AGRANULOCYTOSIS IN BANGKOK, THAILAND: A PREDOMINANTLY DRUG-INDUCED DISEASE WITH AN UNUSUALLY LOW INCIDENCE

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Abstract. Agranulocytosis, a syndrome characterized by a marked reduction in circulating granulocytes, is strongly associated with medical drug use in Europe and the United States. Unregulated use of common pharmaceutical agents in developing countries has been suspected of causing large numbers of cases of agranulocytosis and deaths, especially among children. To elucidate the incidence and etiology of agranulocytosis in Thailand, a population-based case-control study of symptomatic agranulocytosis that resulted in hospital admission was conducted in Bangkok from 1990 to 1994. An attempt was also made to study the disease in Khonkaen (in northeastern Thailand) and Songkla (in southern Thailand), but there were insufficient cases in the latter regions, and the analysis was confined to subjects from Bangkok. In that region, the overall incidence of agranulocytosis was 0.8 per million per year; there were no deaths. As expected, the incidence was higher in females (0.9 per million), and it increased with age (4.3 per million beyond age 60). Among 25 cases and 529 controls the relative risk estimate for a combined category of all suspect drugs was 9.2 (95% confidence interval = 3.9–21), and the proportion of cases that could be attributed to drug use was 68%. For individual drugs and drug classes the data were sparse; within these limitations, the strongest association appeared to be with antithyroid drugs. One case and three controls were exposed to dipyrone, a drug known to cause agranulocytosis; with such scanty data the risk could not be evaluated. Exposure to pesticides or solvents was not associated with an increased risk. This is the first formal epidemiologic study of agranulocytosis in a developing country. As in the West, most cases are attributable to medical drug use. However, the incidence of agranulocytosis in Bangkok, and apparently, in Thailand as a whole, is unusually low, and the disease does not pose a public health risk.

Agranulocytosis is a hematologic syndrome of acute onset in which the numbers of circulating neutrophils decrease, often abruptly and to undetectable levels, leading to a markedly increased susceptibility to bacterial infection, serious local infection or sepsis, and, even in the era of antibiotics, a significant risk of mortality.1,2 In the early years of the twentieth century American investigators recognized a strong association between agranulocytosis and medical drug use.1,3 Demographic features of agranulocytosis patients have supported the relationship with drug use: patients were often of higher economic class, Caucasian, and women, categories that in the 1920s included individuals who were more likely to use newer pharmaceutical products.1,3 Decades of case reporting led to the compilation of “black lists” of incriminated drugs. Early on dipyrone, a pyrazolone with antipyretic and analgesic properties that was commonly used in the United States, came under suspicion,4,5 and in 1977 it was banned because it was thought to cause agranulocytosis too commonly to be safe.6 However, in other parts of the world, the drug continues to be used.

Drug use was a specific etiology that was formally assessed in a large, multinational study performed in Europe and Israel in the 1980s7,8 to determine which drugs were associated with agranulocytosis, and for those drugs that were, the incidence rates attributable to exposure. Aplastic anemia was also studied; in contrast to agranulocytosis, it is a disease characterized by primary failure of hemopoiesis in the bone marrow, and the onset is usually insidious, evolving over a period of several months. However, drugs also play a role in its etiology, and it was logistically feasible to study both diseases at the same time. The study, termed the International Agranulocytosis and Anemia Study (IAAAS), had a prospective, population-based, case-control design, and resulted in the quantitation of relative and absolute risks of the two bone marrow diseases attributable to the use of drugs.

For agranulocytosis, the majority of cases appeared to be secondary to drug use, and the IAAAS documented associations for dipyrone,7 sulfonamides,9 nonsteroidal anti-inflammatory agents,7 thyrostatics,10 cardiovascular drugs,11 and a miscellaneous array of other agents.8 In all, it was estimated that 65% of the occurrences of agranulocytosis could be attributed to drugs.3

The IAAAS also documented the incidence of aplastic anemia in Europe and Israel, as well as the relationship of
the disease to drug exposure. In Asia, it was suspected that aplastic anemia was more common than elsewhere in the world, and for that reason a new study of that condition was undertaken in Thailand based on the methodology developed in the IAAAS. The specific aims were to determine the incidence of aplastic anemia, and to explore possible associations with drugs, pesticides, industrial exposures, and viruses. Agranulocytosis was not initially intended to be a subject of investigation in Thailand. However, some clinical investigators published concerns that dipyrone, a drug still commonly used in the developing world, was dangerous to patients, especially children, and might significantly affect mortality because of the known high risk of agranulocytosis as a complication of drug therapy. Therefore, shortly after commencing the epidemiologic study of aplastic anemia in Thailand we expanded its scope to include an investigation of the incidence and drug etiology of agranulocytosis; as in the IAAAS, the methods had been designed for the study of both dyscrasias, and minimal changes to the protocol were needed. In addition to drugs, the roles of pesticides and industrial solvents were investigated.

METHODS

The protocol for this study was approved in advance by the Institutional Review Boards of Boston University School of Medicine, Mahidol University, Khonkaen University, and Prince of Songkla University. Consent forms were reviewed and approved by these boards, and informed consent was obtained from all participants. The study was conducted from 1990 to 1994. It initially included all cases of agranulocytosis identified in Bangkok and its immediately surrounding suburbs (a denominator population of 9.51 million), Khonkaen in northeastern Thailand (7.40 million), and Songkla in southern Thailand (4.41 million). The cases were identified by means of systematic procedures used to actively seek and identify all patients with symptomatic agranulocytosis that resulted in hospital admission. However, only four cases were identified in Khonkaen, and two in Songkla. Such small numbers were insufficient to enable the estimation of incidence rates or to carry out case-control comparisons, and further consideration was therefore restricted to cases and controls identified in Bangkok.

Potential cases of agranulocytosis were patients with a granulocyte count $\leq 0.5 \times 10^9/L$, or a total white blood cell count of $\leq 3.0 \times 10^9/L$. To ascertain potential cases, regular contact was maintained with all hospitals in each study region at intervals of no more than two weeks. The contacts included regular visits to each institution and telephone calls to designated individuals to inquire about possible cases. In the larger hospitals, the results of all bone marrow aspirations were reviewed to ensure that no cases had escaped detection.

Controls were selected from among patients admitted for conditions judged to be unrelated to medication use, including trauma, acute infections (e.g., pneumonia), acute abdominal emergencies (e.g., appendicitis), and other conditions, such as cataract; they were matched to the potential cases in a ratio of 4:1 for age ($\leq 2$, $2-5$, $6-14$, $15-24$, $25-44$, $45-64$, and $\geq 65$ years), sex, and time of interview (within three months). The same exclusions that applied to the cases were also used for controls.

Specially trained nurses or physicians administered a standard questionnaire. Agranulocytosis is a disease of acute onset characterized by sore throat, chills, and headache in which if death due to overwhelming infection does not occur, the granulocyte level returns to normal within 28 days. The decrease in the granulocyte count occurs suddenly, either while the causal drug or its metabolites are still present in the body, or soon thereafter. To cover all potentially relevant drug use, detailed information on exposures up to 28 days before admission was recorded.

To aid recall, patients were questioned about a list of 33 indications (such as pain, headache, infection, anxiety, insomnia, heart failure, high blood pressure, chest pain, hormonal disorders, and other indications for drug therapy). To further prompt recall, especially among controls, the interviewers then mentioned a list of trade names of products available in Thailand that included commonly used drugs, as well as drugs specifically suspected as possible causes of agranulocytosis. The list of suspect drugs was originally compiled by a panel of hematologists in the IAAAS, based on a comprehensive review of the hematologic literature; the list was also supplemented with those drugs that were found to be significantly associated with agranulocytosis in the IAAAS.

Information was also obtained on major illnesses, history of allergy, years of education, personal and family income, and histories of exposure to radiation, industrial chemicals, and pesticides. Possible viral causes of agranulocytosis were not investigated in this study.

The clinical course of the disease, all hematologic data, and other relevant clinical laboratory information was recorded for all potential cases; blood smears, bone marrow aspirates, and biopsy sections were obtained whenever available. When data collection was completed, the information on the potential cases was reviewed by two hematologists (SI and AP), without knowledge of drug exposure status, to confirm the diagnosis.

Final included cases had a granulocyte count $\leq 0.5 \times 10^9/L$, with a hemoglobin level $\geq 10$ g/dL, and a platelet count $\geq 100 \times 10^9/L$. Patients undergoing chemotherapy, radiotherapy, or immunosuppressive therapy and those with chronic neutropenia or systemic diseases that can be associated with neutropenia (e.g., systemic lupus erythematosus, leukemia, malignant lymphoma) were excluded. If a bone marrow aspirate or biopsy specimen was not available, the diagnosis was accepted only if the neutrophil count returned to normal within 30 days.

All controls were included in the analysis, regardless of whether they were initially matched to potential cases of agranulocytosis or aplastic anemia. This was done because the selection procedures, interview, and other aspects of data collection were identical for all controls, and it was desirable to maximize the informativeness of the data.

Data analysis. An index day was defined for all subjects with adequate information on the timing of clinical onset. For the agranulocytosis cases, the index day was the first day on which sore throat, fever, or chills occurred, as reported by the patient. For controls with trauma it was the day of the accident; for those with acute conditions it was...
the day on which symptoms commenced; for those with elective admissions for treatment of long-standing conditions (e.g., cataract) it was the day of admission.

The effects of drug use in the seven days before the index day were assessed because drug-induced agranulocytosis is characterized by a short induction period. Use that took place on or after the index day, or more than a week previously, was ignored. Since detailed information on drug exposures was only recorded for the 28 days prior to admission, the analysis was confined to those cases and controls whose index day was within 21 days of admission, so that a full seven-day period of drug exposure information would be available for all subjects. For all comparisons the reference category consisted of cases and controls not exposed to the drug under consideration in the week before the index day. Individual drugs, and when appropriate, classes of drugs (e.g., beta-blocking agents, benzodiazepines) were evaluated.

Table 1: Annual incidence of agranulocytosis in Bangkok, August 1990–December 1994

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No. of cases</th>
<th>Population size (thousands)</th>
<th>Annual rate per million</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–14</td>
<td>0</td>
<td>2,302</td>
<td>0.0</td>
</tr>
<tr>
<td>15–24</td>
<td>6</td>
<td>2,086</td>
<td>0.7</td>
</tr>
<tr>
<td>25–39</td>
<td>5</td>
<td>2,816</td>
<td>0.4</td>
</tr>
<tr>
<td>40–59</td>
<td>9</td>
<td>1,700</td>
<td>1.2</td>
</tr>
<tr>
<td>≥ 60</td>
<td>9</td>
<td>604</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Table 2: Drug use in the week before the index day among 25 cases of agranulocytosis and 529 controls

<table>
<thead>
<tr>
<th>Drug*</th>
<th>Cases</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol</td>
<td>8 (32)</td>
<td>38 (16)</td>
<td>0.13</td>
</tr>
<tr>
<td>Salicylates</td>
<td>4 (16)</td>
<td>48 (9)</td>
<td>0.43</td>
</tr>
<tr>
<td>Antithyroid agents</td>
<td>4 (16)</td>
<td>36 (7)</td>
<td>0.25</td>
</tr>
<tr>
<td>Phenylpropanolamine</td>
<td>2 (8)</td>
<td>22 (4)</td>
<td>–</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>3 (12)</td>
<td>5 (1)</td>
<td>–</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>2 (8)</td>
<td>5 (1)</td>
<td>–</td>
</tr>
<tr>
<td>H2 antagonists</td>
<td>2 (8)</td>
<td>3 (0.6)</td>
<td>–</td>
</tr>
<tr>
<td>Sulfonamides</td>
<td>2 (8)</td>
<td>3 (0.6)</td>
<td>–</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>4 (16)</td>
<td>2 (0.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>2 (8)</td>
<td>2 (0.4)</td>
<td>–</td>
</tr>
<tr>
<td>Nitrates</td>
<td>2 (8)</td>
<td>2 (0.4)</td>
<td>–</td>
</tr>
<tr>
<td>Antithyroid drugs</td>
<td>6 (24)</td>
<td>0 (0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>3 (12)</td>
<td>0 (0)</td>
<td>–</td>
</tr>
<tr>
<td>Any suspect drug</td>
<td>19 (76)</td>
<td>134 (25)</td>
<td>–</td>
</tr>
</tbody>
</table>

* Chlorpheniramine (2 cases, 28 controls), hydroxyzine (1, 0), dimenhydrinate (1, 0), clemizole (0, 2), cinnarizin (0, 1), doxylamine (0, 1), brompheniramine and unknown antihistaminic (0, 3), diazepam (2 cases, 5 controls), clorazepate (1, 0). NSAIDs = nonsteroidal anti-inflammatories. Diclofenac (1 case, 2 controls), indobufen (1, 0), piroxicam (0, 2), gafenicine (0, 1). Cimetidine (1 case, 2 controls), ranitidine (1, 1). Trimethoprim/sulfamethoxazole (2 cases, 2 controls), sulfanilamide (0, 1). Propafenone (4 cases, 1 control), bisoprolol (0, 1). Diltiazem (1 case, 1 control), nifedipine (1, 0), felodipine (0, 1). Atenolol (2 cases, 1 control), pentaerythritol (0, 1). Propylthiouracil (4 cases, 0 controls), methimazole (2, 0).

1 Age- and sex-adjusted relative risk = 9.2 (95% confidence interval, 3.9–21).

RESULTS

Incidence. The overall incidence of agranulocytosis in Bangkok was 0.7 per million per year (Table 1). There were no cases less than 15 years of age (denominator population = 2.3 million); beyond that age, the annual incidence increased from 0.7 per million at ages 15–24 to 3.4 per million at an age ≥ 60. Among females and males the overall annual incidence rates were 0.8 and 0.6 per million, respectively (ratio = 1.33).

Case-control comparisons. Distribution of drug use in Bangkok in the week before the index day among the 25 cases and 529 controls are given in Table 2. The table includes all drugs taken by at least two cases, and the data are ordered according to descending frequency of exposure among the controls. All drugs listed in the table were taken more commonly by the cases, but significant differences were observed only for antithyroid drugs and beta-blockers.

Paracetamol (acetaminophen) was the most commonly used drug, with exposure rates among the cases and controls of 32% and 16%, respectively. Six of the eight exposed cases had used other drugs known to increase the risk of agranulocytosis, and some used more than one; these included chlorpheniramine (2 cases), tetracycline (1), dipyridamole (1), propylthiouracil (1), diflunisal (1), diclofenac (1), and phenytoin (1). Among four cases exposed to salicylates, all took one or more other suspected drugs, including indobufen (1 case), paracetamol (1), diclofenac (1), propranolol (1), nifedipine (1), phenytoin (1), and furosemide (1). The four antithyroid exposures among the cases comprised a heterogeneous group of agents (chlorpheniramine, hydroxyzine, and dimenhydrinate); concomitant exposures to other impli-
cated drugs included paracetamol (3 cases), tetracycline (1), phenytion (1), trimethoprim/sulfamethoxazole (1), and ranitidine (1).

A strong association was observed for antithyroid drugs \( (P < 0.0001) \); however, four of the six exposed cases also used other suspect drugs, including propranolol (3 cases), diazepam (2), erythromycin (1), digoxin (1), and paracetamol (1). All of the four cases exposed to beta-blockers, the other significantly associated drug group \( (P < 0.0001) \), also took other suspect drugs, including propylthiouracil (2 cases), diazepam (2), aspirin (1), methimazole (1), erythromycin (1), and digoxin (1). A combined category of all suspect drugs was also evaluated. As explained earlier, the list included all drugs generally accepted by hematologists as causes of agranulocytosis. In addition to the drugs shown individually in Table 2, the following suspect drugs were each taken by one case: dipyrone (there were also three exposed controls), tetracycline (2 controls), erythromycin (no controls), digoxin (one control), furosemide (two controls), and metoclopramide (one control). The overall prevalence of exposure to suspect drugs was 76% among the cases and 25% among the controls, giving an age- and sex-adjusted relative risk estimate of 9.2 (95% confidence interval = 3.9–21). For all suspect drugs taken together, the estimated etiologic fraction \( (P = 0.0001) \), (the proportion of cases attributable to exposure) was 68%.

Chemicals other than drugs were also evaluated. Fourteen cases (56%) and 334 controls (63%) were exposed to pesticides in the month before the index day. No cases and 24 controls (5%) had been exposed to organic solvents.

**DISCUSSION**

The present study indicates that the incidence of agranulocytosis in the ambulatory population in Bangkok is exceedingly low, at 0.7 per million per year. That rate can be contrasted with an incidence of 3.4 per million per year (range = 1.5–5.5) in the eight regions that participated in the IAAAS. As has been reported in numerous studies, the disease occurs more commonly in women, and the incidence increases with age: below the age of 15 years there were no cases, whereas at ages ≥ 60 years the incidence was 3.4 per million per year.

The unusually low annual incidence of agranulocytosis of 0.7 per million can be contrasted with the relatively high rate for aplastic anemia in Bangkok of 3.7 per million derived from the parallel study. In the IAAAS, an inverse relationship between the incidence of agranulocytosis and aplastic anemia was also observed among the eight regions that participated. The present findings fit this pattern.

The death rate also appears to have been low: there were no deaths observed among the 29 incident cases, whereas the expected number based on the 10% fatality observed in the IAAAS was three. In Bangkok, hospital care is accessible to the entire population, regardless of socioeconomic status. Thus, it is unlikely that many deaths would have been missed: patients with conditions such as septicemia or other overwhelming infections would usually have been hospitalized before they died. Occasionally, however, in the presence of long-standing diseases such as congestive heart failure, chronic obstructive lung disease, or chronic renal failure, death may mistakenly have been attributed to the underlying disease when the proximate cause may in fact have been agranulocytosis.

It is also unlikely that the low incidence of agranulocytosis in Bangkok can be explained to any major degree by failure to ascertain surviving cases, since the symptoms tend to be severe, and to result in hospitalization. White blood cell counts were routine admission procedures in all of the institutions covered, and an intensive net was cast in this study to capture all cases of neutropenia. However, we cannot exclude the possibility that there may have been some cases that were so mild that they recovered without hospitalization. If so, the present study still documents a lower incidence than in the IAAAS since the methods of case ascertainment were identical.

There were sufficient data in the present study only to determine the incidence of agranulocytosis in Bangkok. However, the incidence rates in Songkla and Khonkaen, although unmeasurable, were clearly low, suggesting that it is reasonable to generalize the findings to Thailand as a whole.

In all, over a 4.5-year period covering more than 40 million person-years of experience in Bangkok, only 29 cases of agranulocytosis were observed, none of them less than 15 years of age. Thus, it is clear that agranulocytosis does not constitute a major public health problem in Thailand. Nonetheless, about 68% of the cases that did occur could be attributed to drugs, a proportion that is strikingly similar to the overall etiologic fraction of 65% estimated for drug use in the IAAAS. There did not appear to be associations with exposure to household or other pesticides, which are very commonly used in Thailand. With limited data, there was no evidence to implicate solvents.

The explanation for a lower incidence of agranulocytosis in Thailand in the face of a similar high level of drug attributability as in other regions is not obvious, especially since the use of potentially causal drugs is also similar (25% of the controls in Thailand versus 27% in the IAAAS countries). Another possibility is that there may be differences in genetic or other cofactors, but the present study provides no direct information to elucidate that question.

Despite the high overall etiologic fraction, when it came to the evaluation of individual drugs or drug categories, the present study had very limited statistical power because of the paucity of cases. In addition, there was confounding by the concomitant use of multiple known causal drugs—so much so that full control for their confounding effects was not feasible. With that caveat, the most powerful association, as in other studies, was with antithyroid drugs, and indeed this was the only specific drug class, other than paracetamol, to which more than four cases had been exposed. The other significant association, with beta-blockers, was consistent with the 2.5-fold increase in risk reported for propranolol from the IAAAS, but all the exposed cases took other suspect drugs. Some other expected associations with drugs such as sulphonamides, nonsteroidal anti-inflammatory drugs, digoxin and furosemide also appeared to be present, but because of limited numbers these were not subjected to statistical testing.

One drug, dipyrone, is of particular interest because it appears to be strongly associated with agranulocytosis in certain regions of the world, but for reasons that are not
understood, much less so in other regions: in the IAAAS the relative risk associated with its use in Barcelona, Berlin, Ulm, and Milan was increased more than 15-fold; in contrast, in Israel, Budapest, and Sofia it was hardly increased.\(^7\) However, even in the regions with a high relative risk, the excess risk attributable to exposure was very low, less than one case per million users in a week. In the present study, only one case and three controls were exposed to dipyrone, and it was not possible to evaluate this drug. Thus, a substantial increase in the relative risk could have escaped detection. Whatever the risk may be, it is clear that very few cases are being caused by the drug, contrary to a recent suggestion that a public health problem exists.\(^8\)

We conclude that in Thailand, as in other parts of the world, the risks posed by agranulocytosis, both for the public health overall and for individuals exposed to various drugs, are exceedingly small.

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