Original Articles

POLYMORPHONUCLEAR LEUKOCYTE FUNCTIONS IN CHILDREN WITH CYANOTIC AND ACYANOTIC CONGENITAL HEART DISEASE

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ABSTRACT

Eighteen cyanotic congenital heart disease (CCHD) and 17 acyanotic congenital heart disease (ACHD) patients in the age range of 2 months to 10 years along with their age and nutrition matched controls were studied for bactericidal, chemotactic and phagocytic functions. Bactericidal and phagocytic functions were significantly depressed in CCHD ($p < 0.001$) as well as ACHD group ($p < 0.001$) compared with controls. Chemotactic function was not significantly affected in either. Arterial oxygen content (as a measure of hypoxia) was calculated for each patient and correlated with each immune parameter by univariate linear regression analysis. In CCHD patients linear correlation of borderline significance ($p = 0.07$) was found between arterial oxygen content and bactericidal activity, but no correlation could be established with phagocytic and chemotactic functions. No correlation was obtained between hematocrit and any of the immune parameters. In ACHD patients no correlations were obtained between the immune parameters and arterial oxygen content or hematocrit. Iron deficiency anemia, known to affect bactericidal function, did not seem to affect the immune parameters in CCHD and ACHD groups. Altered oxygen content of the blood owing to hypoxia in CCHD patients may be an important etiological factor in the genesis of bacteremia and cerebral abscess. The affection of immune functions in ACHD cannot be adequately explained.

Key words: *Congenital heart disease, Bactericidal index, Phagocytic index, Chemotactic index, Polymorphonuclear leukocytes.*

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Received for publication: May 22, 1992;
Accepted: January 15, 1993

The congenital heart disease present mainly in the pediatric age group. Infective complications are an important cause of morbidity and mortality in these patients(1). In cyanotic congenital heart disease (CCHD), brain abscess occurs in 2% of patients after 2 years of age(2-6). Infective endocarditis is not infrequent in patients with Tetralogy of Fallot(6). In acyanotic congenital heart disease (ACHD) patients, infective endocarditis (2-3.7% of ventricular septal defect patients)(7) and recurrent lower respiratory tract infections(8,9) are commonly seen.

Brain abscesses are frequent in CCHD with decreased pulmonary blood flow, classically explained(2,5,6) by: (*i*) brain cellular hypoxia created by the cyanotic state and hyperviscosity of polycythemic blood(10), leading to infarction and encephalomalacia –serving as an inviting nidus for colonization by bacteria, and (*ii*) bypassing of the lung phagocytic mechanism of blood borne bacteria.

In ACHD with left to right shunts, increased pulmonary blood flow causes 'flooding of the lung', presumably predisposing to disruption of the local immune and mucocilliary clearance mechanisms. Pulmonary compliance is reduced(11). With heart failure and pulmonary venous congestion, incidence of respiratory infections increases(8). No clear immune mechanisms have been postulated for the same. Infective endocarditis in most ACHD and CCHD patients has been ascribed mainly to cardiac hemodynamic factors(12,13). The present study examines the role of leukocyte functions in congenital heart disease and of chronic hypoxia as a mediator of immune dysfunction in patients with CCHD. The effect of hypoxia on immune functions has been studied in wounds and *in vitro*(14) but not in a systemic disorder like CCHD. There are no studies of this nature in world literature.

Material and Methods

Patient Selection and Clinical Methods

This study involved CCHD and ACHD patients admitted to a general hospital over a period of 2 years. The age range of patients was 2 months to 10 years. Neonates were excluded from the study. Each patient in both the CCHD and the ACHD groups was age-matched and nutrition-matched with a normal control without cogenital heart disease. Nutrition matching was done using weight by application of the Indian Academy of Pediatrics grading of Protein Energy Malnutrition, using 50th percentile of the Harvard Standard. This was particularly necessary to control for the effects of malnutrition on leukocyte functions.

Patients with major infections (diagnosed by complete hemogram, ESR, plain roentgenogram of the chest and where appropriate, blood cultures and urine cultures), congestive cardiac failure, severe malnutrition (Grade IV of IAP classification) and patients on drug therapy especially antimicrobials and steroids were excluded for similar reasons.

Clinical details recorded included anthropometric measurements, history, clinical findings, radiological findings and findings of cardiac investigations (ECG, 2-D Echocardiogram and cardiac catheterization). 2-D Echocardiography was taken as the basic investigation to confirm the clinical diagnosis when cardiac catheterization was not possible.

Hb, PCV and arterial blood gas (for Sat O_2%) were obtained for all patients. Arterial oxygen content in "ml O_2/100 ml blood" units was calculated using the formula $(Hb \times 1.356 \times Sat\ O_2)/100$(15,16). Two ml of plain blood was collected separately from the patients and their paired controls for the following immunological parameters: (*i*) Phagocytic assay, (*ii*) Bactericidal assay, and (*iii*) Chemotactic assay.

Laboratory Methodology

Phagocytic and bactericidal assays(17): For phagocytic and bactericidal assays, approximately three drops (0.1 ml) of plain whole blood were placed on Bovine Serum Alubumin-coated coverslips placed on saline-moistened filter papers in a petridish. A modification of the acridine orange fluorochrome microassay using opsonized *S. aureus* culture stained with acridine orange dye was performed. The coverslips were examined under an ultraviolet fluorescent microscope. The total number of polymorphs that had engulfed the bacteria out of a total of 100 polymorphs counted was expressed as a percentage and called the Phagocytic Index (PI).

Acridine orange fluoresces green when binding dsDNA and orange red with ssDNA. Thus, bacteria seen as green are viable and

those seen red are non-viable. Bactericidal Index (BI) was calculated as follows.

$$BI = \frac{\text{Number of red bacteria in 100 PMN}}{\text{Number of red and green bacteria in 100 PMN}} \times 100$$

Chemotactic assay to assess the Chemotactic Index (CI) was done using the method of Ternowitz *et al.*(18) using Teflon chambers, chemoattractant N-Formyl Methionyl Leucyl Phenylalanine (SIGMA Labs) and filters (0.45 μ SARTORIUS, INDIA: 2.0μ NUCLEOPORE CORP, USA).

Statistical Methods

LOTUS/EPISTAT computer programmes were used to analyse the data. Values of PI, BI and CI in CCHD and ACHD groups were compared with their paired controls and with each other using the Students' unpaired 't' test.

CCHD groups were subdivided based on Hb values–Hb < 15 g/dl and Hb ≥15 g/dl in CCHD group and Hb < 10 g/dl and Hb > 10 g/dl in ACHD group. Immune parameters in these subgroups were compared again using Student's unpaired 't' test in order to study the effect of iron deficiency on the immune parameters.

PI, BI and CI in both CCHD and ACHD groups were computed independently against Hb, PCV and arterial oxygen content employing univariate linear regression analysis.

Results

Eighteen patients of CCHD and 17 patients of ACHD were studied. Age range of the patients and controls was from 2 months to 10 years. In CCHD group, Tetralogy of Fallot had the highest incidence (61%) whereas in the ACHD group, VSD (41.1%) had the highest incidence (*Table I*). Bactericidal and phagocytic indices were significantly depressed in CCHD ($p<0.001$) as well as ACHD group ($p<0.001$) compared with controls. Chemotactic function was not significantly depressed in either the CCHD group ($p=0.09$) or the ACHD group ($p=0.5$) when compared with controls (*Table II*). All the three immune parameters expressed as mean percentage of values revealed no statistically significant difference between the ACHD and CCHD groups.

The mean Hb value in CCHD patients was 15.03 g/dl and in ACHD patients 12.37 g/dl. The mean value of PCV in CCHD patients was 49.22 and in ACHD patients 34.94. The mean value of arterial oxygen content in CCHD patients was 13.28 ml O_2/100 ml blood and in ACHD patients 15.73 ml O_2/100 ml blood.

In patients with CCHD a linear correlation of borderline significance ($p=0.07$) was found between arterial oxygen content and BI (*Fig. 1*) but not with PI and CI. No correlation was found between PCV and the immune parameters.

In patients with ACHD, no correlations were obtained between the immune parameters and arterial oxygen content or PCV.

Iron deficiency anemia did not seem to affect the immune parameters in CCHD and ACHD groups as shown in *Table III*, where the immune parameters have been compared in patients with Hb < 15 g/dl and Hb ≥15 g/dl in the CCHD group and in patients with Hb≤10 g/dl and > 10 g/dl in the ACHD group. The p value for both these correlations was not significant.

Discussion

Of the infections commonly seen in CCHD patients, brain abscesses have been studied by most workers(2,3). Matson and Salam(3) reported 13 cases of brain abscess in patients with CCHD seen between 1946 and 1956 at Harvard Medical School. Their

TABLE I–*Cardiac Malformations in Patients Studied*

(A)	*Cyanotic Congenital Heart Diseases* (n=18)		
	Tetralogy of Fallot	11	61%
	Transposition of great arteries and associated defects.	3	16%
	Other including double outlet right ventricle with pulmonary stenosis, total anomalous pulmonary venous connection	4	23%
(B)	*Acyanotic Congenital Heart Disease* (n=17)		
	Ventricular septal defect	7	41.1%
	Atrial septal defect	3	17.6%
	Aortic stenosis	2	11.8%
	Patient ductus arteriosus	3	17.6%
	Double outlet right ventricle without pulmonary stenosis	1	5.8%
	Valvular pulmonary stenosis	1	5.8%

TABLE II–*Phagocytic Index, Bactericidal Index and Chemotactic Index in Patients with CCHD, ACHD and Their Matched Controls*

	Phagocytic index		Bactericidal index		Chemotactic index	
	CCHD	Controls	CCHD	Controls	CCHD	Controls
Mean	64.17	76.61	58.79	76.08	1.60	1.62
Median	64.5	78	59.6	76.25	1.47	1.45
SD	4.84	3.64	5.2	2.38	0.65	0.87
	($p<0.001$)		($p<0.001$)		($p=0.9$)	
	ACHD	Controls	ACHD	Controls	ACHD	Controls
Mean	66.23	76.47	57.85	76.31	1.42	1.32
Median	67.00	78.00	59.20	75.90	1.10	1.20
SD	4.04	3.24	4.41	2.22	0.50	0.42
	($p<0.001$)		($p<0.001$)		($p=0.5$)	

TABLE III—*Analysis of Hb Values in CCHD and ACHD*

CCHD	Hb <15 g/dl (n=9) Mean ± SD	Hb >15 g/dl (n=9) Mean ± SD
P.I. *	63 ± 4.4	65.33±4.71
B.I. *	57.7±4.19	60.32±5.37
C.I. *	1.64±0.6	1.57±0.6

ACHD	Hb ≤ 10 g/dl (n=3) Mean ± SD	Hb >10 g/dl (n=14) Mean ± SD
P.I. *	65.7 ± 4.49	66.35 ± 3.77
B.I. *	56.7 ± 4.58	58.11 ± 4.16
C.I. *	1.6 ± 0.45	1.38 ± 0.49

* p = not significant.

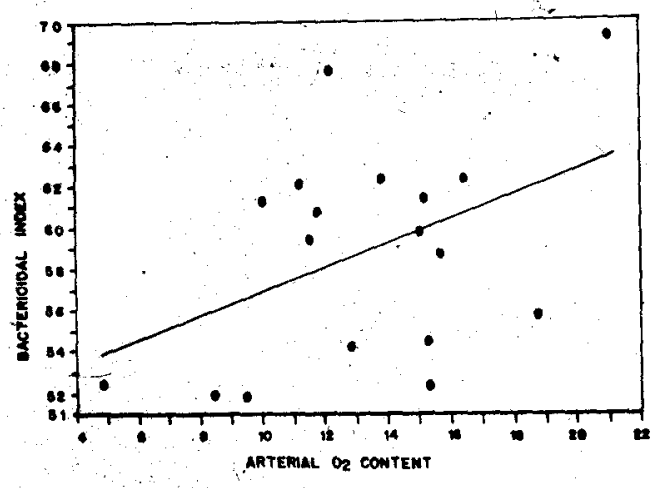

Fig. 1. Correlation of arterial oxygen content with bactericidal index (BI) using univariate regression analysis shows a linear regression of borderline significance (p=0.07). Arterial oxygen content is expressed in 'ml O_2/ 100 ml blood' units. Bactericidal index is expressed as a percentage.

report emphasized on improved prognosis with surgery. The etiological explanations offered have been discussed earlier(2).

Fischbein *et al.*(2) studied 26 cases of CCHD and brain abscess between 1960 and 1973 and compared their data with that of a control group with CCHD without brain abscess. They concluded that both morbidity and mortality of brain abscesses were inversely related to oxygen saturation levels, thus pointing to hypoxia as a major risk factor in the development and prognosis of brain abscess. They also suggested that hyperviscosity associated with polycythemia could be another important risk factor in the development of cerebral abscess.

None of the studies done so far have evaluated the role of defects in limbs of the immune system as a possible risk factor predisposing patients with CCHD to infections. This study evaluated the nonspecific limb of the immune system, namely chemo-

taxis, phagocytosis and intraleukocytic bactericidal activity of polymorphonuclear leukocytes–in CCHD and ACHD patients and attempted to evaluate the role of hypoxia (by measure of arterial oxygen content) in affecting these parameters.

Arora *et al.* have studied immunoglobulins, C_4 and T and B cell percentage in 37 acyanotic and 13 cyanotic children aged 1 to 132 months who were age and sex matched with controls. Cyanotic heart disease patients had higher IgM and B cell percentage versus acyanotic heart disease patients and lower C_4 values(19). This study does not control for PEM. It also does not take into consideration the wide ranges of immunoglobulin levels at differing ages. The study does not probe the role of chronic desaturation and immune function.

It has been conclusively proved by this study that there is a highly significant depression of phagocytic and bactericidal functions in both CCHD and ACHD patients suggesting that these may play a very basic role in the genesis of susceptibility to bacteremia and brain abscess.

The study has also revealed that with a reduced arterial oxygen content in CCHD patients, but not in ACHD patients, the bactericidal activity is correspondingly reduced. *Fig. 1* demonstrates the linear regression with equal scatter of values and the correlation could assume clear significance by a similar analysis of a larger group of patients. This suggests that chronic hypoxia could be the basis for immune dysfunction in CCHD patients. Furthermore, bactericidal capacity is the only parameter where a burst of oxygen is needed(20-22). The depression of phagocytosis in both CCHD and ACHD groups has not significantly correlated with the arterial oxygen content.

It may be possible, therefore, to ascribe immune dysfunction in CCHD patients to hypoxia. In patients with ACHD alternative explanations have to be explored.

Protein energy malnutrition is known to affect bactericidal function(23). Since patients were carefully selected to control for grade of PEM, effects of PEM *per se* can be ruled out as an alternative explanation.

Iron deficiency anemia is also known to depress bactericidal function, but only at Hb values of less than 10 g/dl(23). Analysis based on Hb values do not show any significant role of iron deficiency in affecting the immune parameters. For the ACHD group the cut-off Hb value of 10 g/dl was taken in compliance with the previous studies(24-25). For the CCHD group, Hb value of 15 g/dl would still constitute anemia and hence was taken as a cut-off Hb value. No analysis of iron deficiency anemia is complete without a study of parameters like MCV, serum iron levels or parameters of tissue iron storage like serum ferritin and hence, there are reservations about this particular conclusion.

Other nutritional factors which could be important are zinc(26), pyridoxine deficiency and vitamin A deficiency. Stress(27) as a cause of immune dysfunction is dificult to measure.

A possible cycling of events as a hypothesis for immune dysfunction in CCHD and ACHD is shown in *Fig. 2*. It is difficult to explain the immune function depreciation found in ACHD in this study satisfactorily on the basis of the present data.

If the possible involvement of chronic hypoxia in immunity in CCHD can be substantiated, the therapeutic implications for early surgical intervention are obvious in the reduction of morbidity and mortality from these diseases.

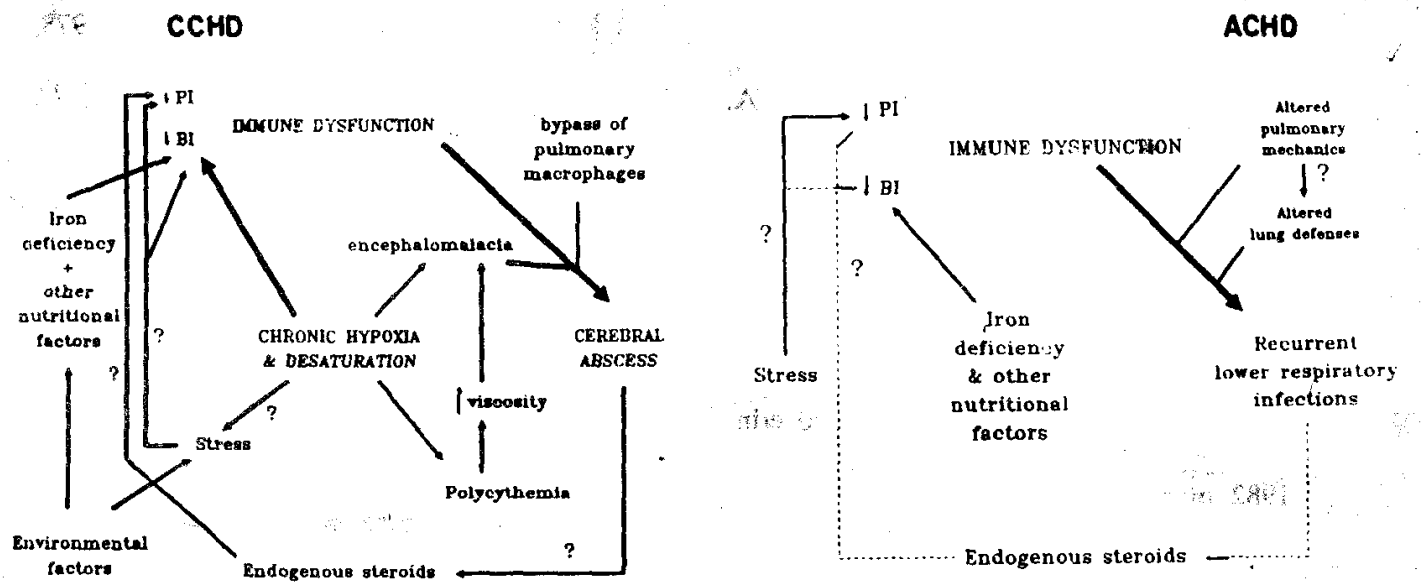

Fig. 2. Possible interplay of factors predisposing to infections in CCHD and ACHD patients.

Acknowledgements

The authors are grateful to Dr. (Mrs) P.M. Pai, Dean, Seth G.S. Medical College for granting permission to publish this article and to Drs. B.V. Dalvi and Karnad of the Departments of Cardiology and General Medicine, respectively for aiding in the statistical analysis.

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