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# Incidence and risk factors of aplastic anemia in Latin American countries: the LATIN case-control study

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## ABSTRACT

### Background
Associations between aplastic anemia and numerous drugs, pesticides and chemicals have been reported. However, at least 50% of the etiology of aplastic anemia remains unexplained.

### Design and Methods
This was a case-control, multicenter, multinational study, designed to identify risk factors for agranulocytosis and aplastic anemia. The cases were patients with diagnosis of aplastic anemia confirmed through biopsy or bone marrow aspiration, selected through an active search of clinical laboratories, hematology clinics and medical records. The controls did not have either aplastic anemia or chronic diseases. A total of 224 patients with aplastic anemia were included in the study, each case was paired with four controls, according to sex, age group, and hospital where the case was first seen. Information was collected on demographic data, medical history, laboratory tests, medications, and other potential risk factors prior to diagnosis.

### Results
The incidence of aplastic anemia was 1.6 cases per million per year. Higher rates of benzene exposure (≥30 exposures per year) were associated with a greater risk of aplastic anemia (odds ratio, OR: 4.2; 95% confidence interval, CI: 1.82-9.82). Individuals exposed to chloramphenicol in the previous year had an adjusted OR for aplastic anemia of 8.7 (CI: 0.87-87.93) and those exposed to azithromycin had an adjusted OR of 11.02 (CI 1.14-108.02).

### Conclusions
The incidence of aplastic anemia in Latin America countries is low. Although the research study centers had a high coverage of health services, the underreporting of cases of aplastic anemia in selected regions can be discussed. Frequent exposure to benzene-based products increases the risk for aplastic anemia. Few associations with specific drugs were found, and it is likely that some of these were due to chance alone.

Key words: aplastic anemia, incidence, risk factors, benzene.

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## Introduction

Aplastic anemia (AA) is a hematologic condition characterized by bone marrow hypoplasia or aplasia resulting in pancytopenia. It is a severe disease and its etiology has been attributed to medications,[1,2] chemicals,[1-3] and environmental factors.[4] Although bone marrow transplants have increased the survival rate of patients with AA, most people do not have access to this therapy, and fatality rates of the disease remain high.[5]

Several studies published to date disagree as to the etiology of AA, which may be partially explained by their different methodologies.[2] Well-founded studies with good statistical power, assessing the association between AA and drugs and other risk factors are scarce.[1] A recent review of the epidemiology of aplastic anemia shows that most cases of aplastic anemia appear to be secondary to the immunological destruction of the hematopoietic cells. The risk of development of autoimmune diseases has been linked to host genetics, and a few risk factors have been identified that affect the immune response and the susceptibility of the hematopoietic target cell.[6]

Methodologically well-founded studies have found an incidence of AA ranging from 1.4 to 14 cases per million people,[7-9] with higher rates in Asian countries than in Western ones.[9,10] A study conducted in Southern Brazil, from 1999 to 2000, reported an incidence of 2.4 cases per million per year.[1] This wide variation in incidence among regions is generally thought to be due to environmental, rather than genetic factors.[7,11-13]

Based on data from several publications indicating that environmental factors play a major role in the development of AA[2], the fact that the risk factors have not yet been well-described for our context, and because treatment is not widely available in developing countries, this study has been carried out since 2002,[14] with the purpose of providing more information for prevention of the disease. The LATIN study is an international case-control study designed to identify risk factors for agranulocytosis and AA, including drugs, other diseases, and environmental factors, using a methodological approach similar to that used in previous studies.[2,3] Its secondary objective was to estimate the incidence rates of both agranulocytosis and AA in some Latin American countries.[14] This report focuses on the risk factors and incidence rate of AA.

## Design and Methods

### Design and settings

The LATIN study[14] is an international, multi-center, case-control study. Due to the vast size of Brazil (covering an area of more than 8.5 million km$^2$ and with a population in 2007 of nearly 190 million)[15,16] and its high regional population diversity, it is not possible to assess the incidence of AA at a national level. Therefore, this study included seven sites in representative areas of six Brazilian regions, plus two additional study sites, one in Argentina (Buenos Aires) and one in Mexico (Monterrey).

In the pilot phase of the LATIN study, an active search for AA cases was carried out in the Brazilian states of Paraná, Minas Gerais, Goiás, Pernambuco and Amazonas, and in the city of Ribeirão Preto (state of São Paulo) and adjacent cities. The geographical area covered was extremely large. Following the pilot phase (after April 2003), the area covered by each study center was then restricted, including only regions with better medical systems, to avoid underreporting of cases. Therefore, instead of covering whole states, a small region formed by the cities adjacent to each study center was defined as their catchment area. The only exception was the center of Curitiba, for which the catchment area continued to be the whole state of Paraná. This reduced the study area in Brazil from 2.25 million km$^2$ to 295 thousand km$^2$. (Figure 1). Buenos Aires (Argentina) and Monterrey (Mexico) joined the study after the pilot phase.

For all the sites, an active search of AA patients was carried out on a weekly basis, among hematology clinics in predefined areas. Records of death due to hematologic diseases were also obtained from government agencies on an annual basis. An informed consent was obtained from all the patients and controls. The local and national research ethics committees approved this study.

### Participants

#### Cases

All the patients with acquired AA included in the study had been living in the area covered by the study site for more than three months, and had undergone peripheral blood testing and bone marrow study. All the patients were submitted to peripheral blood count, bone marrow aspiration, and/or bone marrow biopsy. All the patients under two years of age were submitted to a specific test to exclude Fanconi anemia. The eligible patients were those who met at least two of the three


**Figure 1.** Location of Brazilian study sites.

peripheral blood count criteria below, together with a compatible bone marrow study:[17]

1. White cells < $3{,}5\times10^9$/L;
2. Platelets < $50\times10^9$/L;
3. Hemoglobin < 10.0 g/dL or hematocrit < 30%.

The bone marrow biopsy and/or myelogram had to show hypocellularity, but no fibrosis or leukemic, lymphomatous or carcinomatous infiltration. Cases with hypocellular myelodysplasia were also ruled out.

Cases and controls were also excluded from the study if they had other severe hematologic diseases (neural tube defects, neoplasias, megaloblastic anemia) or systemic diseases usually associated with neutropenia or pancytopenia (such as lupus, HIV infection, and hypersplenism) or if they had previously undergone any organ transplantation, chemotherapy, radiotherapy or immunosuppressive therapy. Felty's syndrome, Kostmann's syndrome, Shwachman-Diamond syndrome, neutropenias of the autoimmune, isoimmune, chronic hypoplasic or cyclic types, reticular dysgenesis (dyskeratosis congenita) and dyskeratoses were also conditions for exclusion.

To exclude alternative diagnoses, the final acceptance of cases for inclusion in the study required a characteristic hypocellular bone marrow biopsy (marrow cellularity <30%) without gross marrow fibrosis and absence of infiltration by leukemic, lymphomatous, or cancer cells.

Patients who had received chemotherapy, immunotherapy, or radiotherapy were also excluded.

All cases of AA were evaluated by an Independent Event Validation Committee (IEVC), consisting of expert hematologists, who had access to the laboratory results, bone marrow tests (biopsy and myelogram) and medical history summaries, but they were not given access to exposure-related information. Only cases that had been IEVC validated were included in the study.

### *Controls*

For each case of AA, four controls, individually matched by sex, age group and hospital where the case was first seen, were enrolled. The controls consisted of patients hospitalized for conditions not related to AA, the most common being respiratory infection (usually pneumonia) (23.2%), acute gastrointestinal infection (10.8%), genitourinary disease (7.7%), trauma (6.2%), and other acute infections (19%), such as conjunctivitis, cellulitis, abscess and dengue.

Cases or controls were excluded when the patient presented other hemopathies affecting the bone marrow, such as myelodysplasia, megaloblastic anemia; lymphoproliferative or myeloproliferative diseases; systemic diseases causing pancytopenia, such as systemic erythematous lupus; neoplasias with bone marrow invasion; HIV infection, or a past history of transplantation. Children aged under two years, those with congenital diseases, and those who developed AA during chemotherapy, radiotherapy or immunosuppressive therapy were also excluded. Patients who were unable to be interviewed, and who did not have a relative or guardian who could answer the questions on their behalf, were included in the incidence rate estimates, but not the case-control study. For patients under 12 years, the parents or guardians were interviewed.

The information needed to characterize the disease, aplastic anemia, or the control illness, was collected using standardized forms, which were the same for the cases and the controls. However, most of the information was collected directly from the patients' medical records, or from the patients' doctor.

### *Exposures*

Information on the following exposures was collected, and their relationship with aplastic anemia assessed in statistical analyses: 1) previous diseases: rheumatoid arthritis and viral hepatitis A, B or C; 2) occupation: main occupation; 3) contact with animals: cattle, horses, pigs and birds; 4) exposure to chemical products: agricultural pesticides and herbicides; veterinary and household pesticides; petroleum derived solvents, such as benzene, leather glues, and others; 5) exposure to radiation: accidental, diagnostic, therapeutic, occupational exposure; 6) medicines: any medicine used in the last year, except for nutritional products. Specially trained study monitors (nurses or pharmacists) conducted interviews from January 2002 to December 2005 using a standardized structured data collection tool to collect information on exposure to the above. The data collection tools were the same for the cases and the controls. The 12 months preceding the index date were defined as the risk and/or exposure period. Since AA is an insidious condition, it is difficult to determine the exact date of onset, and drugs may have been used to treat the initial symptoms of AA, rather than having a cause-effect relationship. Therefore, it was decided to exclude exposures reported between one and 30 days prior to the index date. The reference date for the data collection period was defined as the day on which the patient was admitted to the first hospital or health service where care was provided in connection with the signs and/or symptoms of AA. The interviewer first asked the subjects about his/her use of medications and chemical products (pesticides and solvents). If the subject was unable to recall the use of these substances, a standardized photograph album of products that could potentially cause AA was shown as a memory prompt. The printed case report forms used in the interviews were transcribed to an internet-based central electronic database.

## *Reducing bias*

During the study design phase, the researchers from all the LATIN centers produced a list of possible biases that could occur in each procedure, and the actions to prevent them were carried out.

In the selection of patients, differential diagnostic tools were implemented, to ascertain that the cases were true aplastic anemia patients, and that the controls were truly healthy individuals. A list of diagnoses unrelated to hematologic diseases was produced, and used as a criterion for the inclusion of controls. The diagnostic criteria were standardized, and hematologists confirmed the diagnoses: a committee of specialists (IEVC) gathered to review all the biopsy and bone marrow aspirates, before reaching an agreement.

To reduce the possibility of missing some cases, in addition to the active search in clinics and hospitals,

death certificates were obtained from the local health and social agencies, and cases in which aplastic anemia was reported as the cause of death were investigated. After confirmation, these were included in the calculation of incidence. The research committee also assessed patients with suspected aplastic anemia who were involved in ongoing diagnostic investigation for hematologic diseases at health centers.

A Research Manual was produced to enable the data collected by the interviewers to be standardized. A photo album showing pictures of medications, drugs and pesticides was shown during the interview, to help the patients identify the products they had used.

### Statistical methods

Incidence rates were calculated, based on the premise that all residents in the areas covered by the study were at risk. For each region, the number of inhabitants-year was calculated based on the total inhabitants, for each year the center in question took part in the study.

Quantitative variables were described as means, medians, standard deviations, and quartiles. Categorical variables were expressed as absolute and relative frequencies. Univariate and multivariate conditional logistic regressions were used to investigate the association between potential risk factors and disease occurrence. Potential risk factors with univariate $p$ values lower than 0.20 were included in the multivariate conditional logistic regression model. The odds ratios (OR) and 95% confidence intervals (CI) were estimated. The formula used to calculate the population attributable risk percentage[18] was:

$$PAR\% = [(OR-1)/OR] \times 100 \times \text{exposure prevalence among cases}$$

Statistical analysis was performed using Logxact 7 (Cytel Inc., Cambridge, MA, USA) and SAS (SAS, Inc., Cary, North Carolina, USA).

**Table 1.** Aplastic anemia: incidence rate by age and sex, 2002-2005.

| Sex | Cases | Persons-year | Incidence (/million persons-year) |
|---|---|---|---|
| Male | 121 | 67362881.0 | 1.8 |
| Female | 103 | 69853101.7 | 1.47 |

| Age group (years ) | Cases | Persons-year | Incidence (/million persons-year) |
|---|---|---|---|
| 2-9 | 23 | 24966989.0 | 0.92 |
| 10-19 | 67 | 27246079.0 | 2.46 |
| 20-29 | 60 | 24136494.38 | 2.49 |
| 30-39 | 20 | 21837781 | 0.92 |
| 40-59 | 31 | 26542110.0 | 1.17 |
| 60+ | 23 | 12271664.0 | 1.87 |
| Total | 224 | 137001117.4 | 1.64 |

## Results

Two hundred and eighty-one cases of AA were initially included during the study period, comprising patients cared for in the study regions (Figure 2). Of these, 227 (81%) patients had their diagnosis confirmed by the IEVC. Three were excluded because although they had received care at the participating sites, they did not live within the geographical area defined for the study.

Of the 224 patients with AA included in the incidence estimate, the incidence was somewhat higher among males; 1.80 per million, compared to 1.48 per million among females. The age distribution showed three peaks of incidence: 10 to 29 years, and over 60 years, with rates of 2.5 and 1.9 per million, respectively (Table 1). The annual incidence rate of AA for all the regions studied was 1.64 per 1,000,000. The highest rate was seen in Recife (Table 2). A total of 173 cases and 692 controls were included in the study of risk factors for AA. In the individual assessment of drugs, a statistically significant high OR was found for the use of azithromycin and chloramphenicol (Table 3). No other association was significant.

With regard to solvents and pesticides, a statistically significant high OR was found for exposure to agricultural pesticides and high exposure to pyrethroids and benzene. High OR, with borderline statistical significance was found for exposure to organophosphorates and herbicides (Table 4). The final model (Table 5) evidenced a positive association with frequent benzene exposure (≥30 exposures per year) with OR of 3.9 (95% CI=1.7-9.3). In multivariate analysis, chloramphenicol use showed high, though not statistically significant risk

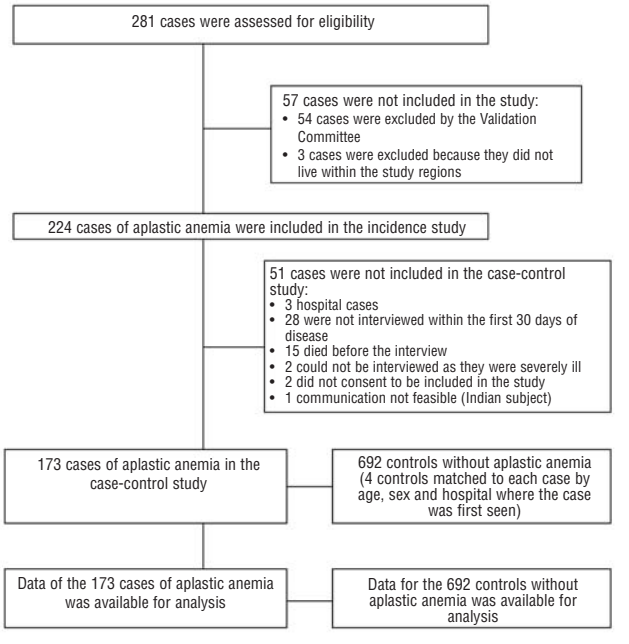


**Figure 2.** Patients and control flows in the LATIN study.

(OR=8.7, 95% CI=0.9-87.9), and azithromycin use showed a statistically significant high OR (OR=11.0, 95% CI=1.1-106.3).

The attributable risk[19] of frequent benzene exposure (≥30 exposures per year) was 5.4%, while that of azithromycin exposure was 1.4%.

## Discussion

The incidence of AA was 1.6 cases per 1,000,000 a year. The rates were found to be slightly different between the study sites, but none of them reported more than 2.5 cases per 1,000,000 a year. This rate is similar to that found in Brazil by Maluf *et al.* in 2002.[1] It is low compared to that found in Thailand,[20,21] where AA has been monitored for about ten years. Other countries studying the incidence of AA found higher rates than those seen in the LATIN study.[22] Data from Mexico show an incidence of 1.7 cases per 1,000,000, while in Japan and Korea, a rate of 11.0.[9] was found. Our data are similar to those found in European countries and Israel, as reported in the International Agranulocytosis and Aplastic Anemia Study (IAAAS).[23] However, the marked variations in the frequency of aplastic anemia among European cities, or among regions of Thailand and China, reported in more recent papers, remain largely unexplained.[6]

The incidence of AA in this study may have been affected by underreporting. In some regions the participants in the study had difficulty accessing the health services, and some cases may have remained undiagnosed. Also, it has been proposed that people living in certain regions with a higher incidence have a genetic

**Table 2.** Aplastic anemia: incidence rate by study site, 2002-2005.

| Region | Cases | Persons-year | Incidence rate (cases per million individuals-year) |
|---|---|---|---|
| Paraná | 73 | 37,450,901 | 1.95 |
| Ribeirão Preto | 12 | 8,888,574 | 1.35 |
| Minas Gerais | 48 | 41,512,773 | 1.16 |
| Goiânia | 26 | 16,859,834 | 1.54 |
| Recife | 45 | 17,519,071 | 2.57 |
| Manaus | 13 | 6,619,363 | 1,96 |
| Buenos Aires | 4 | 3,350,600 | 1,19 |
| Monterrey | 3 | 4,800,000 | 0.63 |
| Total | 224 | 137,001,116 | 1.64 |

**Table 3.** Exposure to individual medicines among aplastic anemia cases and controls: univariate analysis.

| Medicine | Cases (n=173) n (%) | Controls (n=692) n (%) | Odds ratio (95% CI) | *p* value |
|---|---|---|---|---|
| Azithromycin | 3 (1.7) | 1 (0.1) | 12.0 (1.2-115.4) | 0.03 |
| Captopril | 3 (1.7) | 29 (4.2) | 0.3 (0.1-1.2) | 0.10 |
| Cefalexin | 4 (2.3) | 6 (0.9) | 2.7 (0.8-9.5) | 0.13 |
| Cetoprofen | 2 (1.2) | 2 (0.3) | 4.0 (0.6-28.4) | 0.17 |
| Ciproteron | 3 (1.7) | 3 (0.4) | 4.0 (0.8-19.8) | 0.09 |
| Chloramphenicol | 3 (1.7) | 1 (1.1) | 12.0 (1.3-115.4) | 0.03 |
| Chlorpheniramine | 7 (4.1) | 48 (6.9) | 0.6 (0.2-1.3) | 0.15 |
| Dexchlorpheniramine | 2 (1.2) | 19 (2.8) | 0.3 (0.06-1.6) | 0.18 |
| Dihydroergotamine | 3 (1.7) | 2 (0.3) | 6.0 (1.0-35.9) | 0.05 |
| Dypirone | 95 (54.9) | 413 (59.7) | 0.8 (0.54-1.14) | 0.21 |
| Scopolamine | 3 (1.7) | 30 (4.3) | 0.4 (0.11-1.25) | 0.11 |
| Metoclopramide | 5 (2.9) | 8 (1.2) | 2.6 (0.8-8.4) | 0.10 |
| Orphenadrine | 26 (15.0) | 117 (16.9) | 0.5 (0.26-1.04) | 0.07 |
| Penicillin | 5 (2.9) | 9 (1.3) | 2.2 (0.7-6.6) | 0.15 |

*The exposure period considered was the period between 31 and 365 days.*
*CI: confidence interval.*

**Table 4.** Exposure to pesticides and solvents among aplastic anemia cases and controls: univariate analysis.

| Product | Cases (n=173) n (%) | Controls (n=692) n (%) | Odds ratio (95% CI) | *p* value |
|---|---|---|---|---|
| By setting of use | | | | |
| Agricultural pesticide | 12 (6.9) | 23 (3.3) | 2.2 (1.1-4.7) | 0.03 |
| Home use pesticide | 70 (40.5) | 240 (34.7) | 1.3 (0.9-1.9) | 0.14 |
| By chemical group | | | | |
| Organophosphorates (frequency of exposure) | | | | |
| ≤6 exposures/year | 6 (3.5) | 20 (2.9) | 1.2 (0.5-3.0) | 0.71 |
| 7-29 exposures/year | 2 (1.2) | 11 (1.6) | 0.8 (0.2-3.5) | 0.72 |
| ≥30 exposures/year | 5 (2.9) | 7 (1.0) | 3.0 (0.9-10.1) | 0.07 |
| Pyrethroids (frequency of exposure) | | | | |
| ≤6 exposures/year | 30 (17.3) | 103 (14.9) | 1.2 (0.8-2.0) | 0.38 |
| 7-29 exposures/year | 11 (6.4) | 73 (10.6) | 0.6 (0.3-1.2) | 0.17 |
| ≥30 exposures/year | 23 (13.3) | 57 (8.2) | 1.8 (1.0-3.1) | 0.04 |
| Benzene and benzene-based products (frequency of exposure) | | | | |
| ≤6 exposures/year | 13 (7.5) | 87 (12.6) | 0.6 (0.3-1.1) | 0.08 |
| 7-29 exposures/year | 3 (1.7) | 22 (3.2) | 0.5 (0.2-1.8) | 0.29 |
| ≥30 exposures/year | 12 (6.9) | 11 (1.6) | 4.2 (1.8-9.8) | 0.0009 |
| Herbicides | 8 (4.6) | 15 (2.2) | 2.4 (0.9-6.0) | 0.07 |

*The exposure period considered was the period between 31 and 365 days.*
*CI: confidence interval.*

**Table 5.** Risk factors for aplastic anemia: multivariate analysis.

| Risk factors | Odds ratio | 95% CI | *p* value |
|---|---|---|---|
| Chloramphenicol | 8.7 | 0.9-87.9 | 0.07 |
| Benzene and benzene-based products (rate of exposure) | | | |
| ≤6 exposures/year | 0.6 | 0.3-1.1 | 0.10 |
| 7-29 exposures/year | 0.6 | 0.2-1.9 | 0.35 |
| ≥30 exposures/year | 3.9 | 1.7-9.3 | 0.002 |
| Azithromycin | 11.0 | 1.1-106.3 | 0.04 |

*CI: confidence interval. Every odds ratio adjusted by the other variables in the table.*

predisposition[9] to AA. Our team is carrying out further studies to explore these hypotheses. Case distribution by gender was similar, corroborating with most studies on the epidemiological profile of AA. In agreement with the findings in Thailand,[20] we also found that the incidence of the disease follows a bimodal distribution: young adults and older people.

In the present study, a few factors were associated with increased risk of AA, notably frequent exposure to benzene, as shown in previous studies. However, the estimated attributable risk for benzene exposure showed that if this substance were removed from the environment, cases of AA would drop by around 5.4%. Benzene is a reagent used in the production of drugs, paints, leather glues, rubbers, leather products, linoleum, waterproof products, enamels and varnishes, and as a solvent of wax, grease, resins, oils and tar. It was widely used in the dry cleaning, printing and engraving industries. Due to its health risks, the use of benzene has now been restricted, and it has been replaced by other solvents containing little or no benzene.[24]

The hematotoxic properties of benzene have been known for more than a century,[25-29] and this chemical has been the most convincingly associated with the development of AA.[4,30,31] A high number of individuals who have been exposed to benzene over periods of months or years have developed aplasia, with bone marrow cells becoming replaced with fat. There seems to be a dose-dependent relationship[25,32] and latency time between exposure and hematologic manifestations ranging, on average, from four months to four years.[33,34]

In the multivariate analysis, the association with exposure to any type of pesticides and AA found in the univariate analysis eventually disappeared. The variety of chemical structures involved is etiologically puzzling. A mechanism of direct toxicity and exposure to agricultural pesticides may be a surrogate for further exposures, such as to an infectious agent transmitted by insects or animals, or through water or soil.

Exposure to drugs which are known to be causative agents of AA (with the exception of chemotherapy and immunosuppressive agents) seems to play a minor role in the etiology of AA in our context. No association was found even with dypirone, a very commonly used drug in Brazil and Latin America. Azithromycin was the only drug to show a statistically significant positive association with AA (OR 11.0; 95% CI 1.1-106.3). Chloramphenicol, on the other hand, showed an OR of 8.7 (95% CI 0.9-87.9), which was high but not statistically significant. However, inaccurate risk estimates for azithromycin and chloramphenicol, evidenced by very wide 95% confidence intervals, and low use of chloramphenicol and azithromycin in the sample studied compared with the other drugs, calls for careful interpretation of the study results.

Chloramphenicol has often been described as a causative agent of AA.[35-40] Between 1950 and 1965, chloramphenicol was believed to have caused 44% of all drug-induced cases of AA, and 22% of all cases of AA. The time span between exposure and symptom onset was relatively short; around two months.[24] Nevertheless, the relationship between chloramphenicol dose and AA is not yet fully understood, and there is no consensus on this relationship.[4,7,38,41,42] A reduced number of cases of AA would be expected after a decline in chloramphenicol use, but this has yet to be proven. In our study, the positive association found between chloramphenicol use and development of AA was not statistically significant, possibly due to the low exposure to this drug in our sample. In some studies, like IAAAS[2] and LATIN, the prevalence of chloramphenicol use had fallen so low, due to fears of AA, that it was no longer possible to document an association. Several authors believe it is difficult to find a significant association between chloramphenicol use and AA because study subjects are usually exposed to multiple drugs and multiple factors[1,35] and are often unable to accurately remember all drugs to which they have been exposed.[4,7,42]

To minimize this difficulty, the LATIN study subjects were shown a photo album of products as a mnemonic aid. Despite this, finding a significant association was nevertheless impaired by the difficulty in determining the time span between exposure to the substance and onset of the disease (which has not yet been clearly defined) and by the very nature of AA, a complex disease with multiple causes and predisposing factors which is difficult to manage.

## Conclusions

The incidence of AA in Latin America countries is low, i.e., 1.6 cases per 1,000,000 individuals per year, which corroborates with that found in European countries. Frequent exposure to benzene-based products increases the risk of AA. A weak relationship was seen between drug use and AA, and those associations found for some drugs are highly likely to be random. However, even if true associations did exist, these would explain only a small proportion of cases of AA.

## Authorship and Disclosures

EM, NH and ABC: study design, acquisition of data, and writing and final approval of the version to be published; ÁAJ, JE-N: study design, critical review of the article and final approval of the version to be published; RPF, IGHL-M, DG, CLS, DdOWR, LNdMP, LGMR, MP, SL, AAFR, EDV, ATK, EOdMC, MCTP, HMdS, JRB: design of the criteria for selection of patients, acquisition of data, critical analysis and interpretation of data, case ascertainment, review and approval of the final version of the manuscript. RP: study design, draft of the manuscript and final approval of the version to be published.

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