Blood Group Changes in Leukaemia

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Since Van Loghem, Dorfmeier, and van der Hart (1957) described a change in the A blood group of a patient with myeloblastic leukaemia, which could probably be attributed to the disease, many investigators have reported alterations in the A, B, and H antigens of the ABO system; D and E of the Rh; M and N of the MN; Le^a of the Lewis; and I and i of the I blood groups (Race and Sanger, 1962; Renton, Stratton, Gunson, and Hancock, 1962; Salmon and Salmon, 1963; Salmon, 1964). Although these investigations have contributed to the elucidation of several aspects of this problem (especially the research done by Salmon and his group), many other points remain obscure. In particular no information is available from an unselected sample of leukaemics concerning: (a) the general prevalence of the changes; and (b) whether they affect preferentially one system of blood groups or not. The many reports concerning ABO changes may simply be due to the fact that in this system the abnormalities are more easily detected due to the reciprocal relation existing between antigens and 'natural' agglutinins. The purpose of the present investigation was to provide data on these questions and on other aspects of these changes. A first paper describing in detail one of the cases studied has already been submitted for publication elsewhere (Ayres, Salzano, and Ludwig, 1966).

Subjects and Methods

A search for leukaemics was made in laboratories of clinical analyses, blood banks, hospitals, and among physicians in Porto Alegre, the Capital of the Brazilian State of Rio Grande do Sul, and the following major towns in the interior of the State: Pelotas, Passo Fundo, Lagoa Vermelha, Santa Maria, Rio Grande, Bagé, Caxias, Uruguaiana, Livramento, Alegrete, Vacaria, Camaquã, São Lourenço do Sul, Bento Gonçalves, Guaiiba, and São Marcos. Field work was restricted to the period between June and November 1965. In these

six months we found and studied 51 people with leukaemia. If the incidence of the disease is of the order of 3 per 100,000 per year, as estimated for Chile (Meza Massad, Larrain, and Moróder, 1964), the number of leukaemics potentially available for study at this time would be 84 (assuming the State population as 5,600,000 persons); therefore, our sample should represent about 60% of all people with leukaemia living in the State at the time of the investigation. All were classified as White (in one, however, the presence of Negroid genes was suspected). Since the prevalence of Negroes and Mulattoes in Rio Grande do Sul in the 1950 census was 11%, we would have expected to find about 9 Negro leukaemics during the period of the investigation. The fact that none was classified in this ethnic group indicates that, as was verified elsewhere, the prevalence of leukaemia among them may be lower than that among Whites. But it should also be pointed out that since the Negroes living in the State usually belong to a lower social class, the probability of finding these cases among them is correspondingly decreased.

A significant portion of the sample (23) is made up of personal patients of one of us (O.K.L.). Detailed clinical and haematological information about them was available and similar data were obtained for the other patients through the use of standardized forms. Qualitative determinations of blood groups were performed according to methods currently in use in our laboratory (Salzano, 1961). Any antigenic abnormality disclosed or suspected in this first screening was checked by the use of the cell counting method of quantitative haemagglutination described by Filitti-Wurmser and Jacquot Armand (1947). The level of the plasma α and agglutinins was determined by titration and the salivary secretions were tested for secretion of A, B, H, and Le^a substances. The inhibition titre of the A, B, and H substances in the salivary was also determined. All tests were performed with suitable controls. All available members of the patients' immediate families (parents, sibs, children) were also tested.

The criterion used to decide if there was a change was established through preliminary experiments. Salmon (1959) had already verified that the precision of the cell counting method we used is of the order of 5% and our results agree with his findings (Table I). To be on the safe side, however, we decided to consider the counting abnormal only if the departure between the patient and control values exceeded 10%.

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TABLE I
REPEATED COUNTS IN KNOWN MIXTURES OF CELLS
BY A QUANTITATIVE HAEMAGGLUTINATION TEST

Experiments	Cell Mixtures (%)					
	100 A Zero O	80 A 20 O	60 A 40 O	40 A 60 O	20 A 80 O	Zero A 100 O
1	100	85	65	42	20	0
2	99	81	66	47	25	0
3	99	80	55	43	23	0
4	100	82	57	46	19	0
5	100	81	66	49	27	0
Average	99.6	81.8	61.8	45.4	22.8	0
Standard deviation	0.5	1.9	5.3	2.8	3.3	—

Note: Technique of Filitti-Wurmser and Jacquot-Armand (1947). It consists essentially in placing 0.1 ml. cell mixture with 0.2 ml. antiserum in one tube and another 0.1 ml. cell mixture with 0.2 ml. phosphate buffer (pH 7.3) in another, the latter solution acting as a control. After mixing they are placed in a shaker for 20 minutes at 25°C. A sample of both solutions is then taken and the number of free (unagglutinated) cells counted. The percentage of agglutinated cells is always calculated in relation to the number of free cells present in the control mixture.

Results

Nature of Sample. Table II presents some general characteristics of the sample of leukaemics studied, whether their blood was tested more than

once, and the percentage of cases in which family studies could be carried out. Twenty-five patients had chronic leukaemia, the others showed acute forms. About half of the leukaemics were males. The acute cases, with three exceptions, occurred in the first two decades of life; the chronic lymphoid ones in patients of 41 years and older, while the chronic myeloid patients showed a very wide age distribution. Eighteen leukaemics lived in Porto Alegre, the others in the interior. In 22 it was possible to perform more than one test to detect possible changes. In only 23 of the 51 patients could family investigations be carried out.

Haematological and Clinical Studies (Table III). Haemoglobin levels were on the average 57% of normal, with the acute myeloblastic cases showing the lowest values. The red blood cell counts were about 3.1×10^6 , with the acute myeloblastic cases, as expected, showing the lowest numbers. The highest percentage of leucoblasts was observed among the acute lymphoblastic patients.

The more common triad of symptoms was adenopathy, hepatomegaly, and splenomegaly. Most of them had been treated with corticoid and antimetabolite drugs. Twenty-four had received no

TABLE II
SOME CHARACTERISTICS OF SAMPLE STUDIED

Characteristics of Sample	Type of Leukaemia					Total
	Acute Lymphoblastic	Acute Myeloblastic	Chronic Lymphoid	Chronic Myeloid	Other*	
Male	7	6	6	8	—	27
Female	6	5	1	10	2	24
Age (yr.)						
0-10	9	5	—	—	—	14
11-20	4	5	—	2	—	11
21-30	—	—	—	1	—	1
31-40	—	—	—	4	1	5
41-50	—	1	1	4	—	6
51-60	—	—	3	6	1	10
61-70	—	—	2	1	—	3
71-80	—	—	1	—	—	1
Residence						
Porto Alegre	7	4	1	5	1	18
Cruz Alta	1	1	—	—	—	2
Passo Fundo	2	—	1	—	—	3
Pelotas	—	—	—	1	—	1
São Lourenço do Sul	—	—	1	1	—	2
Other	3	6	3	11	1	24
Frequency of tests in patients						
Tested only once	9	6	6	6	2	29
More than one test	4	5	1	12	—	22
Family studies						
Both parents and sibs	4	2	—	—	—	6
Both parents	3	1	—	1	—	5
Spouse and children	—	—	3	2	—	5
Children	—	—	1	3	—	4
One parent and sibs	1	1	—	—	—	2
One parent	—	1	—	—	—	1
None	5	6	3	12	2	28

*One monocytic and one haemocytoblastic. Both were acute forms.

TABLE III
HAEMATOLOGICAL AND CLINICAL INFORMATION ON PATIENTS WITH LEUKAEMIA

Characteristics of Sample	Type of Leukaemia					
	Acute Lymphoblastic	Acute Myeloblastic	Chronic Lymphoid	Chronic Myeloid	Other	Total
No. studied	13	11	7	18	2	51
<i>Haematological determinations</i>						
Hb (% of normal) average	60	42	72	66	28	57
range	23-98	30-53	31-92	40-80	20-36	20-98
RBC ($\times 10^6$) average	3.1	2.3	3.4	3.6	1.3	3.1
range	1.7-5.0	1.2-3.2	2.1-5.0	2.8-4.1	1.3-1.4	1.2-5.0
Leucocytes average	22,376	23,945	193,000	196,805	37,800	106,608
range	200-95,000	2,800-60,000	41,000-390,000	16,000-698,000	15,600-60,000	200-698,000
Leucoblasts (%) average	45	36	10	4	7	23
range	2-89	1-86	1-44	1-15	5-10	1-86
<i>Symptoms</i>						
Adenopathy, haemorrhage, hepatomegaly, splenomegaly	1	—	1	—	—	2
Adenopathy, hepatomegaly, splenomegaly	2	1	2	1	1	7
Other combinations of 3 symptoms	2	3	—	1	1	7
Adenopathy and splenomegaly	—	1	4	2	—	7
Adenopathy and hepatomegaly	1	2	—	4	—	7
Other combined 2 symptoms	5	2	—	1	—	8
Splenomegaly	—	—	—	8	—	8
Other isolated symptoms	2	2	—	1	—	5
<i>Treatment</i>						
Corticoid drugs	2	5	—	1	—	8
Antimetabolite drugs	1	—	—	8	1	10
Corticoids and antimetabolites	7	5	4	1	—	17
Radiotherapy	—	—	—	3	—	3
Antimetabolites and radiotherapy	—	—	—	2	—	2
None	3	1	3	3	1	11
Transfusions	6	9	2	3	1	21
Average no. of transfusions received	4	11	3	3	1	7
Without transfusions	5	2	2	14	1	24
No information concerning transfusions	2	—	3	1	—	6
<i>Duration of treatment (days)</i>						
Corticoids average	59	121	128	48	—	94
range	1-180	4-330	1-180	7-90	—	1-330
Antimetabolites average	59	55	156	52	9	70
range	1-180	16-90	1-365	15-120	—	1-365
Radiotherapy average	—	—	—	1,117	—	1,117
range	—	—	—	950-1,300	—	950-1,300
<i>Interval between onset of disease and 1st blood group test (days)</i>						
average	99	135	844	1,119	165	555
range	7-300	60-270	240-2,370	30-3,680	60-270	7-3,680

transfusions and 21 had received an average of 7 transfusions; as expected the acute cases had received more transfusions than the chronic ones. The patients had on the average been treated for two or three months with antimetabolites and corticoids when the first tests were done. The interval between the onset of the disease and our first blood group tests ranged from 1 week to 9 months in the acute cases, and 1 month to 11 years in the chronic ones.

Blood and Saliva Typing. Results of the ABO, MN, Rh, and I blood group typings, as well as of the saliva tests done for ABH and Le^a secretion, are presented in Table IV. Only in the ABO and MN systems were there significant departures from dis-

tributions found among healthy persons (saliva samples, however, could be obtained from 26 patients only). The deviations in the ABO blood groups involved a deficiency of O. The A phenotype was slightly raised but not in a significant way. The relative incidence of A : O phenotypes was 1.4.

The deviation in phenotype distribution in the MN system is due to a significant excess of MN individuals; this excess is somewhat diminished if we use the MN distribution of leukaemic patients who have not received transfusions, plus those 26 patients about whose genetic constitution we can be sure (through the study of their parents). We cannot be sure, therefore, that the deviation is not due to transfused blood, and so do not attach importance to this finding.

TABLE IV

ABO, MN, Rh, AND I BLOOD GROUPS, ABH AND Le^a SALIVA SECRETION TYPES IN PATIENTS WITH LEUKAEMIA AND IN CONTROLS

Phenotypes	No. with Leukaemia	No. Normal*
ABO system		
O	31.4	47.1
A ₁	37.3	35.1
A ₂	7.8	7.0
B	17.6	8.4
A ₁ B	5.9	1.8
A ₂ B	—	0.6
No. studied	51	333
MN system		
M	21.6	30.6
MN	70.6	48.4
N	7.8	21.0
No. studied	51	333
Rh system		
CCDEe	3.9	1.8
CCDee	15.7	23.0
CcDEe	19.6	12.4
CcDee	27.4	29.2
CcdDee	2.0	0.9
ccDEE	—	0.9
ccDee	2.0	3.5
ccDEe	17.6	11.5
ccddEe	—	1.8
ccdde	11.8	15.0
No. studied	51	113
I system		
No. I (+)	100.0	—
No. studied	25	—
Secretion of ABH		
Secretors	82.1	78.6
Non-secretors	17.9	21.4
No. studied	28	28
Secretion of Le^a		
Secretors	89.3	92.9
Non-secretors	10.7	7.1
No. studied	28	28

* Data of Salzano (1963) and F. M. Salzano, M. V. Suñé, and M. Fe lauto (unpublished) for the blood group studies; for the saliva investigations the control sample was obtained from unrelated, healthy members of the leukaemics' families.

Homogeneity tests were performed with regard to the phenotype distribution in all systems (except I) in patients and normal subjects. In 2 cases significant departures from random deviations were observed: in the ABO system ($\chi^2=10.6$; 4 d.f.; $p<0.05$), and in the MN system ($\chi^2=9.8$; 2 d.f.; $p<0.01$).

Other Saliva and Plasma Investigations.

Plasma agglutinin titres and saliva inhibition titres from leukaemic patients and normal subjects are presented in Table V. As can be seen, the deviations between the values obtained in these two categories of individuals are slight. The plasma anti-A from leukaemic patients showed an average titre of 1:29, and those from normal subjects, 1:26; the corresponding values for anti-B were 1:36 and 1:33. The same occurs with the saliva determinations. The average inhibition titre of the A substance present in the saliva of leukaemic patients was 1:9 and in normal subjects, 1:11; analogous values for the B substances are 1:1 and 1:3; and for the H substance 1:4 and 1:8.

Despite the fact that in general the leukaemic patients showed more antibodies in the plasmas and fewer in the saliva, analyses of variance in the different series did not disclose any significant deviation.

Antigen Changes. Table VI presents the quantitative determinations of agglutination in leukaemic cells suspect after the qualitative tests of presenting antigenic abnormalities. Changes in which the possible influence of the transfusions received could not be eliminated are omitted. As can be seen in Table VI the modifications did not seem to affect any type of leukaemia preferentially. In the acute lymphoblastic cases, I and H + I partial changes were observed. In the acute myeloblastic ones, one complex instance of A (A₁) + B + H change was found; the abnormalities in this case affected also at least the C antigen of the Rh system. A full report about the studies performed in the blood and saliva of this leukaemia patient has already been submitted for publication (Ayres *et al.*, 1966). In the chronic lymphoid patients an interesting change in A₁ (without affecting the A agglutination) was detected, with a corresponding increase in H substance; in another patient a partial change in I was encountered. Among the patients with chronic myeloid leukaemia there was a partial, reversible change of H and two modifications in the I substance. In the acute haemocytoblastic case studied an abnormality in the H substance was detected.

Discussion

Before we discuss our findings it is worth considering what factors can influence the results obtained in research such as this, where the purpose was primarily to obtain an estimate of the prevalence of changes in blood group antigens in patients with leukaemia. As indicated earlier changes in the ABO system are easier to establish, due to the antigen—'natural antibody' relations present in this blood group. In other systems the detection may depend on family studies. Further, in some antigens with almost universal prevalence, i.e. I, it is easy to eliminate the effect of transfusions, since the transfused blood would presumably contain normal amounts of the antigen. This is not true for other blood groups, and the frequency with which patients with leukaemia are exposed to transfusion seriously limits the possibility of detection of abnormalities. The ideal situation would be to obtain a sizeable group of patients who had never received transfusions. This,

TABLE V

PLASMA AGGLUTININ TITRES AND SALIVA INHIBITION TITRES AS DETERMINED BY SERIAL DILUTION TESTS IN PATIENTS WITH LEUKAEMIA AND IN CONTROLS

Characteristics Tested		Type of Leukaemia					Total
		Acute Lymphoblastic	Acute Myeloblastic	Chronic Lymphoid	Chronic Myeloid	Other	
<i>Plasma agglutinin titres</i>							
<i>Anti-A</i>		(N=12)	(N=4)	(N=5)	(N=18)	(N=2)	(N=41)
Patients	average	25	57	8	32	65	29
	range	4-128	4-128	4-16	2-128	2-128	2-128
	average	23	20	22	28	32	26
Controls	range	8-32	16-32	16-32	16-64	—	8-64
		(N=17)	(N=15)	(N=3)	(N=27)	(N=1)	(N=63)
	average	31	44	4	34	128	36
Patients	range	2-128	2-128	2-8	1-128	—	1-128
	average	31	27	21	39	16	33
	range	16-64	16-64	16-32	16-128	—	16-128
<i>Saliva inhibition titres</i>							
<i>A substance</i>		(N=1)	(N=5)	(N=2)	(N=6)	—	(N=14)
Patients	average	4	8	5	13	—	9
	range	—	2-16	2-8	2-16	—	2-32
	average	16	9	36	5	—	11
Controls	range	—	1-16	8-64	2-16	—	2-64
		(N=1)	(N=2)	(N=2)	—	(N=1)	(N=6)
	average	2	1	1	—	1	1
Patients	range	—	—	—	—	—	1-2
	average	8	3	2	—	1	3
	range	—	2-4	—	—	—	2-8
<i>H substance</i>		(N=4)	(N=7)	(N=3)	(N=8)	(N=1)	(N=23)
Patients	average	2	9	1	11	2	4
	range	1-4	1-32	—	1-64	—	1-32
	average	4	4	6	11	4	8
Controls	range	2-8	2-16	1-16	1-64	—	1-64

N=number of individuals studied (always the same for patients and controls).

TABLE VI

ANTIGENIC CHANGES IN LEUKAEMIC SUBJECTS AS DETERMINED BY CELL COUNTING TECHNIQUE

Type of Leukaemia	Antigens (% of agglutination)									
	A		A ₁		B		H		I	
	Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls
Acute lymphoblastic	—	—	—	—	—	—	95	100	81†	100
Acute lymphoblastic	—	—	—	—	—	—	88†	100	46†	98
Acute myeloblastic*	0‡	100	—	—	0‡	100	100	100	—	—
	0‡	100	—	—	0‡	100	100	100	—	—
	0‡	100	—	—	0‡	100	100	100	—	—
	0‡	100	—	—	0‡	100	100	100	—	—
	19‡	100	18‡	100	0‡	100	78‡	100	—	—
Chronic lymphoid	99	100	45‡	96	—	—	14‡	3	—	—
Chronic lymphoid	99	100	—	—	99	99	—	—	81‡	99
Chronic myeloid†	—	—	—	—	—	—	68‡	96	—	—
	—	—	—	—	—	—	99	100	—	—
Chronic myeloid	97	100	—	—	—	—	—	—	78‡	100
Chronic myeloid	84§	99	—	—	—	—	—	—	89§	98
Acute hemocytoblastic	—	—	—	—	—	—	75‡	100	—	—

* Five independent samples from the same individual, collected at different times. † Two independent samples from the same person. ‡ Differences considered significant. § The A change is probably due to transfusions. Since the transfused cells supposedly contained normal amounts of I the difference of 9% observed among the patient's cells and the control was considered significant.

however, would seriously bias the sample towards those patients in whom the leukaemia had just started. The frequency with which repeated tests are made in the same subject is also important: a second or third test could disclose abnormalities not present in the first determination. On the other hand they can establish at what point of the disease

process these changes start and if they are or are not reversible. Through this knowledge important hints can be obtained about the nature of these abnormalities. Since all these factors can influence any data about the incidence of these changes, it is important that samples collected for this purpose be defined in a very detailed way.

The changes that we have observed do not seem to be more prevalent in any type of leukaemia. Therefore, the first impression that the abnormalities were more frequent in the myeloblastic type (Salmon, 1964) is not confirmed by new data.

Since we have found 9 cases of changes in 51 leukaemic patients, we can guess, as far as the general prevalence of the abnormalities is concerned, that about 1 in every 5 people with this disease may be affected in one of the systems we have studied. The more common modifications were related to antigens I and H. In the latter case two of these changes occurred in association with A₁ abnormalities and in three instances they occurred alone. This gives a prevalence of H changes in leukaemic O individuals of 3/16, quite close to the figure (8/42) obtained by Salmon and Salmon (1965) in another series. As for the I changes our figure of 5 partial abnormalities in 25 individuals tested is somewhat lower than the ones found by McGinniss, Schmidt, and Carbone (1964) who found 5 partial and 10 total modifications in the I antigen of 40 patients with leukaemia; and those of Schmidt, Barile, and McGinniss (1965) who reported 38 I negatives among 124 patients.

Several authors have investigated the relation between leukaemia and blood groups in an attempt to find an increased susceptibility to the disease by persons with a given phenotype. The data have been recently reviewed by Shirley and Desai (1965) who concluded that group A subjects may be more susceptible. In our series no increased incidence of A was found. But the observed lack of O persons gives as a result a relative incidence of A : O phenotypes of 1.4. Shirley and Desai (1965) observed that in 13 of 16 series this relative incidence was also greater than one, and our results agree with those reviewed by them in this sense. But our series is small and there are so many factors involved in these postulated associations that we prefer to regard the problem as unsolved.

Summary

In order to detect possible changes in antigens related to the ABO, MN, and Rh blood group systems, studies were carried out on 51 patients with leukaemia, representing about 60% of all patients with this disease living in the State of Rio Grande do Sul, Brazil, in a six-month period. A search was also made for changes in their α and β agglutinins. The I antigen was studied in the blood of 25 patients; saliva specimens were secured for 28

subjects and tested for secretion of the ABH and Le^a substances.

Of the 51 patients tested, 9 showed antigenic abnormalities in their blood, the more common being related to the H and I antigens. The changes do not seem to be more prevalent in any type of leukaemia. No abnormalities were detected in the saliva and plasma studies.

Thanks are due to the many physicians who referred cases to us, and particularly to Dr. Fani M. Job who showed a special interest in our investigation. Mr. Girley V. Simões helped in the field investigations and Lic. Maria C. Mallmann in some of the laboratory tests. Dr. H. Fudenberg furnished the anti-I and Drs. P. Levine and H. Gershowitz the anti-Le^a antisera used in the study. One of us (M.A.) is on leave of absence from the Departamento de Puericultura e Pediatria da Faculdade de Medicina da Universidade Federal do Pará, with a post-doctoral fellowship provided by this University. This work has been supported in part by the Rockefeller Foundation, Conselho Nacional de Pesquisas, Conselho de PHS of the University of Rio Grande do Sul and PHS research grant RG-08238 from the Division of General Medical Sciences, Public Health Service, U.S.A.

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