidine and clozapine had already been uncovered in clinical trials.

As in previous studies, we found a significant risk associated with the use of dipyrone. In a separate report, we described this association in detail. As in the IAAAS, we found a borderline risk associated with the use of dicoledon. We cannot exclude bias by indication.
related to diclofenac use, because the drug is often used as a short-course analgesic in Spain.

Spironolactone was also associated with an increase of risk. The IAAAS had not found an association with agranulocytosis, probably because its prevalence of use in the 1980s was much lower than it is now. However, it has been incriminated in several anecdotal reports. The practical implication of this finding is that spironolactone should be the first suspected cause in case of agranulocytosis in a patient with heart failure not receiving any other suspected drug.

Erythromycin had been taken by 4 cases and no controls; a risk associated with the whole class of macrolides was suggested in the IAAAS, but not in another case-control study with a limited number of patients. We believe that the risk associated with its use is very low, if it exists at all.

We found a low relative risk associated with β-lactam antibiotics. Neutropenia associated with short courses of oral β-lactam therapy at lower doses is extremely rare, and we cannot entirely exclude bias by indication in this association.

Our results are based on experience systematically gathered by a multicenter collaborative network for 22 years, during which a large case-control database on agranulocytosis and aplastic anemia has been assembled. The cases have been thoroughly ascertained, and annual incidence rates have been stable throughout the study period. We cannot exclude the possibility that a patient occasionally may have died of agranulocytosis without receiving medical care or without having had a white blood cell count. However, the study population is covered by a high-quality, universal, free health care service, and it is unlikely that patients with overwhelming infection were not admitted to a hospital and have 1 or more blood cell counts. We therefore believe that significant underascertainment is unlikely. Ninety-six (35%) of 273 eligible patients had to be excluded from the case-control study with a limited number of patients. We therefore believe that significant underascertainment is unlikely. Ninety-six (35%) of 273 eligible patients had to be excluded from the case-control study with a limited number of patients.

Abbreviations: CI, confidence interval; NSAIDs, nonsteroidal anti-inflammatory drugs; OR, odds ratio.

Table 1. Drug Exposures Within the Week Before the Index Day

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conditional Analysis</th>
<th>Unconditional Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases/Controls</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td></td>
<td>(n = 245/1530)</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>37/60</td>
<td>1.39 (0.67-2.88)</td>
</tr>
<tr>
<td>Dipyridamole (metamizole sodium and metamizole magnesium)</td>
<td>30/9</td>
<td>25.76 (8.39-79.12)</td>
</tr>
<tr>
<td>Propyphenazonone</td>
<td>7/13</td>
<td>2.30 (0.35-15.32)</td>
</tr>
<tr>
<td>Acetylaminophen</td>
<td>41/50</td>
<td>1.54 (0.88-3.52)</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>10/11</td>
<td>3.86 (1.00-15.00)</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>12/7</td>
<td>2.82 (0.66-12.12)</td>
</tr>
<tr>
<td>Other NSAIDs</td>
<td>9/17*</td>
<td>1.39 (0.41-4.77)</td>
</tr>
<tr>
<td>Ticlopidine hydrochloride</td>
<td>20/1</td>
<td>103.23 (12.73-837.44)</td>
</tr>
<tr>
<td>Calcium dobesilate</td>
<td>9/1</td>
<td>77.84 (4.50-1346.20)</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>6/4</td>
<td>19.97 (2.27-175.89)</td>
</tr>
<tr>
<td>Antithyroid drugs</td>
<td>13/1</td>
<td>52.75 (5.82-478.03)</td>
</tr>
<tr>
<td>Phenytoin and phenytoin sodium</td>
<td>2/1‡</td>
<td>5/6</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>5/1</td>
<td>10.96 (1.17-102.64)</td>
</tr>
<tr>
<td>Sulfonamides</td>
<td>11/5§</td>
<td>8.04 (2.09-30.99)</td>
</tr>
<tr>
<td>β-Lactam antibiotics</td>
<td>27/17¶</td>
<td>4.71 (1.74-12.77)</td>
</tr>
<tr>
<td>Erythromycin stearate and erythromycin ethylsuccinate</td>
<td>4/0‡§</td>
<td>5/5</td>
</tr>
<tr>
<td>Other</td>
<td>30/1**</td>
<td>97.25 (12.18-776.40)</td>
</tr>
</tbody>
</table>

Cases/Controls: 177/586
OR (95% CI): 1.66 (1.01-2.73)

Conditional Analysis

Unconditional Analysis

Abbreviations: CI, confidence interval; NSAIDs, nonsteroidal anti-inflammatory drugs; OR, odds ratio.

- Includes oximes in 1 case and 4 controls; ibuprofen (1 case and 4 controls); ketoprofen (2 controls); flurbiprofen (1 control); naproxen (2 cases); acyclovir (3 controls); tenoxicam (1 case); phenytoin (2 cases and 1 control); and meloxicam (1 control).
- Includes piroxicam (1 case and 4 controls); ibuprofen (1 case and 4 controls); ketoprofen (2 controls); naproxen (2 cases and 5 controls); acyclovir (1 case and 4 controls); tenoxicam (1 case); phenytoin (2 cases and 1 control); and meloxicam (1 control); and rofecoxib (1 control).
- Includes sulpiride (3 cases), ipecac (1 case), phenergan (4 cases), and metoclopramide (1 case).
- Includes sulfa (1 case), sulfadiazine (2 controls), sulfalene (sulfamethopyrazine) (2 controls), sulfamethoxazole (8 cases and 4 controls), and sulfadiazine (1 case).
- Includes erythromycin (4 cases), and metronidazole (2 cases).
- Includes amoxicillin (17 cases and 28 controls), ampicillin (5 cases and 4 controls), benzylpenicillin (6 cases and 2 controls), and phenoxymethylpenicillin (1 case).
- Includes aprindine hydrochloride (6 cases), cinoxapine (6 cases), clopidogrel (1 case), sodium carboxylate (1 case), and erethromycin (4 cases), gold salts (1 case), mianserin hydrochloride (1 case), phentermin (2 cases and 1 control), and pyrithyldione (8 cases).

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control analysis for various reasons that do not seem to be related to exposure to any particular drug, except death before the interview (15 patients). If the risk of a particular drug was associated with an especially high death rate, this would result in risk underestimation. Unfortunately, our methods do not allow identification and estimation of the risk associated with cytostatics, immunosuppressants, and drugs of hospital use. Similarly, our exclusion criteria, which were set up to avoid confounding, preclude risk estimation in special groups of patients, such as those with active cancer, various hematological conditions, AIDS, or lupus erythematosus, and risk associated with the drugs specifically used in their treatment.

To avoid exposure misclassification, 3 approaches were used. First, accurate information on the timing of drug exposure and of the onset of the first symptoms was carefully recorded with the structured questionnaire, and the interviewer prepared a narrative with details on the events leading to hospital admission. Second, this was carefully reviewed by a hematologist who was blinded with respect to drug use, to establish the index day, ie, the day of the first symptom of the disease. Third, to exclude that incorrectly reported or unreported symptoms could have induced use of a drug (eg, an analgesic or an antibiotic) that might be erroneously classified as having been taken within the etiologic period, an additional analysis was made by sliding the 7-day etiologic time span window 2 days before the index day established by the blind review. The results of this analysis confirmed the overall findings.

Controls were patients with conditions judged to be unrelated to prior drug use. It was reassuring that the prevalence of the use of the drugs of interest were those expected on the basis of the general drug consumption patterns in the study area (data not shown), and that the distributions of use of drugs were generally similar across the major diagnostic categories of the controls.

Three approaches were used to minimize information bias due to differential recall between cases and controls. First, the patients were interviewed within 28 days of admission. Second, exposure information was obtained directly from patients with a structured questionnaire, including several carefully worded questions. Third, to avoid information bias, an analysis was performed where only use for 3 or more days during the preceding week (which is presumably less subject to differential recall) was considered. This analysis confirmed the overall findings.

As our results show, agranulocytosis is rare, and two thirds of the cases are caused by a limited number of drugs. Monitoring blood cell counts in patients undergoing long-term treatment with certain drugs (eg, ticlopidine, antithyroid drugs, antiepileptic drugs) during the first few months of treatment may be appropriate to prevent intercurrent infections, because the risk is partly dose related in susceptible patients. However, agranulocytosis induced by other drugs (eg, dipyrone) is often an acute and unpredictable immunological condition, and in this situation surveillance of blood cell counts may be unjustified.

### CONCLUSIONS

Agranulocytosis is rare and serious. Its epidemiological surveillance by an independent multicenter network allowed identification of previously unknown causes of the disease and evaluation of the risks associated with various known causes. Although almost all drugs have been implicated at least once in the etiology, in our setting, more than two thirds of the cases were attributable to a few products.

Accepted for Publication: October 15, 2004.
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Funding/Support: The network for the surveillance of blood dyscrasias was initially part of the IAAAS, which was carried out from 1980 to 1986 and was funded by Hoechst AG, Frankfurt, Germany. From 1987 to 2000 the network did not receive any external financial support, except that of personnel from the public health provider organization Institut Català de la Salut, Barcelona, Spain, and partial funding by the Agencia Española del Medicamento, Madrid, Spain. Since January 2001, our institute has received partial funding from the Agencia Española del Medicamento, Madrid, and Aventis and Boehringer Ingelheim, Ridgefield, Conn.

Disclaimer: Data analysis and interpretation and the preparation of the present report have been independent of all sponsors.

Acknowledgment: We thank the patients who participated in the study and the following hematologists who collaborated in case reporting: Eugènia Abella, PhD, Esther Alonso, MD, Ramon Ayats, PhD, Carles Besses, PhD, Alba Bosch, MD, Salut Brunet, PhD, Núria Crespo, MD, Isabel De Diego, MD, Alicia Domingo, MD, Javier Estella, MD, Marta García, MD, José Angel Hernández, Antoni Julià, PhD, Ramón López, MD, Pedro Marín, PhD, Fuensanta Millà, MD, Jesús Moll, MD, Tomás Navarro, PhD, Benet Nomdedéu, PhD, Teresa Olivé, MD, Juan José Fuensanta Millà, MD, Jesús Moll, MD, Tomás Navarro, PhD, Benet Nomdedéu, PhD, Teresa Olivé, MD, Juan José Fuensanta Millà, PhD (Service of Hematology, Hospital Vall d’Hebron), reviewed and confirmed the cases of agranulocytosis.

REFERENCES