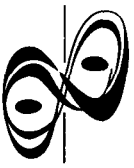

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Genetic and Environmental Effects on Blood Cells

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Abstract. In a sample of 105 concordant sex MZ and DZ twin pairs, the following characteristics were measured: red cell count, haemoglobin concentration, package cell volume, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, reticulocytes, platelets, white cell count and the six types of leucocytes, lymphocytes, monocytes, band and segmented neutrophils, eosinophils and basophils. The statistical model employed in the univariate twin analysis allows for three sources of variation: genetic (h^2), shared environmental (c^2) and specific environmental influences (e^2). A genetic component was significant for red cell count, haemoglobin and mean cell haemoglobin (0.64, 0.60 and 0.46 respectively), with heritable variation suggested for package cell volume, mean cell volume, mean cell haemoglobin, lymphocytes and monocytes. Shared environmental variation was only present for neutrophils.

Key words: Heritability, Shared and specific environmental component, Leucocytes, Red cells

INTRODUCTION

This work is part of a twin study on quantitative characteristics of blood, including serum and cells. The genetic and environmental components of the serum variables have been studied previously [2], [3]. The present analysis was carried out to estimate the same components for blood cell variables, using a sample of normal monozoygotic and dizygotic twin pairs.

The variables related to the blood cells have been employed to evaluate people's health and to detect pathological conditions, which have been extensively studied. However, all the individuals in the present sample are normal. Evidence of a genetic effect on red cell count and mean cell volume has been found [8], and genetic and non-

shared environmental influences on white cell counts, and indices related to circulating red cell mass and platelet number and size, have also been detected [10].

In this present paper, the following 15 variables were studied: red cell count (RBC), haemoglobin concentration (Hb), packed cell volume (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), reticulocytes, platelets, white cell count (WBC), and the following types of leucocytes: lymphocytes, monocytes, band and segmented neutrophils, eosinophils and basophils.

SUBJECTS AND METHODS

105 pairs of monozygotic (MZ) and same sex dizygotic (DZ) twins aged between 13 and 49 years were recruited for a serological characteristics study [3]. All of them live in the Sao Paulo area, and the material was collected and analyzed simultaneously for both members of a pair. No significant differences in age distributions among the four groups (24 MZ male, 23 DZ male, 34 MZ female, 24 DZ female) were found.

The RBC and WBC measurements were made using a Microcell Counter. The haemoglobin concentration was obtained by the oxi-haemoglobin spectrophotometer reading (Wintrobe), and the packed cell volume was determined by an International Micro Centrifuge. The variables mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were calculated from the first three measures. The platelets were obtained by the Rees-Ecker method. Reticulocytes were counted according to Dacie and Lewis (1968). Zygosity was determined by two independent methods: blood genetic markers (ABO, Rh, MNSs, Duffy, P, Kell and serum haptoglobins) and by dermatoglyphic analysis [1].

There were no significant differences in total variance of these hematological variables between MZ and DZ concordant sex twin pairs. The leucocyte variables, with the exception of monocytes and basophils, were log-transformed to normalize the distributions. All variables were standardized before the analysis.

Statistical Model

The genetic and environmental model employed in the univariate twin analysis recognizes three sources of variation: that due to genetic differences between individuals (h^2), that due to shared environmental influences (c^2), and that due to non-shared, or specific, enviromental influences (e^2). The models were fitted to observed 2x2 variance/covariance matrices of the MZ and DZ twin pairs, calculated separately for males and females, in order to assess the importance of sex differences in the mode of inheritance.

A maximum likelihood function was employed to evaluate goodness of fit and the LISREL package [6] was used to fit the model to the data. The following maximum-likelihood function was minimized:

$$F = \sum_{i=1}^{m} (N_i - 1.0)[\text{In } |E_i| - \text{In } |S_i| + \text{tr } (S_i E_i^{-1}) - p_i],$$

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where m is the number of observed and expected covariance matrices; p_i is the order of the $i^{th.}$ matrix; and S_i and E_i are observed and expected covariance matrices, the S_i having (N_i-1.0) degrees of freedom. This function is distributed asymptotically as a chi-square statistic, having the following degrees of freedom:

$$df = \sum_{i=1}^{m} [p_i(p_i + 1)/2] - n$$

where n is the number of estimated parameters. Functions difference between full and reduced models also yield χ^2 values, which have degrees of freedom equal to the difference between the number of parameters estimated by the two models. The testing procedure followed was to fit a full model, allowing different parameters for males and females initially, and then to fit reduced models and employ chi-square tests of the differences between models to arrive at a parsimonious description of the data. The method is described in detail elsewhere, [5], [9].

RESULTS

The means and standard deviations of the 15 variables, for men and women separately, are shown in Table 1. The means were significantly different between the sexes for all variables derived from red cell information (RBC, Hb, PCV, MCV, MCH, MCHC) with the exception of reticulocytes, but not for platelets and white cells (all types of leucocytes). However, the variances were not significantly different between sexes, even for the red cell variables. There were no significant differences in the variances of monozygotic and dizygotic twins. The covariance matrices were calculated for monozygous and dizygous twin pairs (male and female separately).

For all variables, we first fitted model 1, where the parameters h, c and e were estimated for males and females separately, and then model 2 was fitted, where the sexes were pooled. The χ^2 differences between models 1 and 2 were non-significant in all cases. Subsequently, the following constrained models were fitted: model 3, where $h = 0$, c and e estimated, model 4, where $c = 0$, h and e estimated, and model 5, where $h = c = 0$ and only e estimated. The resulting χ^2 of these models were then compared with the chi-squares for model 2. The results of the estimated parameters and χ^2 comparison for red cell variables are shown in Table 2, and those for white cells and platelets in Table 3. In both Tables it can be seen that models 1 and 2 fitted well for all variables with probabilities greater than 7%.

In Table 2, for all variables, the best fitting model is model 4, where h and e are estimated, and c is fixed to zero, i.e., shared environment has no significant influence. Moreover, the results of fitting model 5 allow us to reject the hypothesis that only the specific environment (e) is responsible for these variables, with the exception of reticulocytes. Thus, the red cell variables appear to be determined solely by genetic factors (h) and the specific environment (e). For red cell count (RBC), haemoglobin concentration (Hb) and mean cell haemoglobin concentration (CHCM), the estimated genetic components ($h^2 = 0.64$, 0.60 and 0.46, respectively) are statistically significant.

Since the phenotypic correlations between the first three variables (RBC, Hb and PCV) are very high (average 0.96), which indicates that these variables are probably

Table 1 - Mean and Standard Deviation (SD) for men and women (N = 105 pairs)

Variable	Units	Range	Men		Women	
			Mean	SD	Mean	SD
RBC	cell/l $\times 10^{12}$	3.8-5.9	5.10	0.31	4.74	0.31
Hb	g/100ml	10-17	15.2	0.99	13.9	0.71
PCV	%	0.34-0.52	0.46	0.03	0.42	0.03
MCV	fl	84-94	89.1	1.1	88.8	1.3
MCH	pg	26-32	29.7	0.6	29.3	0.6
MCHC	g/100ml	30-52	33.2	0.6	32.9	0.6
Reticulocytes	%	2-19	0.8	0.3	0.8	0.4
WBC	cell/l $\times 10^{9}$	3-12	6.99	1.47	6.96	1.70
(ln)			4.23	0.20	4.21	0.24
Lymphocyte	cell/mmc	850-5100	2244	651	2170	732
(ln)			7.68	0.29	7.63	0.33
Monocyte	cell/mmc	98-1020	376	160	374	177
Eosinophil	cell/mmc	40-1860	275	251	218	251
(ln)			5.30	0.78	5.01	0.80
Basophil	cell/mmc	0-112	27	34	24	33
Band	cell/mmc	40-1380	245	223	291	233
(ln)			5.19	0.78	5.36	0.82
Segmented	cell/mmc	1560-8520	3822	1084	3853	1279
(ln)			8.21	0.27	8.21	0.21
Platelet	cell/l $\times 10^{9}$	220-470	297	39	301	37

different measures of the same trait, we estimated the first principal component of the correlations among them, which we named erythron. We subsequently performed the same sequence of analyses as before for this measure. The results shown in Table 4 indicate a highly significant genetic component for this trait ($p<0.001$).

In Table 3, the results for leucocytes appear to be less uniform, as some variables seem to be subject to genetic influences, while others show shared environmental influences, or only specific environmental effects. WBC, lymphocytes, monocytes and eosinophils indicate the presence of some genetic component, since the most parsimonious model is number 4, although the estimated parameter h^2 is not significant. But, for band and segmented neutrophils, the best model is number 3, where the genetic effect is small or zero, indicating that only the specific and shared environment are influencing these traits. For segmented neutrophils, we found a shared environmental component (c^2) of 0.49, and when c was fixed to zero (model 4), the χ^2 was significant ($\chi^2=4.59$, $p<0.05$). For eosinophils, athough the best model is model 4, model 5 ($h=c=0$) does not differ significantly from model 2, which could indicate that this variable is determined by specific environment only, with little or no genetic effects. In the case of basophils also, it seems that specific environment alone is responsible for variation in the number of cells, as model 5 does not differ significantly from model 2.

Table 2 - Model comparisons for the variables from the red cells

Variable	Model	h^2	c^2	e^2	χ^2	df	p	Dif.χ^2	df	p
RBC	1 (male)	.54	.09	.37	9.64	6	0.14			
	(female)	.67	.00	.33						
	2	.64	.01	.34	11.12	9	0.26	1.48	3	ns
	3	–	.53	.47	16.98	10	0.07	5.86	1	<.025
	4	.65	–	.35	11.12	10	0.34	0.00	1	ns
	5	–	–	1.0	71.62	11	0.00	10.50	2	<.001
Hb	1 (male)	.67	.00	.33	8.38	6	0.21			
	(female)	.52	.00	.48						
	2	.60	.00	.40	10.14	9	0.33	1.76	3	ns
	3	–	.47	.53	15.22	10	0.12	5.08	1	<.025
	4	.60	–	.40	10.14	10	0.42	0.00	1	ns
	5	–	–	1.0	42.92	11	0.00	32.78	2	<.001
PCV	1 (male)	.49	.13	.38	14.09	6	0.29			
	(female)	.34	.30	.35						
	2	.44	.19	.37	15.53	9	0.07	1.44	3	ns
	3	–	.55	.45	18.48	10	0.04	2.95	1	ns
	4	.64	–	.36	16.01	10	0.09	0.47	1	ns
	5	–	–	1.0	56.18	11	0.00	40.65	2	<.001
MCV	1 (male)	.55	.00	.45	2.00	6	0.92			
	(female)	.34	.03	.62						
	2	.41	.00	.59	2.93	9	0.96	0.93	3	ns
	3	–	.31	.69	4.80	10	0.90	1.87	1	ns
	4	.41	–	.59	2.93	10	0.98	0.00	1	ns
	5	–	–	1.0	14.70	11	0.19	11.77	2	<.005
MCH	1 (male)	.31	.00	.69	5.48	6	0.48			
	(female)	.52	.11	.37						
	2	.49	.04	.47	8.79	9	0.45	3.31	3	ns
	3	–	.39	.61	11.06	10	0.35	2.27	1	ns
	4	.51	–	.49	8.80	10	0.55	0.01	1	ns
	5	–	–	1.0	36.84	11	0.00	27.05	2	<.001
MCHC	1 (male)	.07	.16	.77	10.06	6	0.12			
	(female)	.63	.00	.37						
	2	.46	.00	.54	14.02	9	0.12	3.96	3	ns
	3	–	.31	.69	18.09	10	0.05	4.03	1	<.05
	4	.46	–	.54	14.02	10	0.17	0.00	1	ns
	5	–	–	1.0	28.22	11	0.00	14.20	2	<.001
Reticuloc.	1 (male)	.29	.00	.71	1.91	6	0.93			
	(female)	.00	.15	.85						
	2	.13	.07	.80	2.61	9	0.97	0.70	3	ns
	3	–	.18	.82	2.71	10	0.98	0.10	1	ns
	4	.21	–	.79	2.66	10	0.98	0.05	1	ns
	5	–	–	1.0	5.84	11	0.88	3.23	2	ns

Models:
1 - h, c, e; male and female separately
2 - h, c, e; sexes pooled
3 - c, e, h = 0
4 - h, e, c = 0
5 - e, h = c = 0

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Table 3 - Model comparisons for variables from the white cells and platelets

Var.	Model	h^2	c^2	e^2	χ^2	df	p	Dif.χ^2	df	p
WBC	1 (male)	.60	.00	.40	11.71	6	0.07			
	(female)	.24	.24	.52						
	2	.37	.14	.49	14.10	9	0.11	2.39	3	ns
	3	–	.41	.59	15.63	10	0.11	1.53	1	ns
	4	.53	–	.47	14.42	10	0.15	0.32	1	ns
	5	–	–	1.0	34.39	11	0.00	20.29	2	<.001
Lymphoc.	1 (male)	.34	.00	.66	2.44	6	0.88			
	(female)	.29	.16	.55						
	2	.42	.00	.58	3.77	9	0.92	1.33	3	ns
	3	–	.30	.70	5.52	10	0.85	1.75	1	ns
	4	.42	–	.58	3.77	10	0.95	0.00	1	ns
	5	–	–	1.0	17.21	11	0.10	13.44	2	<.005
Monoc.	1 (male)	.48	.00	.52	8.08	6	0.23			
	(female)	.29	.00	.71						
	2	.37	.00	.63	9.50	9	0.39	1.42	3	ns
	3	–	.24	.76	11.98	10	0.28	2.48	1	ns
	4	.37	–	.63	9.50	10	0.48	0.00	1	ns
	5	–	–	1.0	40.53	11	0.00	31.03	2	<.001
Eosinop.	1 (male)	0.03	.17	.80	3.37	6	0.76			
	(female)	.24	.00	.76						
	2	.24	.00	.76	3.59	9	0.93	0.22	3	ns
	3	–	.18	.82	3.97	10	0.93	0.38	1	ns
	4	.24	–	.76	3.59	10	0.96	0.00	1	ns
	5	–	–	1.0	7.13	11	0.78	3.54	2	ns
Basop.	1 (male)	.28	.00	.72	4.56	6	0.60			
	(female)	.00	.15	.85						
	2	.00	.07	.93	7.48	9	0.58	2.92	3	ns
	3	–	.07	.93	7.48	10	0.68	0.00	1	ns
	4	.06	–	.94	7.70	10	0.65	0.22	1	ns
	5	–	–	1.0	7.95	11	0.71	0.47	2	ns
Band	1 (male)	.00	.35	.65	4.42	6	0.62			
	(female)	.00	.44	.56						
	2	.00	.40	.60	4.73	9	0.85	0.31	3	ns
	3	–	.40	.60	4.73	10	0.90	0.00	1	ns
	4	.41	–	.59	7.79	10	0.65	3.06	1	ns
	5	–	–	1.0	22.18	11	0.02	17.45	2	<.001
Segm.	1 (male)	.56	.01	.43	7.97	6	0.24			
	(female)	.00	.52	.48						
	2	.00	.49	.51	11.16	9	0.26	3.19	3	ns
	3	–	.49	.51	11.16	10	0.26	0.00	1	ns
	4	.53	–	.47	15.74	10	0.10	4.59	1	<.05
	5	–	–	1.0	38.33	11	0.00	27.17	2	<.001
Platelet	1 (male)	.19	.36	.44	9.99	6	0.12			
	(female)	.01	.46	.53						
	2	.10	.41	.49	10.60	9	0.30	0.61	3	ns
	3	–	.49	.51	10.71	10	0.38	0.11	1	ns
	4	.52	–	.48	12.36	10	0.26	1.76	1	ns
	5	–	–	1.0	38.32	11	0.00	27.72	2	<.001

Table 4 - Model comparisons for erytron

Var.	Model	h^2	c^2	e^2	χ^2	df	p	Dif.χ^2	df	p
Erytron	1 (male)	.61	.05	.34	10.81	6	0.10			
	(female)	.53	.05	.41						
	2	.59	.04	.37	11.78	9	0.22	0.97	3	ns
	3	–	.00	1.0	50.22	10	0.00	39.41	1	<.001
	4	.63	–	.37	11.80	10	0.29	0.02	1	ns
	5	–	–	1.0	47.69	11	0.00	36.88	2	<.001

DISCUSSION

The results of these analyses indicate that the variables related to red cells were influenced by genetic factors, with no evidence for shared environment. In addition, the results for the composite measure erythron suggest that number of red cells, packed cell volume and haemoglobin concentration are related to the same common factors, genetic and environmental.

However, the different types of white cells gave differing results; some of them appear to have a genetic component, while others show a shared environment effect, or merely a specific environmental one. These results indicate clear-cut genetic influences on red cell variables, but for the different types of white blood cells genetic influence is not so clear. Leucocytes are divided into two families, mononuclear (lymphocytes and monocytes) and polymorphonuclear (neutrophils, eosinophils and basophils), also called granulocytes [7]. It seems that lymphocytes and monocytes, which are more related to immunological response, take some time to appear in the circulation, as a consequence of an immunization by antigens. This group of leucocytes can live for months or even years, and is subject to genetic and specific environmental influences. On the other hand, band and segmented neutrophils, which are subject to a faster response when stimulated by an antigen, are subject to shared and specific environmental control, but not to genetic influences. Eosinophils and basophils, which are related to the individual hypersensitivity reactions, are solely determined by specific environment effects.

Our results for red cell variables agree with the results of earlier analyses [10] in most instances, except in the case of platelets, for which a heritability of 0.86 was previously found, while we found a non-significant $h^2 = 0.10$. Those results for the several types of leucocytes which appear to have a plausible biological explanation, are new in the published literature, and should be replicated with a larger sample.

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