Interactions between drugs and chemicals in industrial societies.
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APLASTIC ANAEMIA (BONE MARROW APLASIA) ASSOCIATED WITH CHEMICAL AGENTS AND DRUGS

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The term aplastic anaemia includes a group of diseases that are characterized by the presence of pancytopenia in peripheral blood and variable hypocellularity of the bone marrow. All pathological situations that lead to pancytopenia cannot be labelled as aplastic anaemia; the disorders listed in Table 1 must be excluded (1).

TABLE 1. CAUSES OF PANCYTOPENIA.

- BONE MARROW APLASIA
- BONE MARROW INFILTRATION
- LEUKAEMIA, MYELODISPLASIA AND MYELOMA
- HYPERSPLENISM AND LIPIDOSES
- MEGALOBLASTIC ANAEMIA
- MYELOSCLEROSIS
- PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA

From Hoffbrand and Pettit, 1984 (1)

Although it is true that the term aplastic anaemia has been generalized, anaemia is not the most representative cytopenia; it can even be absent. Thus an alternative term would be bone marrow aplasia (BMA). BMA rarely selectively

affects only one series of cells, as is the case with erythroblastopenia (selective red cell aplasia). Other forms are usually congenital.

Different classifications of BMA have been formulated, though the one based on etiologic criteria is the most accepted. From an etiologic point of view, two large categories can be considered: idiopathic BMA and secondary BMA.

IDIOPATHIC BMA

This classification includes constitutional and acquired types. At present, all constitutional BMA forms are considered idiopathic. They deal with complex, congenital defects where BMA is an important component, sometimes involving the erythroid series (Blackfan Diamond disease), or the megacaryocytic series (2).

A large group of acquired BMA forms are considered idiopathic, although there seems to be a genetic predisposition with racial and geographical differences. In the case of the acquired types, the number diminishes progressively, as new causes are recognized. In many cases, the cause cannot be established through conventional anamnesis; an extensive investigation is necessary on such aspects as automedication, accidental exposure, domestic use of suspected products, proximity of industrial areas, and coexistence of other diseases.

SECONDARY BMA

A large number of BMA forms are caused by different external agents or associated with known pathological disorders, often immune in nature (Table 2).

From a prognostic point of view, the International Aplastic Anaemia Study Group (3) has defined criteria that permit the classification of BMA in terms of severity (Table 3). The presence of two or three criteria in peripheral blood and one in bone marrow defines the case as severe with a fatal prognostic (4), bone marrow transplantation being the best therapeutic alternative.

TABLE 2. SECONDARY MEDULLARY APLASIA

1- DRUGS

- CHLORAMPHENICOL
- PYRAZOLONE
- GOLD SALTS
- OTHERS

2- CHEMICALS AGENTS

- BENZENE, METABOLITES AND DERIVATIVES
- INSECTICIDES, HERBICIDES
- ORGANIC SOLVENTS
- METALS COMPOUNDS

3- IRRADIATION

4- VIRUSES AND BACTERIA

- HEPATITIS (no A no B) VIRUS
- EPSTEIN BARR VIRUS
- MYCOBACTERIA

5- IMMUNOLOGIC DISORDERS

- SYSTEMIC LUPUS ERYTHEMATOSUS
- RHEUMATOID ARTHRITIS
- GVHD
- THYMOMA

6- OTHERS

- PANCREATITIS
- PREGNANCY

TABLE 3. INTERNATIONAL APLASTIC ANAEMIA STUDY GROUP CRITERIA FOR SEVERE APLASTIC ANAEMIA

SITES	FINDINGS
BLOOD	NEUTROPHILS < 500 / mm^3 PLATELETS < 20000 / mm^3 CORRECTED RETICULOCYTES < 1%
MARROW	SEVERE HYPOCELLULARITY MODERATE HYPOCELLULARITY WITH < 30% OF RESIDUAL CELLS BEING HAEMOPOIETIC

From Camitta et al. 1979 (3)

PATHOGENESIS

The mechanisms by which marrow depression is produced are still unknown. In vitro studies with haemopoietic cell cultures indicate that the mechanisms are numerous and that they develop differently. A series of hypothesis have been described suggesting that BMA can be produced in the manner described in Tables 4 and 5.

TABLE 4. MEDULLARY APLASIA. PATHOGENESIS

1- DIRECT DAMAGE TO STEM-CELLS
2- SUPRESSION OF THE STEM-CELL FUNCTION
3- DEFECT IN THE MARROW MICROENVIRONMENT
4- DISTURBANCES OF HAEMOPOIESIS REGULATION
5- AUTOIMMUNE DAMAGE TO THE MARROW

TABLE 5. MEDULLARY APLASIA. MECHANISMS.

1-	DRUG INDUCED
	- TOXIC OR IMMUNOLOGICAL DAMAGE
2-	INFECTIONS AND VIRUSES
	- INTERFERONS
	- VIRUS-LYMPHOCYTE INTERACTION
3-	CELL-MEDIATED
	- T-LYMPHOCYTES
4-	HUMORAL-MEDIATED FACTORS
	- CIRCULATING OR CELLULAR INHIBITORS OF HEMOPOIESIS
5-	MARROW MICROENVIRONMENT DEFECTS
	- IMMUNOLOGICAL ATTACK
	- FIBROBLAST INFLUENCE
6-	GENETIC DEFECTS
	- UNKNOWN

From Gordon and Barret (6)

DRUG-INDUCED BMA

BMA can be produced by a large number of drugs (excluding cytotoxic drugs) as an adverse effect (7,8). From a quantitative point of view, chloramphenicol, pyrazolones and gold salts are the most important. The frequency of chloramphenicol-induced BMA was estimated as 1:100,000 in the series of Thomas (9) and 1:11,500 to 1:40,000 in Camitta's series (10) (risks of 5-40 times more than that in the unexposed population). The incidence of chloramphenicol-induced BMA has been related to its consumption level in the population (11); therefore the incidence will diminish as its use is limited.

Chloramphenicol can produce reversible pancytopenia during treatment depending on the dose; this effect is related to the inhibition of mitochondrial protein synthesis and decreased cytochrome synthesis in all proliferating cells (12, 13). Pancytopenia appears when chloramphenicol blood levels are surperior to 25 µg/mL (14). In vitro, concentrations of 25 µg/mL decrease DNA synthesis in bone marrow recovered from chloramphenicol-induced BMA. Concentrations of 100 µg/mL or larger suppress DNA synthesis in normal marrows (15). Chloramphenicol can produce persistent marrow depression after the administration of small doses, after a period from 2 weeks to 6 months (13), complete recovery being rare. Chloramphenicol-related BMA could be the result of an idiosyncratic reaction or an immunologic mechanism. Geary (15) and Murray (16) studied chloramphenicol metabolism and observed that the reduction of the p-nitro groups of chloramphenicol gives rise to a large number of toxic metabolites, such as nitrochloramphenicol that would degrade marrow DNA. Thiamphenicol has a methylsulphate group in place of the p-nitro group of chloramphenicol (3).

BMA INDUCED BY CHEMICAL AGENTS

The great expansion of industrial zones during the last fifty years and the indiscriminate, general use of certain natural or synthetised products have given rise as an undesirable consequence to industrial contamination.

Substances that are potentially toxic to bone marrow are rare but small quantities are found in many products. Restrictions in the use of substances such as benzene, will decrease cases greatly. BMA has said to be induced by: 1) benzene and derivatives, 2) organic solvents, 3) insecticides, herbicides and pesticides and 4) metallic compounds (17, 18). Benzene and its derivatives and organic solvents are found widely in industry (refineries, petrochemical complexes, lacquers, varnishes, leather, batteries, cosmetics and flavoring products, industrial and domestic solvents, adhesive materials, etc...) (18).

The incidence of BMA induced by chemical agents is variable. Evaluation of the exposed population is very difficult, as well as the amount of product absorbed since inhalation is the main route of entry. The circumstances of exposure are of considerable epidemiologic interest and can be classified as: 1) individual, domestic exposure (chronic or accidental) due to a faulty utilization of certain occupational or domestic products (hair lacquers, paints, varnishes, perfumes, and insecticides) (19-21); 2) collective exposure in workers of industries not using adequate protective measures (22-25); and 3) individual and voluntary exposure by the use of commercial glues containing toluene and inhaled as "stimulants" (26-27). An interesting epidemiologic feature is that exposure to toxic chemical products is not exclusive to industrial areas; insecticides and herbicides are widely used by farmers.

The toxicity of these products appears after long periods of exposure; acute and massive forms are usually lethal, due to involvement of other organs, with lung aedema, renal failure, and distrubances of CNS.

Benzene has been associated with multiple haematological disturbances, haemolytic anaemia, bone marrow hyperplasia, myeloid metaplasia, lymphopenia, acute non-lymmphoblastic leukaemia, and possibly lymphoma, the more frequent toxic effects being pancytopenia by BMA or myelodisplasia (28-31). In mice, the inhalation of benzene inhibits DNA synthesis in differentiated marrow cells (32) and damages colony forming stem-cells (33). A large number of chromosomal abnormalities, breaks and gaps have been described (34). Studies carried out in animals in vivo show that the exposure to benzene causes marrow lesions because of metabolic products with the formation of phenolic compounds like pyrocatechol (35). For these reasons studies in vitro using benzene yields negative results.

Hydroquinone and its terminal oxidation products (p-benzoquinone) have been shown to be potent suppressors of cellular growth in cultures (31). The suppression of lymphoblastic transformation depends on the content of sulphydryl components produced in lethal concentrations (31). Hydroquinone and p-benzoquinone inhibit RNA synthesis of mouse splenic lymphocytes and growth factors of T-lymphocytes (interleukin 2); these mechanisms explain aplastic anaemia as inhibiting growth factors of haemopoiesis (36).

Organic solvents used in industrial and domestic activities that cause BMA are most frequently ethyleneglycol and its ethers (37-39).

Insecticides, herbicides, pesticides and plaguicides are sometimes used illegally in agriculture. Their misapplication without taking protection measures, can give rise to the inhalation of small quantities potentially capable of causing BMA. Lindane, pentachlorphenol and DDT, are claimed to be responsible for isolated cases (40-47). Insecticides are frequently used as aerosols, using toluene as a solvent; this should be taken into account in the investigation of etiologic agents.

DATA FROM BARCELONA

Some etiologic, epidemiologic, and prognostic data of a series of 205 patients diagnosed of BMA during a period of 23 years (1963-1986) in the University Hospital of Barcelona are presented. Of the 205 patients, 110 (53.7%) are male and 95 (46.3%) female, with a ratio male/female of 1.15. Mean age was 40 years (6-87). From the etiological point of view, 117 (57%) are classified as idiopathic, and 88 (43%) a secondary. Of the latter 40 (45.5%) were induced by drugs, 32 (36.3%) by chemicals and 16 (18.2%) by other causes (Figure 1). Regarding severity (3), 114 (57%) were considered severe and 77 (37%) not severe; 14 were not evaluated. Case incidence by decades and etiology are described in Figure 2. Idiopathic marrow aplasias were distributed by sex as follows: 64 (54.7%) males and 53 (45.3%) females, with a mean age of 33 years (6-87), the ratio male/female was 1.2; 65 cases (55.6%) were severe, 46 (39, 3) not severe and 6 were not evaluated. Secondary marrow aplasia was classified as: drug induced, 40 (45.5%); chemical-induced, 32 (36.3%); other causes, 16 (18.2%). The drugs involved are given in Table 6.

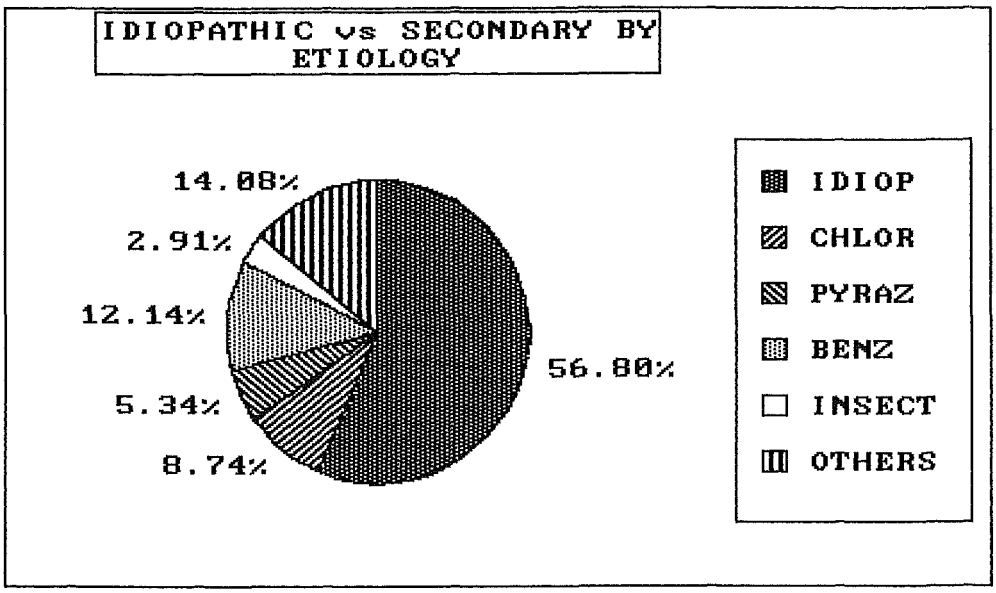

Fig. 1. Distribution of the cases by etiology.

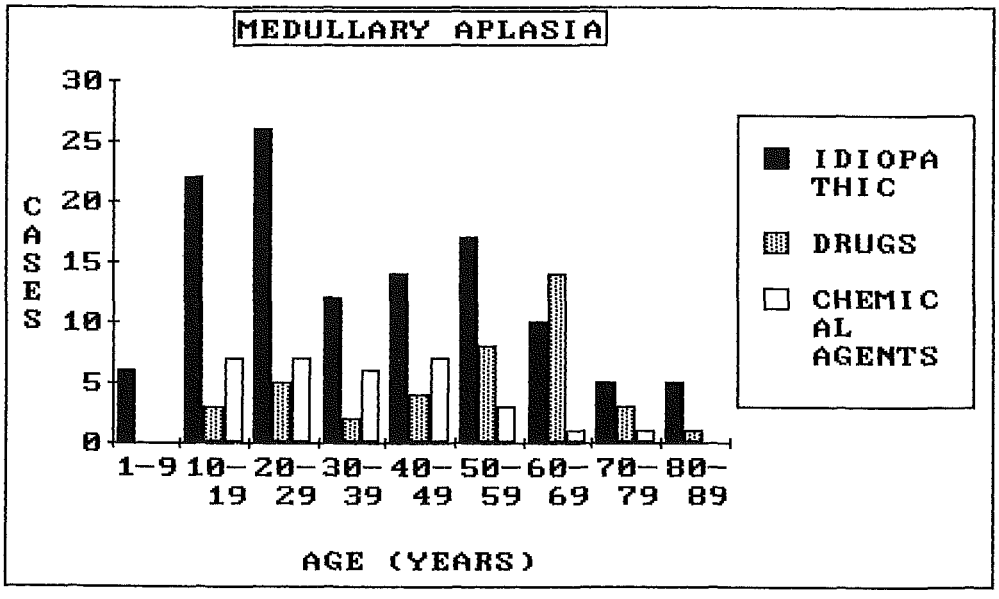

Fig. 2. Idiopathic, drug and chemical related forms. Age distribution.

From a prognostic point of view, the actuarial survival curves do not demonstrate statistically significant differences between the idiopathic and secondary cases, with a median survival around 300 weeks. (Figure 3). There were no significant differences between cases due to chloramphenicol and to pyrazolone or between the benzene-associated or insecticide-related forms.

From these data some features are evident: 1) the mean age of this series is quite inferior to that referred to in others. The bias in our population may be due to the fact that not all patients come from the local geographical area, but are sent from very different areas from marrow transplantation; 2) the cases of idiopathic BMA were more frequent than those is referred to by others; 3) Pyrazolone-induced BMA was seen more frequently in the female, adult population probably because self-medication or the predominance of rheumatic diseases in this group; 4) the chemical agents affect the male working population more frequently; 5) young farmers are exposed to insecticides, the implicated product is generally unknown; 6) in the prevalence study, it can be seen that cases related to chloramphenicol decrease in the later years and the pyrazolone-related cases progress. The restriction of the use of benzene contributes to a decrease in yearly prevalence. In our experience, the etiology does not play an important role in survival.

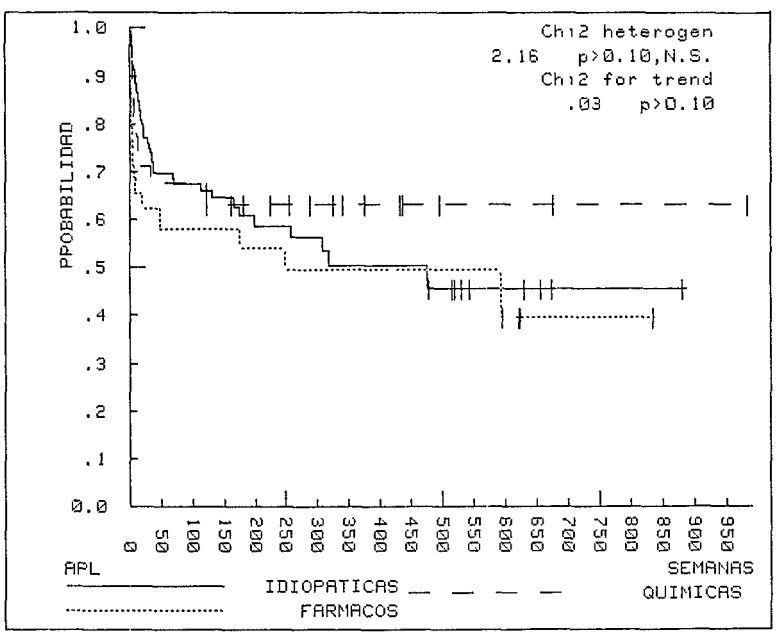

Fig. 3 Actuarial survival curves of idiopathic, drugs and chemicals related forms.

TABLE 6. HCB MEDULLARY APLASIA, DRUG-INDUCED.

TOTAL NUMBER OF PATIENTS	:	40	
CHLORAMPHENICOL	:	18	(45%)
PYRAZOLONE DER.	:	11	(27.5%)
HYDANTOIN	:	2	(5%)
PYRAMIDON	:	2	(5%)
TUBERCULOSTATICS	:	4	(10%)
MEPROBAMATE	:	1	(2.5%)
SULPHONYLUREAS	:	1	(2.5%)
D-PENICILLAMINE	:	1	(2.5%)

Benzene was considered responsable for 25 cases and insecticides for 6. The other cases were due to hepatitis (10 cases), pregnancy (4 cases) and thymoma (2 cases). The case distribution by years and etiologies is of epidemiological interest (Table 7).

TABLE 7. HCB MEDULLARY APLASIA, PREVALENCE BY YEAR.

BENZENE:		
1963-1969		5 cases
1970-1979		14 cases
1980-1986		6 cases
1969-1979	18 - - - - - - - - -	1.63/year
1980-1986	6 - - - - - - - - -	0.85/year
CHLORAMPHENICOL:		
1970-1975		11 cases
1976-1980		4 cases
1981-1986		3 cases
1970-1979	14 - - - - - - - - -	1.40/year
1980-1986	4 - - - - - - - - -	0.57/year
PYRAZOLONES:		
1970-1975		2 cases
1976-1980		4 cases
1981-1986		5 cases
1970-1979	6 - - - - - - - - -	0.6/year
1980-1986	5 - - - - - - - - -	1.4/year

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DISCUSSION - Aplastic anemia

R. Lauwerys

I am wondering on what basis the diagnosis of insecticide-induced aplastic anemia was made. I know that there have been isolated case reports of aplastic anemia caused by lindane or some chlorophenol, but from an epidemiological point of view these data are not very convincing.

L. Hernández-Nieto

The only criteria we used was anamnesis revealing use of insecticides and no other cause, but we cannot exclude an idiopathic origin.

E.E. Ohnhaus

I was also surprised by the high proportion of secondary aplastic anemia in your series. We very rarely can identify a causative agent and even if there is suspicion of an environmental factor to which the patients were exposed, levels are usually very low. Perhaps the limits accepted in Spain are different.

L. Hernández-Nieto

The majority of the cases of chemically-induced aplastic anemia came from uncontrolled, domestic-type workshops, where the environmental levels may be rather high.

L.F. Prescott

I also would like to know how were you able to determine that the drugs you listed were the actual cause of aplastic anemia.

L. Hernández-Nieto

Again, I have to accept that the diagnosis was based only on anamnesis.

H. Vainio

Have you been following these patients with aplastic anemia to see whether they develop leukemia or other late manifestations?

L. Hernández-Nieto

We have seen cases presenting with leukemia. I cannot recall any case of late evolution to leukemia among the patients that we were able to follow up in this series.

J.V. Castell

Can one talk about a general mechanism in drug-induced aplastic anemia?

L. Hernández-Nieto

I do not think so. Probably many factors are involved.

A.L. de Weck

It is of interest to notice that when an immunological mechanism is operating, it seems to produce either agranulocytosis or thrombocytopenia, but very rarely aplastic anemia. So, I have a tendency to consider aplastic anemia as a toxic reaction whereas when a single cell population is affected I strongly suspect an immunological reaction.

R. Lauwerys

I think that it would be useful to report that ethylene glycol monomethyl ether, a solvent that is widely used in the printing industry and in painting, can induce aplastic anemia in animals, and that this bone marrow aplasia is quickly reversible after cessation of exposure.