CHAPTER III

RESULTS

A. Patients.

The clinical and routine laboratory data collected systematically according to a set of criteria were used to catagorize the patients into 3 groups according to the diagnostic criteria described above.

1. Tuberculous group.

Ten patients were diagnosed as having tuberculous effusion. The male:female ratio in this group was 1:1 (table 2) and the mean age was 43±17(range 24-62) The details of the diagnostic criteria of the patients assigned to this group were shown in Table 3 . Diagnosis of tuberculous effusion in the studied group based on the major criteria was not found in any patient. The diagnosis was base on minor criteria only. All patients had exudative pleural fluid with predominant MNC and clinical response to anti-tuberculous treatment within 3 months. Only 6 cases, or 60% of the group, had concomitant lung lesions. 80% of cases were tuberculin skin test positive. Fine needle pleural biopsy revealed granulomatous lesion in 7 cases and one out of these had caseating granuloma. There were 2 patients in whom detected AFB on sputum smears and Mycobacterium tuberculosis were positive from sputum cultures.

Table 2. Sex and age of the 3 study groups.

Patients	N	Sex ratio (male:female)	Average age X±SD	Age range
Tuberculuos group	10	1:1	43±17	24-62
Non-tuberculous group	15	1.5:1	56±15	31-76
Non-specific group	5	1.5:1	42±14	23-58

Table 3. Diagnostic details of the tuberculous group.

	Major criteria	Minor criteria				
	pleural fluid AFB &/or culture	exudative effusion predominant MNC	concomitant lung lesion	PPD skin test (mm.)	Biopsy Granuloma	improvement with Anti-TB Px
1	-	+	+	+(15)	+	+
2	-	+	-	+(12)	+	+
3	-	+	+	-(4)	+ (caseous)	+
4	-	+	+	+(12)	-	+
5	-	+	- **	+(13)	+	+
6	-	+	+ **	+(18)	-	+
7	-	+	+	+(17)	-	+
8	-	+	-	+(15)	+	+
9	-	+	-	+(12)	+	+
10	-	+	+	-(8)	+	+
Total positive	0/10	10/10	6/10	8/10	7/10	10/10
Percent positive	0%	100%	70%	80%	70%	100%

** = Sputum positive for AFB & culture.

Not include in the diagnostic criteria was sputum AFB positivity and positive Mycobacterium tuberculosis sputum cultures. These were positive in 2 patients, one even without demonstrable concomitant lung lesion (patient No. 5)

2. Non-tuberculous group.

Fifteen patients who had other obvious causes for their pleural effusion were summarized in Table 4. All but one of these patients had malignant pleural effusions proved either by pleural biopsy or by cytology smear. One patients had congestive heart failure as the cause of his pleural effusion. The male:female ratio of this group was 1.5:1 and the age range was 31-76 years with mean of 56±15 years (table 2).

3. Non-specific group.

Five patients who could not fulfil diagnostic criteria for tuberculous effusion and had no other obvious pleural effusion were collectively grouped into the non-specific group. The clinical and laboratory finding of these patients are in table 5. All patients had exudative pleural fluids with predominant MNC and clinical response to anti-tuberculous treatment. Only one patient had a positive tuberculin test, and another had caseating granuloma on pleural biopsy. The male:female ratio of this group was 1.5:1 and the average age was 42±14 years (Table 2).

Table 4. Details of clinical diagnosis of the non tuberculous group.

Final diagnosis	Number (total = 15)
Carcinomatous effusion:	
-Squamous cell carcinoma	4
-Adenocarcinoma	5
-Undifferentiated cell carcinoma	4
Lymphoma with pleural effusion	1
Congestive heart failure with pleural effusion	1

Table 5. Detailed clinical and laboratory data of the patients with pleural effusion designated as the " non-specific group".

	Major criteria	Minor criteria				
	pleural fluid AFB &/or culture	exudative effusion predominant MNC	concomitant lung lesion	PPD skin test (mm.)	Biopsy Granuloma	improvement with Anti-TB Px
1	-	+	-	-(0)	-	+
2	-	+	-	ND	-	+
3	-	+	-	ND	-	+
4	-	+	-	ND	+ (caseous)	+
5	-	+	-	ND	-	+

B. Characterization of lymphocytes and lymphocyte subpopulations.

1. White blood cell count and differential cell count (Table 6).

As shown in Table 6, the pleural fluid leukocyte counts and the differential cell counts could not be used to differentiate tuberculous from non-tuberculous (malignant) pleural effusions. The percentage of lymphocytes in pleural fluids was significantly higher than in peripheral blood (Figure 10 & Table 6). How ever, when the absolute lymphocyte count was calculated, There was no significant difference between the absolute number of lymphocytes in the pleural fluid and in the peripheral blood of both tuberculous and non-tuberculous groups (Figure 11). The percentage and absolute number of pleural fluid and peripheral blood lymphocytes of the tuberculous group from figure 10 and 11 are summarized in Figure 12 which is a three-dimensional graphic presentation. The relative numbers of lymphocytes of both pleural fluid and blood in the Y axis are illustrated with the absolute number in the Z axis. In addition, regression coefficient (r) analysis of the leukocyte count, the percentage of lymphocytes and the absolute lymphocyte count between pleural fluid and peripheral blood, showed no corelation in either the tuberculous or the non-tuberculous groups (Table 6 and Figure 13).

Table 6. WBC and differential cell count in pleural fluid and blood.

Patient	WBC (cells/ mm.)	% Neutrophil	% Lymphocyte	% Monocyte	Absolute lympho-cyte count
Tuberculous group					
-pleural fluid (10)**	1910 ±1427 *	4.7±5.5	93.6±5.1	1.6±1.5	1800 ±1300
-blood (9)	7700 ±933	68±7	28.3±6.6	3±2	2100 ±600
Non-tuberculous group					
-pleural fluid (14)	1916 ±2251	28±34	69.4±33.9	2±2	1100 ±800
-blood (11)	8918 ±2300	77±8	20.2±7.6	2±1	1700 ±450
Non-specific group					
-pleural fluid (5)	1080 ±563	13±13	83.6±14	3±1	900 ±500
-blood (3)	7500 ±970	70±2	29.3±0.6	1±1	2200 ±300
Normal control (12)	8600 ±2400	—	36±7	—	3100 ±1200

* = $\bar{X}\pm SD$
** = Number performed

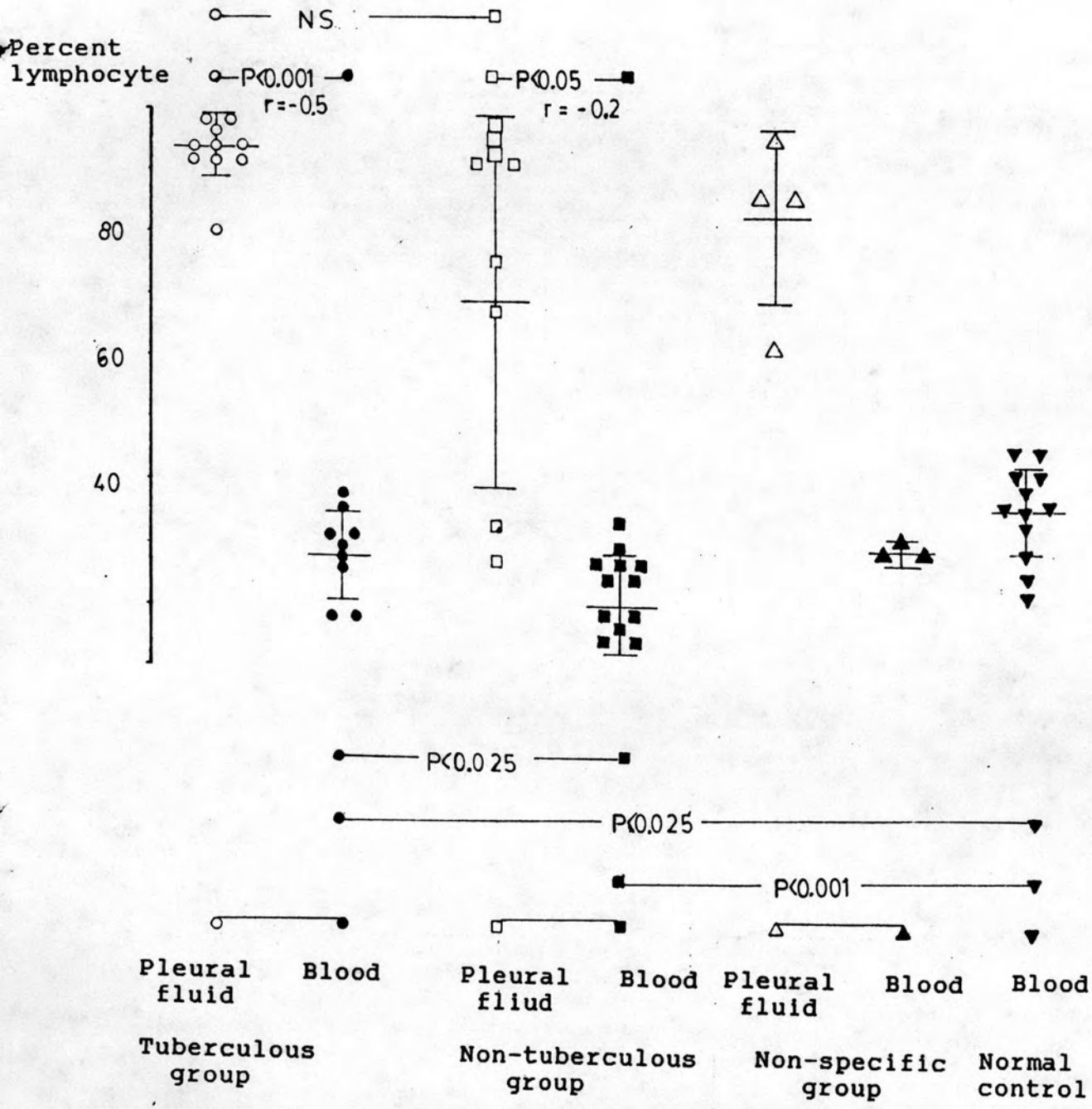


Figure 10.. Percentage of lymphocytes in pleural fluid and peripheral blood.

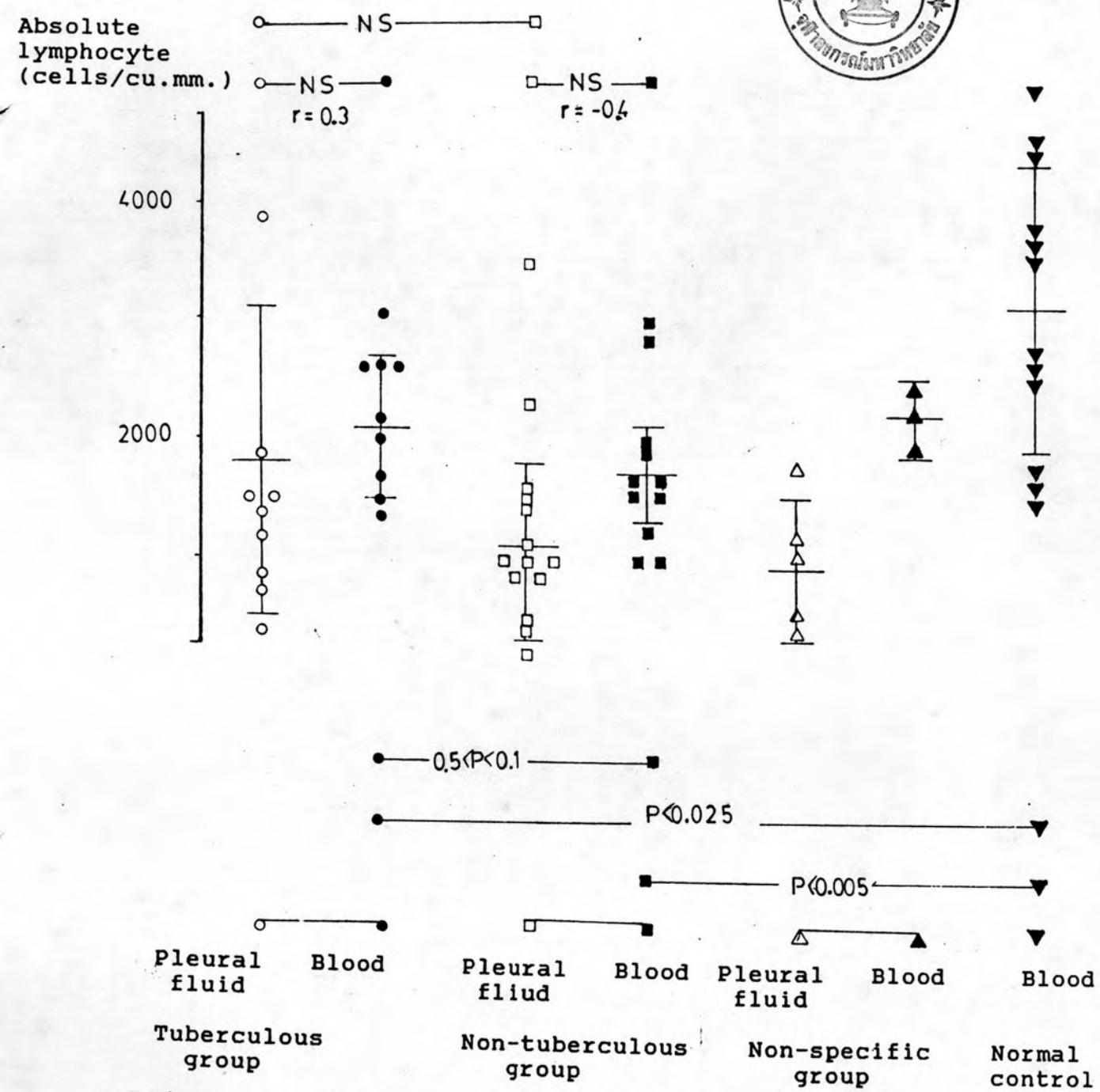


Figure 11. Calculated absolute number of lymphocytes in pleural fluid and peripheral blood.

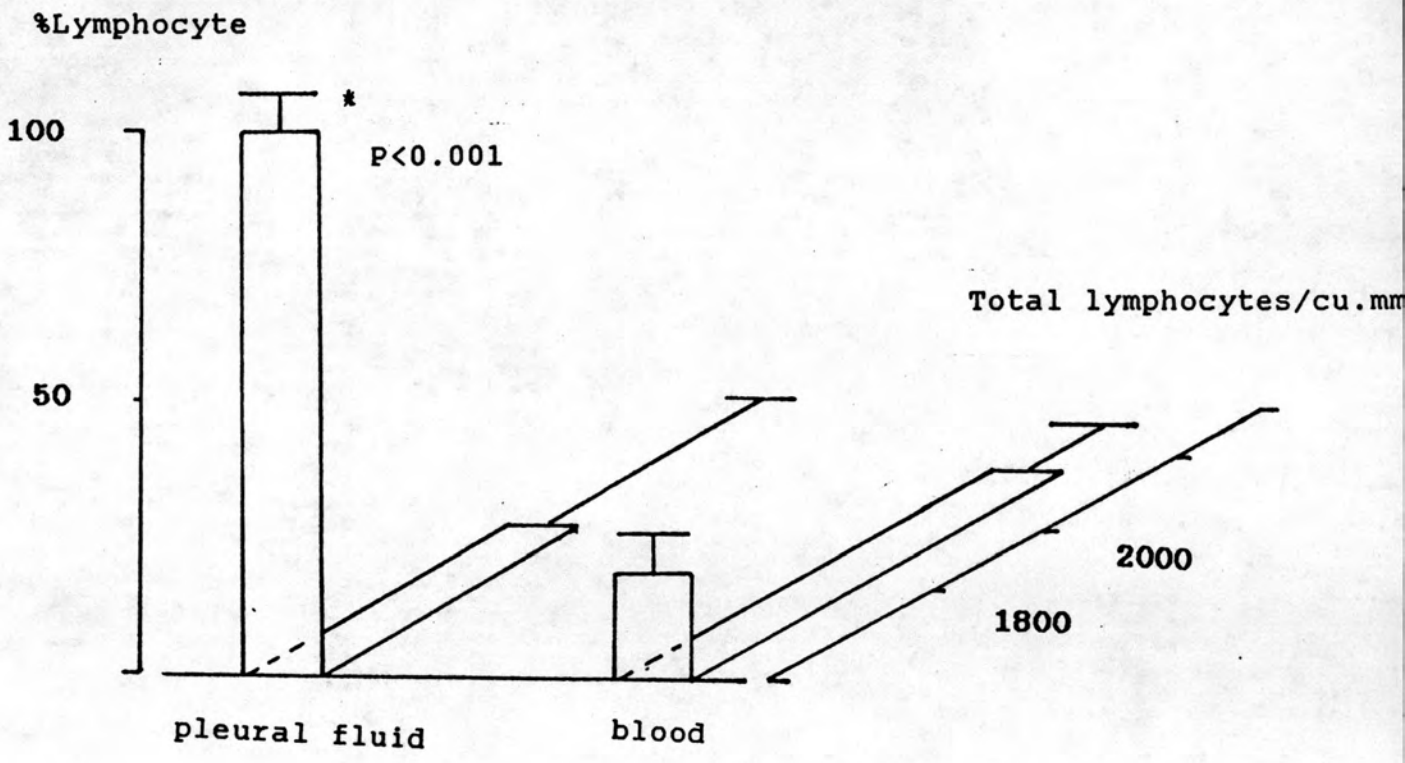


Figure 12. Percentage and absolute number of lymphocytes in the tuberculous pleural fluid and the blood.

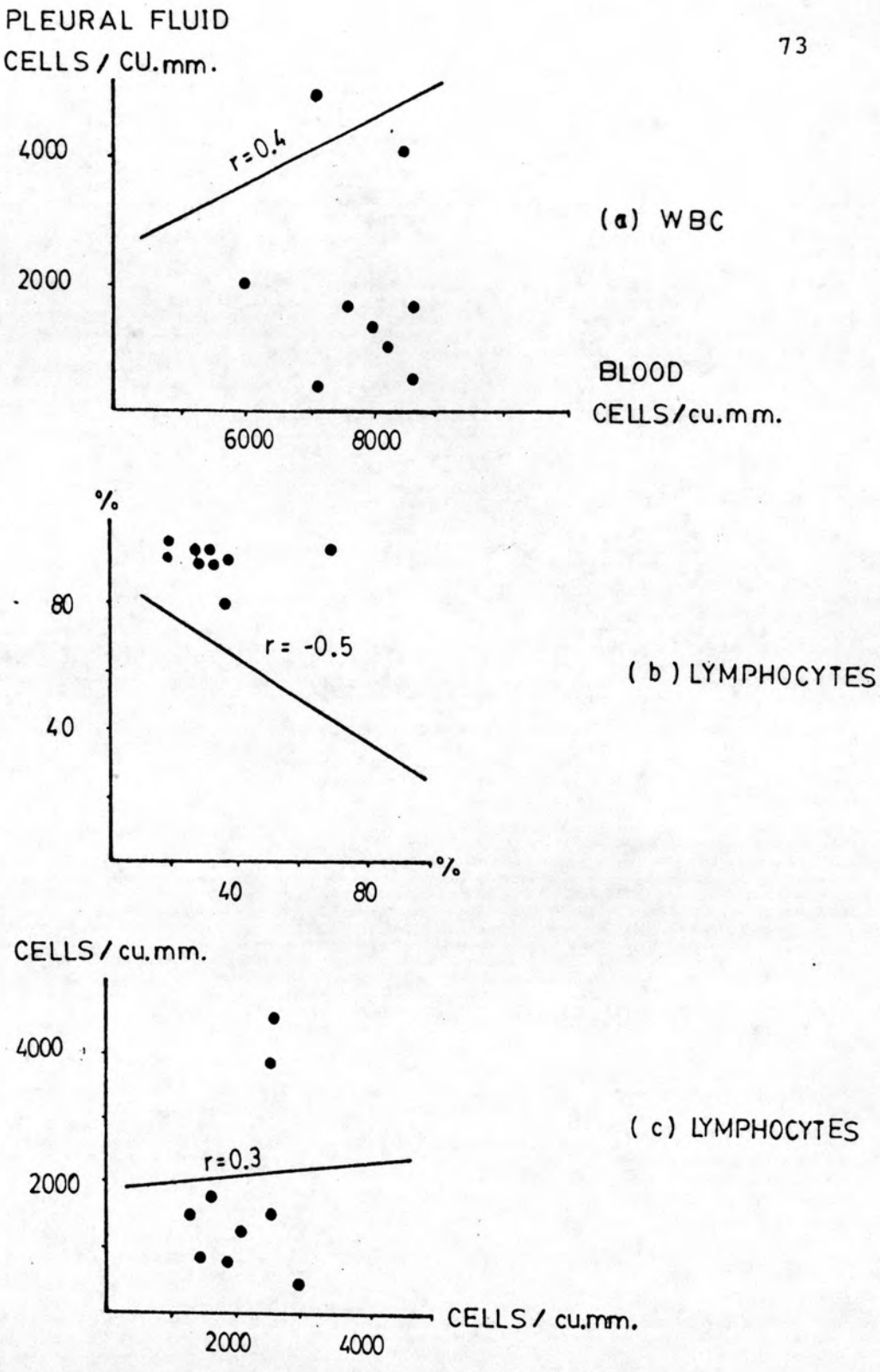


Figure 13. The regression line of tuberculous Pleural fluid WBC, Percent lymphocytes, absolut number of lymphocytes compared with blood.

2. Enumeration of T-lymphocytes.

Similar to the lymphocyte count, the percentages of pleural fluid T-cells in all 3 groups were significantly higher than those of peripheral blood (Figure 14, $P<0.001$) but the absolute number of T-cells in these 2 compartments were not significantly different (Figure 15).Therefore, the analysis of regression coefficient of the percentage and the absolute number of E-RFC+ between PFMC and PBMC of the tuberculous group revealed no significant corelation ($r = 0.5, -0.2$ respectively). These results showed that the majority of tuberculous and malignant pleural fluid lymphocytes were T-cells with equal number of circulating pool T-lymphocytes as shown in Figure 16.

3. Enumeration of T-cell subpopulations.

The percentages of PFMC OKT4+ cells between the tuberculous and the non-tuberculous groups were not significantly different (Figure 17), but were significantly higher than those in the corresponding peripheral blood ($P<0.001$). In addition, the percentage of tuberculous PBMC OKT4+ cells was significantly higher then in the normal controls ($P<0.001$). However, the cell concentration of tuberculous PFMC OKT4+ cells was not different from its PBMC counterpart or from the normal control (Figure 18). Figure 19 depicts the combined relationship of the percentage

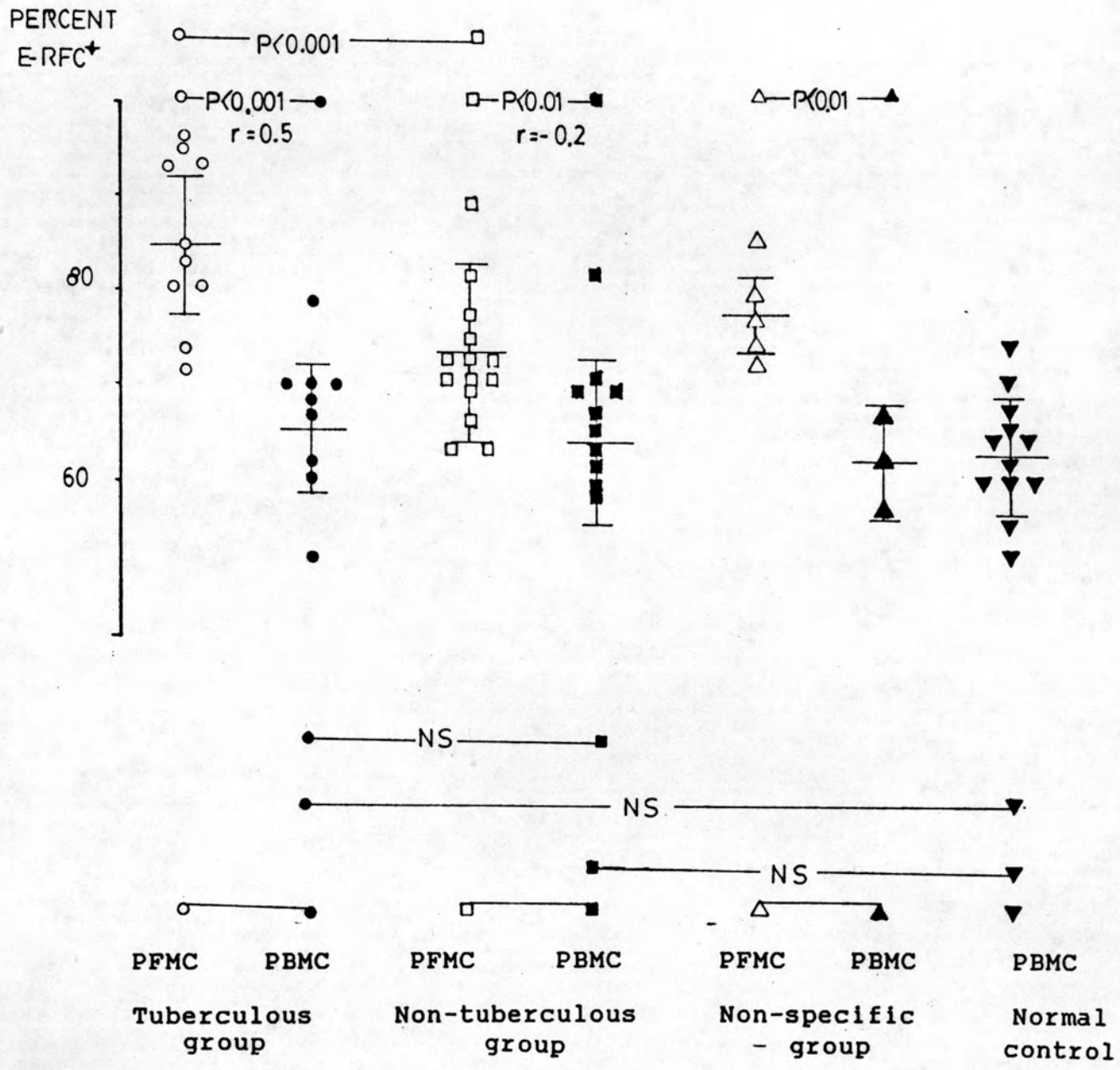


Figure 14. Percentage of E-RFC+ cells in PFMC and PBMC.

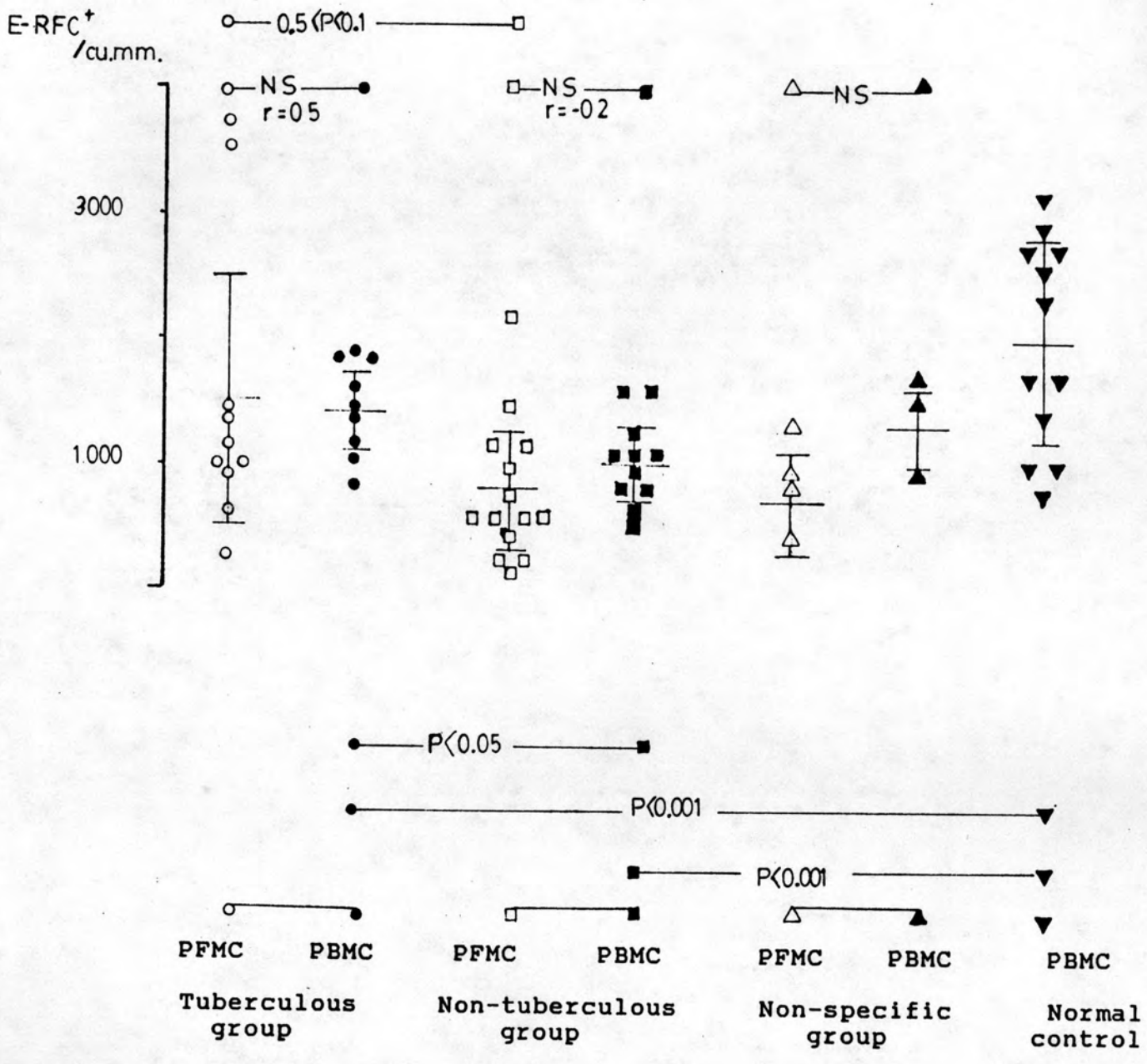


Figure 15. Absolute number of E-RFC+ cells in PFMC and PBMC.

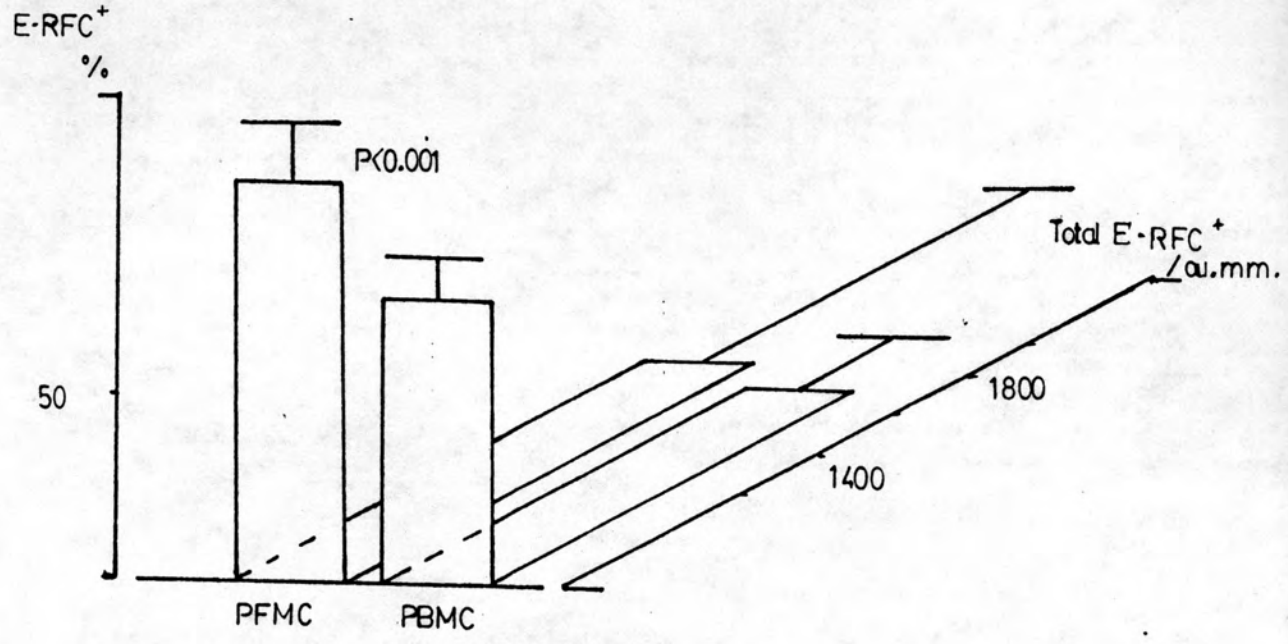


Figure 16. Percentage and absolute number of E-RFC+ cells in pleural fluids and blood from tuberculous patients.

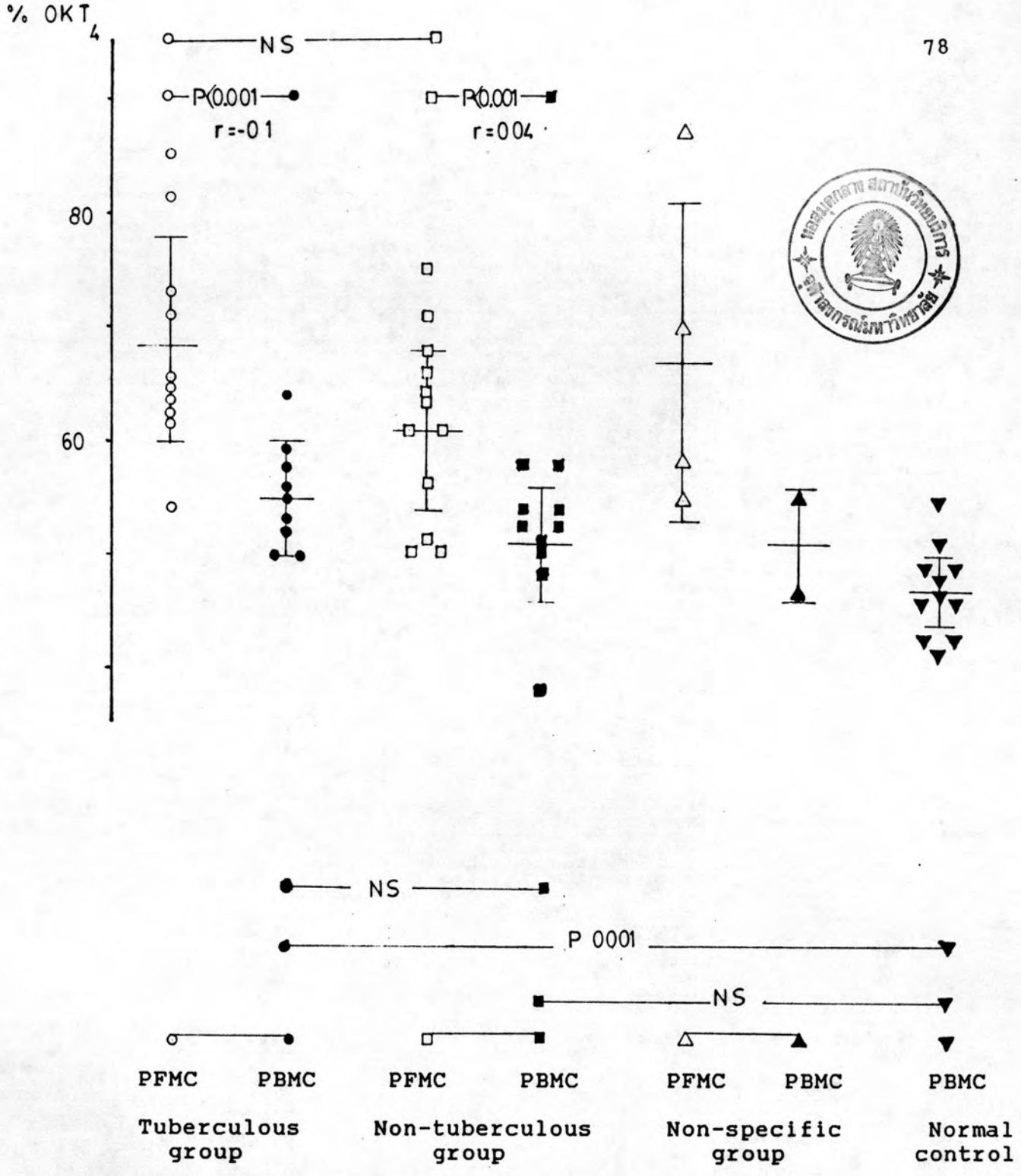


Figure 17. Percentage of OKT 4+ cells in PFMC and PBMC.

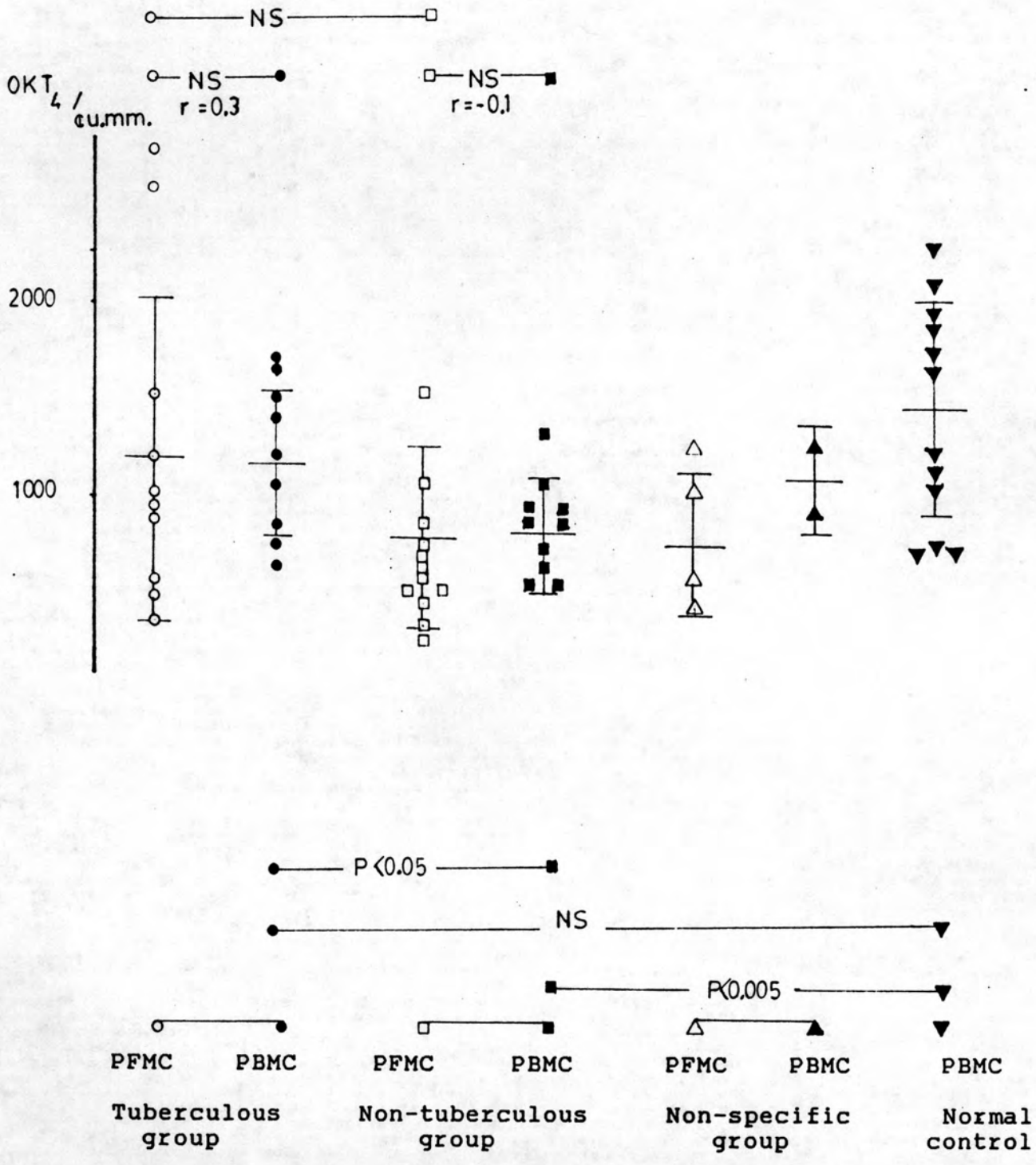


Figure 18. Absolute number of OKT4+ cells in PFMC and PBMC.

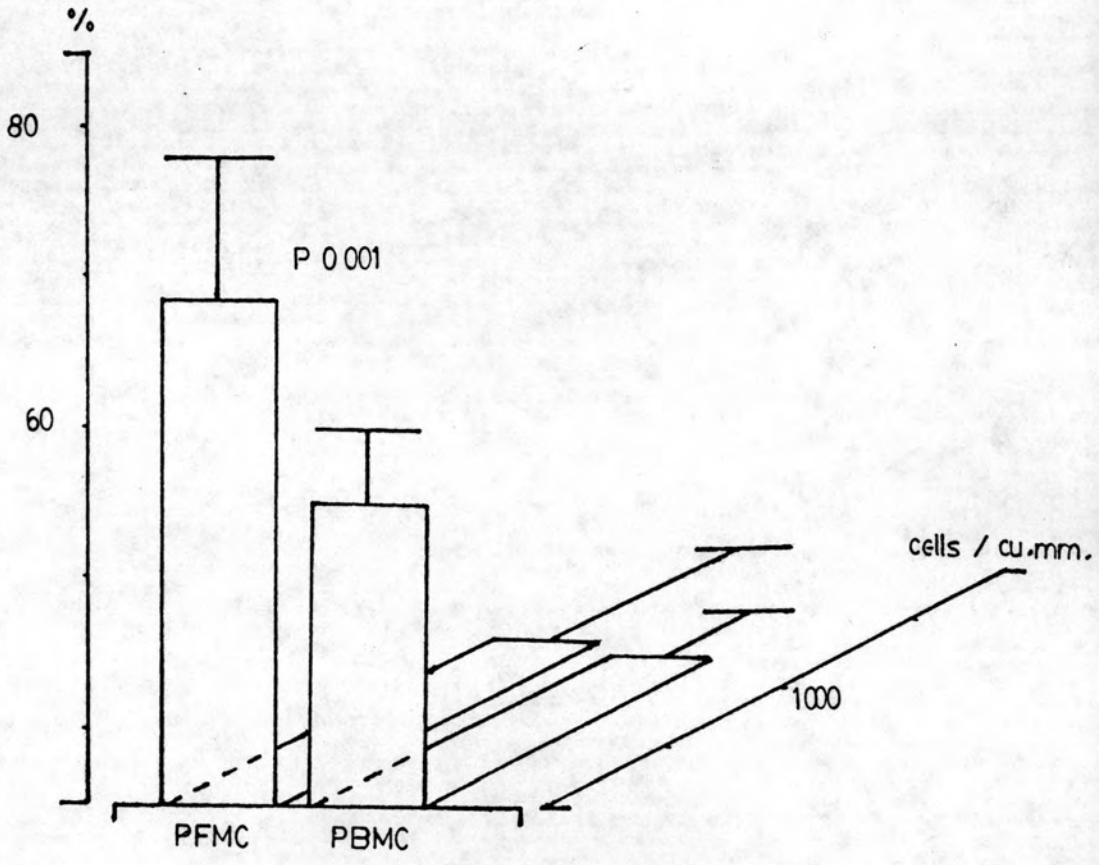


Figure 19. Percentage and absolute number of Okt4+ cells in tuberculous PFMC and PBMC.

and the absolute number of OKT4+ cells in tuberculous PFMC and PBMC. Neverthereless, regression analysis failed to demonstrate any correlation between the percentage and absolute number of OKT4+ cells between PFMC and PBMC of the tuberculous and the non-tuberculous groups (Figure 17 and 18).

In contrat to the results with OKT4+ cells, the percentages of OKT8+ cells in PFMC of both tuberculous and non-tuberculous groups were significantly lower than the corresponding values in the peripheral blood (Figure 20, $P<0.005$). The absolute numbers of OKT8+ cells in PFMC of both tuberculous and non tuberculous groups were also significantly lower than those in PBMC (Figure 21, $P<0.025$ and <0.05 repectively). Only the percentage of tuberculous PBMC OKT8+ cells was significantly higher than that of the normal controls (Figure 20, $P<0.005$) but no significant difference was observed in the absolute numbers of PBMC OKT8+ cells when compared (Figure 21). Such a relationship in the percentage and the absolute number of tuberculous PFMC and PBMC OKT8+ cells is presented in a two dimensional graph shown in Figure 22.

Because of the increase in the number of PFMC OKT4+ cells and the decrease in the PFMC OKT8+ cells of both tuberculous and non-tuberculous groups, the OKT4/OKT8 ratios of pleural fluid lymphocytes in both groups were

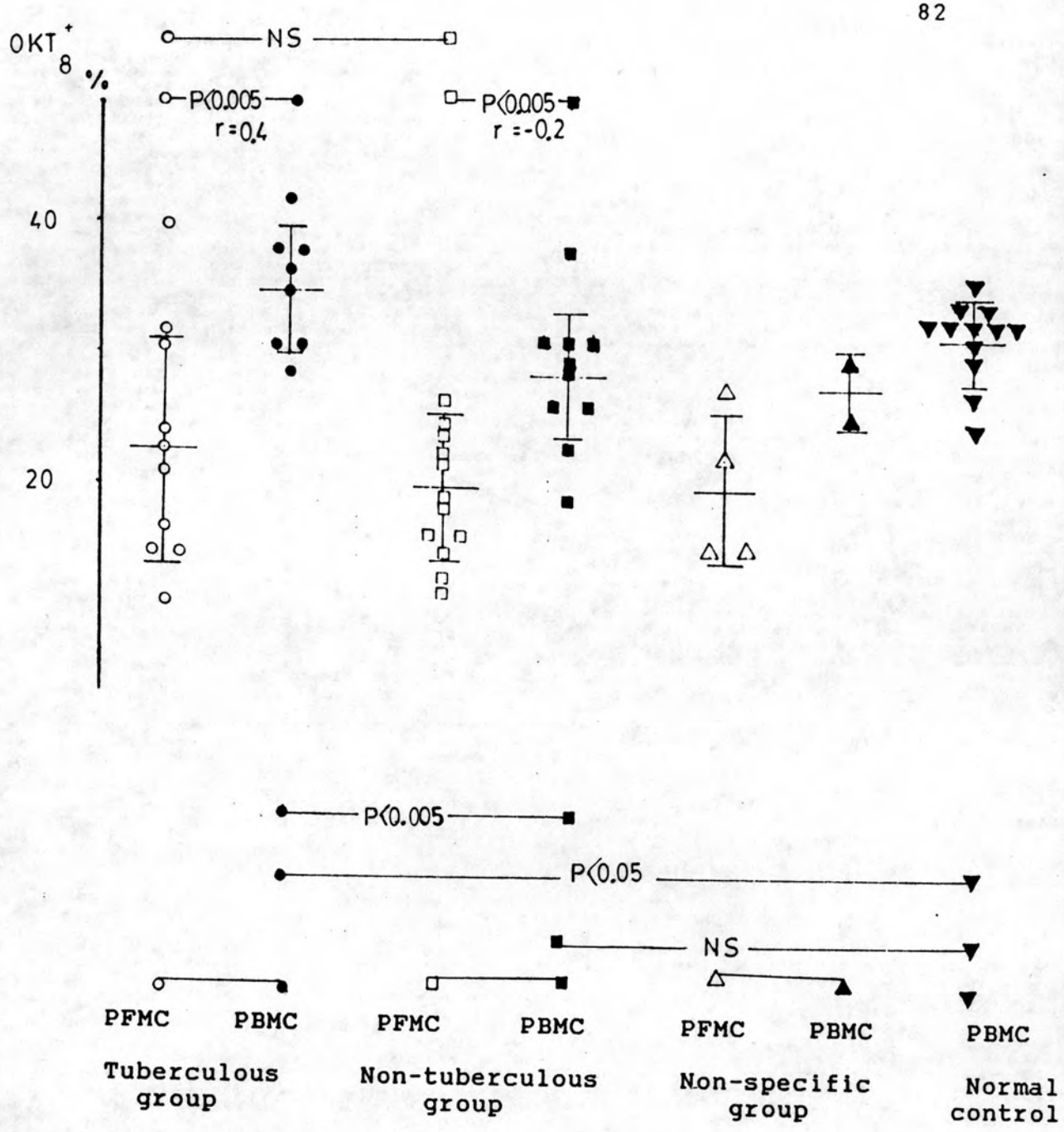


Figure 20. Percentage of OKT8+ cells in PFMC and PBMC.

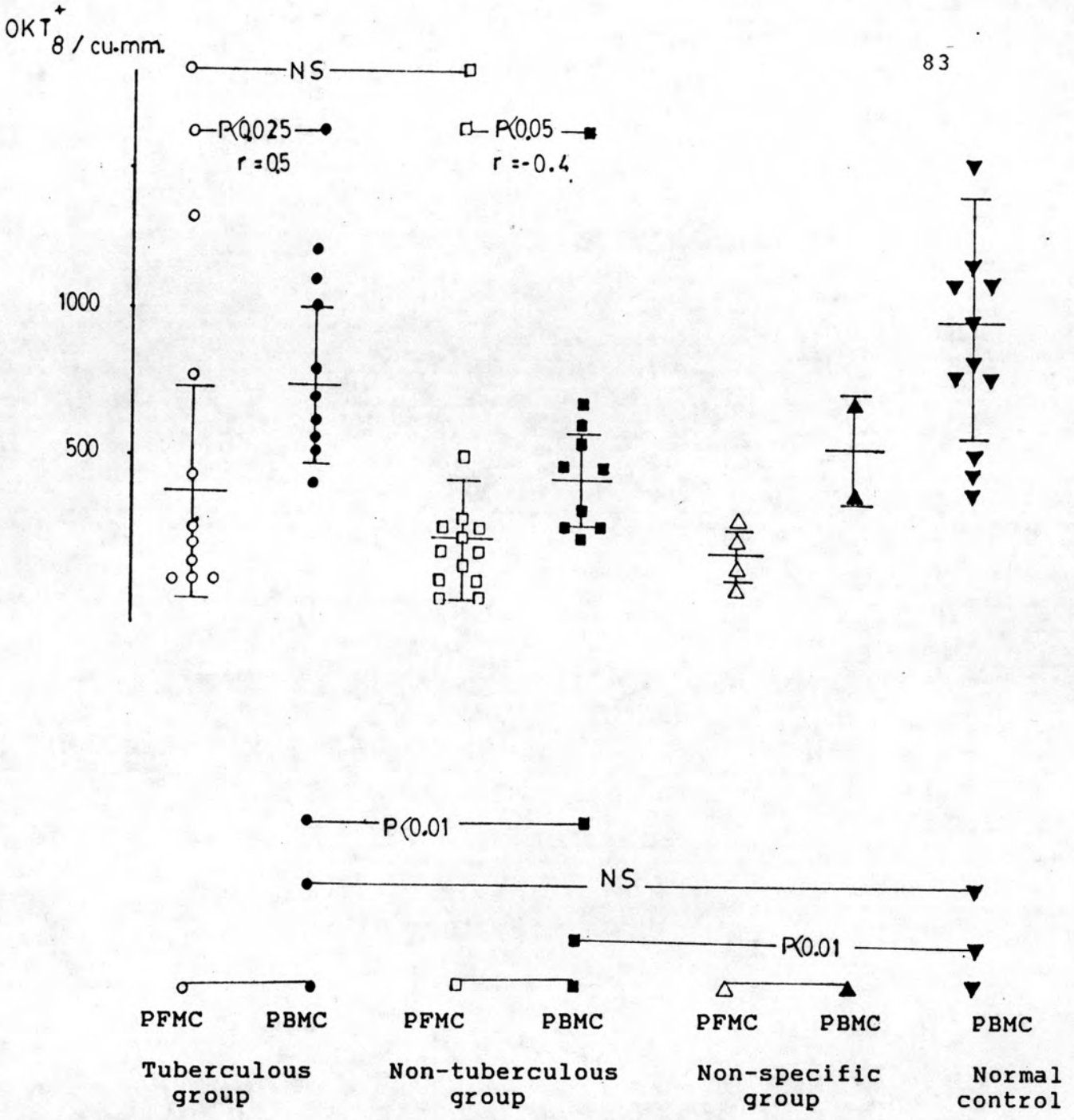


Figure 21: Absolute number of OKT8+ cells in PFMC and PBMC.

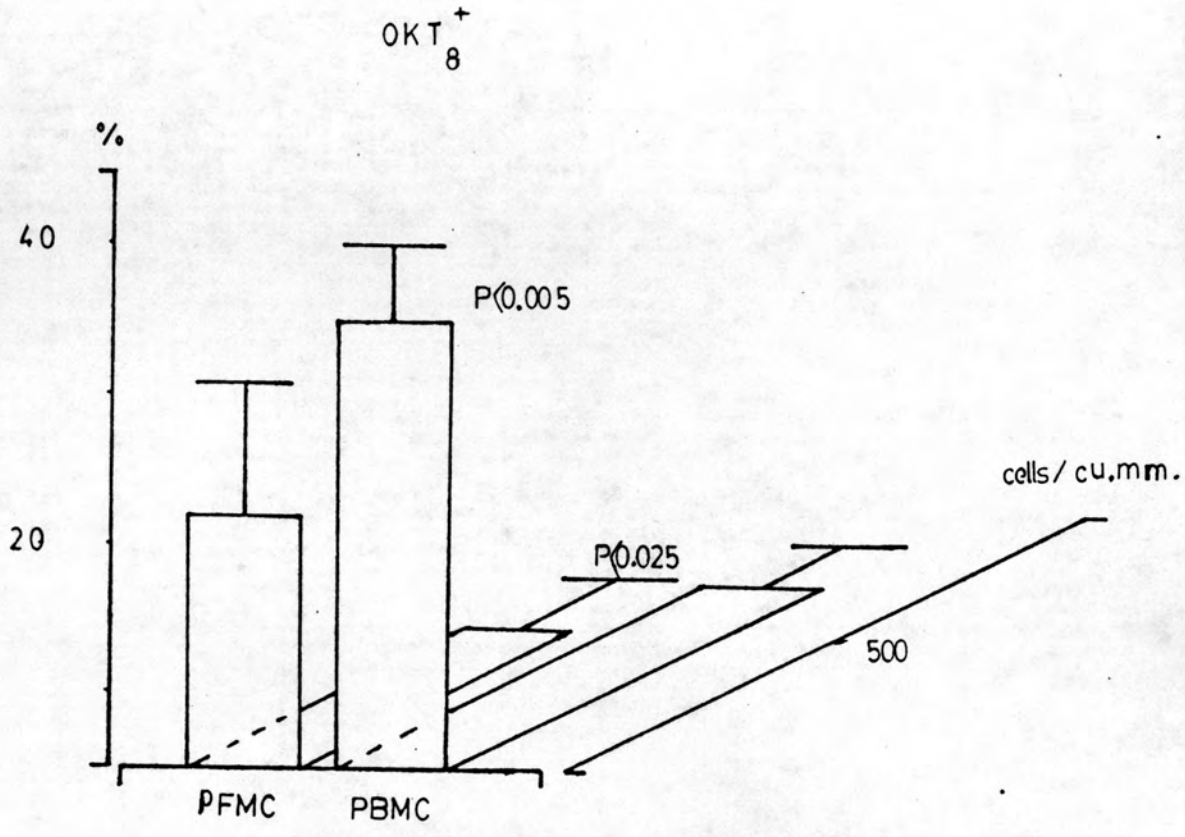


Figure 22. The percentage and absolute number of tuberculous OKT8+ cells in pleyral fluids and blood.

significantly increased when compared to their PBMC counterparts (Figure 23, $P<0.01$ and $P<0.001$ respectively). The OKT4/OKT8 ratio of the tuberculous PBMC was not increased even though the OKT4+ cells were increased (Figure 23). This is due to the simultaneous increase in OKT8+ cells.

4. Enumeration of B-cells and macrophages.

The percentages of surface membrane immunoglobulin positive or B-cells in pleural fluids of both tuberculuos and non-tuberculous groups were significantly decreased as compared to those in peripheral blood (Table 7, $P<0.001$). In addition, the small number of pleural fluid macrophages as determined by non-specific esterase staining was egually seen in all group of patients (table 7).

C. Lymphocyte transformation test.

The result of in vitro PPD stimulated proliferative response of PFMC and PBMC of all patient groups are shown in Figure 24&25 (as $\triangle$cpm.) and in Figures 26&27 (as stimulation index or S.I.). The $\triangle$ cpm. and the S.I. of the PFMC of both the tuberculous and the non-specific groups were significantly higher than the non-tuberculous group in both PPD concentrations (1 and 10 Ug./ml.) and were also higer than their PBMC counterparts. However, these feeble PPD responses of PBMC from the tuberculous and the non-specific groups were still higher than that

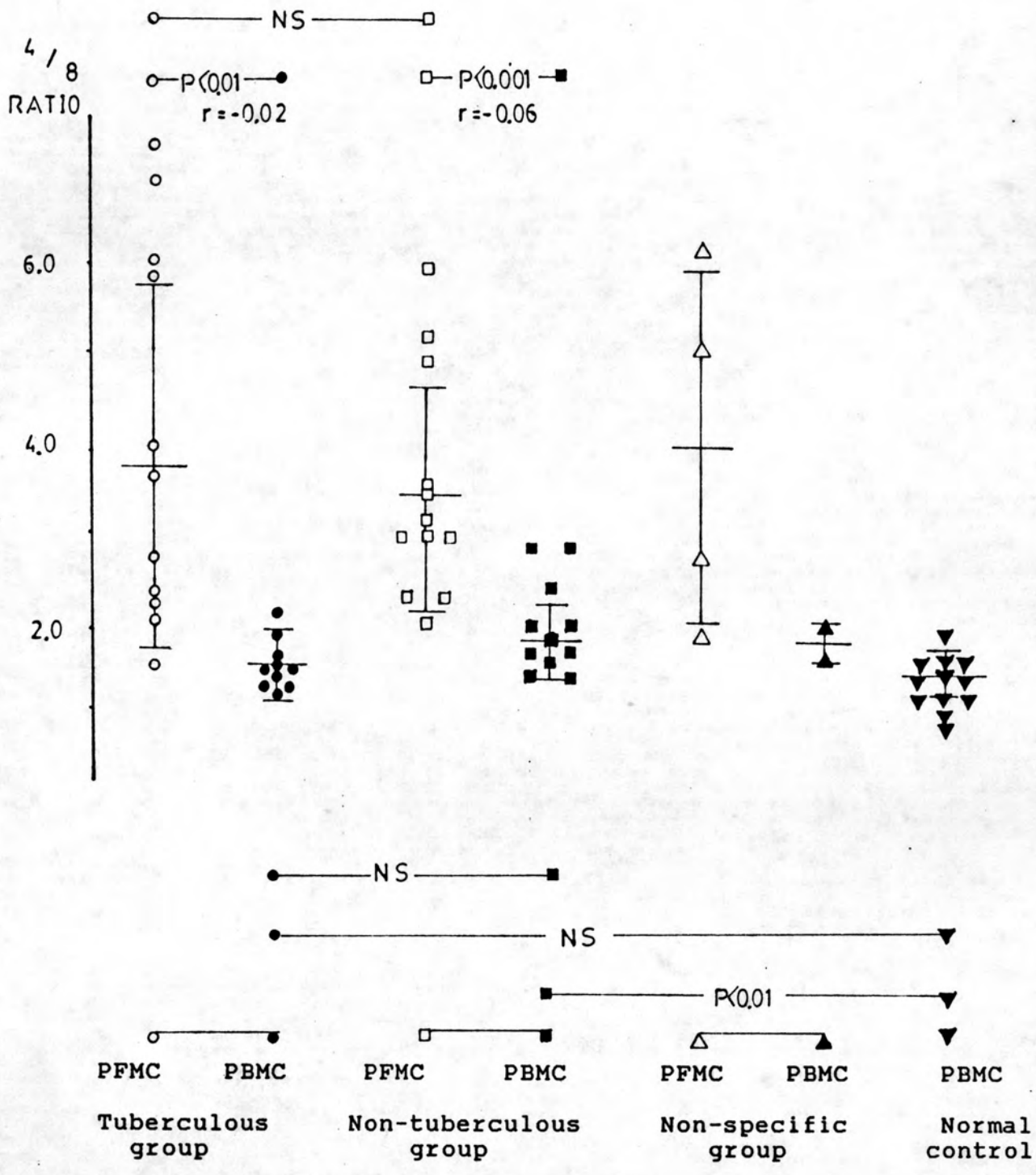


Figure 23. Pleural fluid and peripheral blood OKT4/OKT8 ratios.

Table 7. Percentage of B-cells and macrophages in pleural fluids and peripheral blood.

Patient Group	B-cells (%)	P value	Non-specific esterase+ cells (%)
Tuberculous group			
-pleural fluid	4±4 (7)*	<0.01	3±1 (7)
-blood	12±3 (12)		-
Non-tuberculous group			
-pleural fluid	5±1 (4)	<0.01	3±2 (12)
-blood	13±3 (12)		-
Non-specific group			
-pleural fluid	5±1 (4)	<0.01	4±1 (4)
-blood	14±3 (2)		-

* = $\overline{X}$±SD (N)

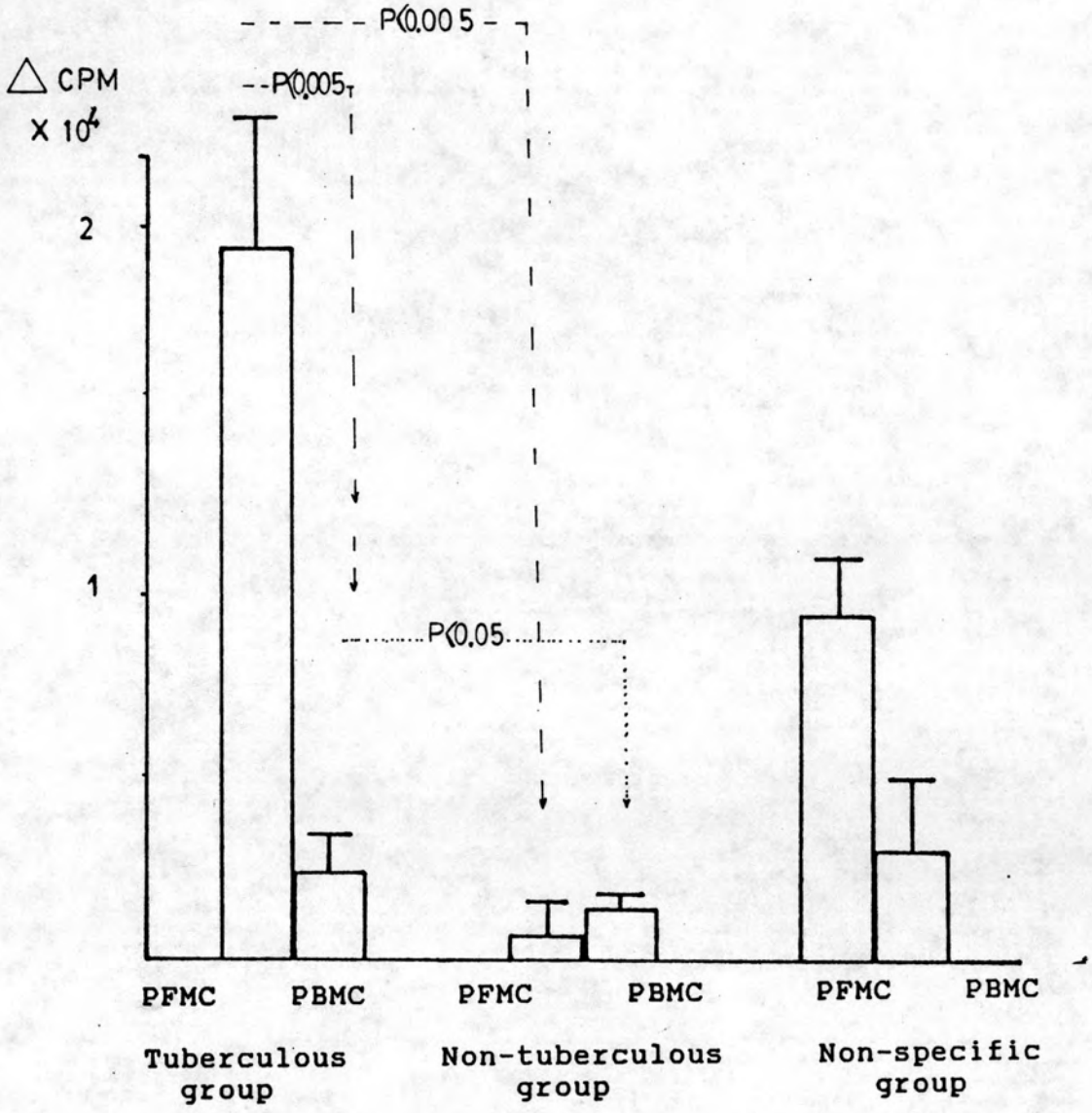


Figure 24. △ cpm. of PPD (Ug./ml.)-stimulated lymphocyte transformation test.

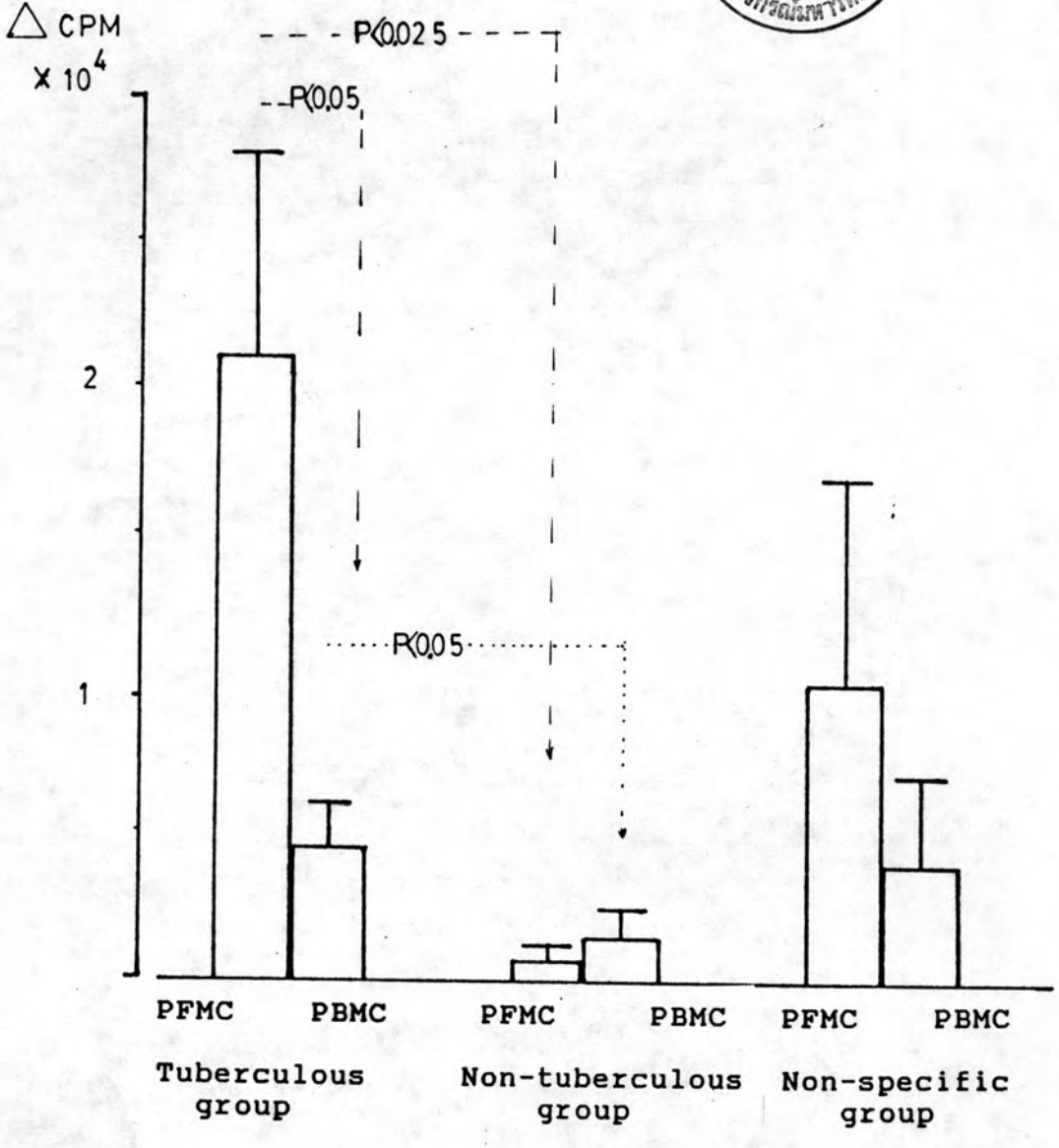


Figure 25. △cpm. of PPD (10 Ug./ml.)-stimulated lymphocyte transformation test.

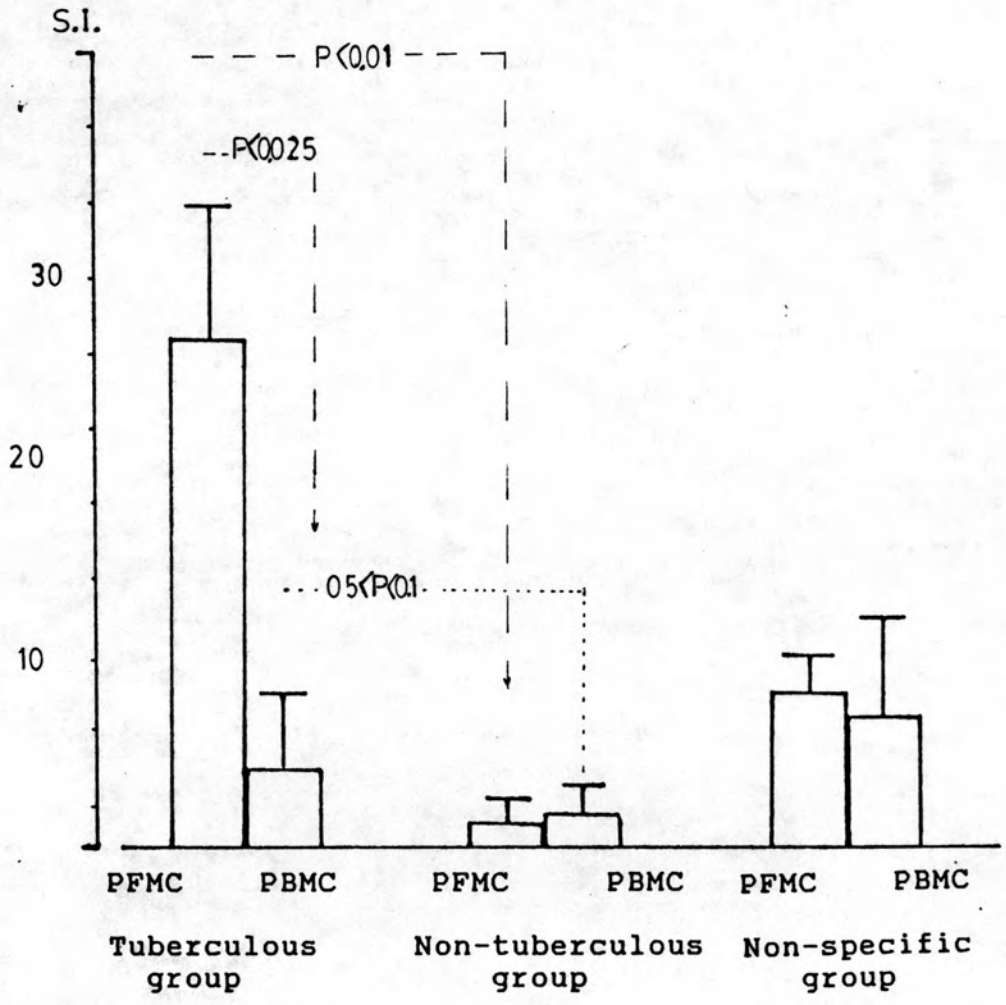


Figure 26. Stimulation Index (S.I.) of PPD (1 Ug./ml.) -stimulated lymphocyte transformation test.

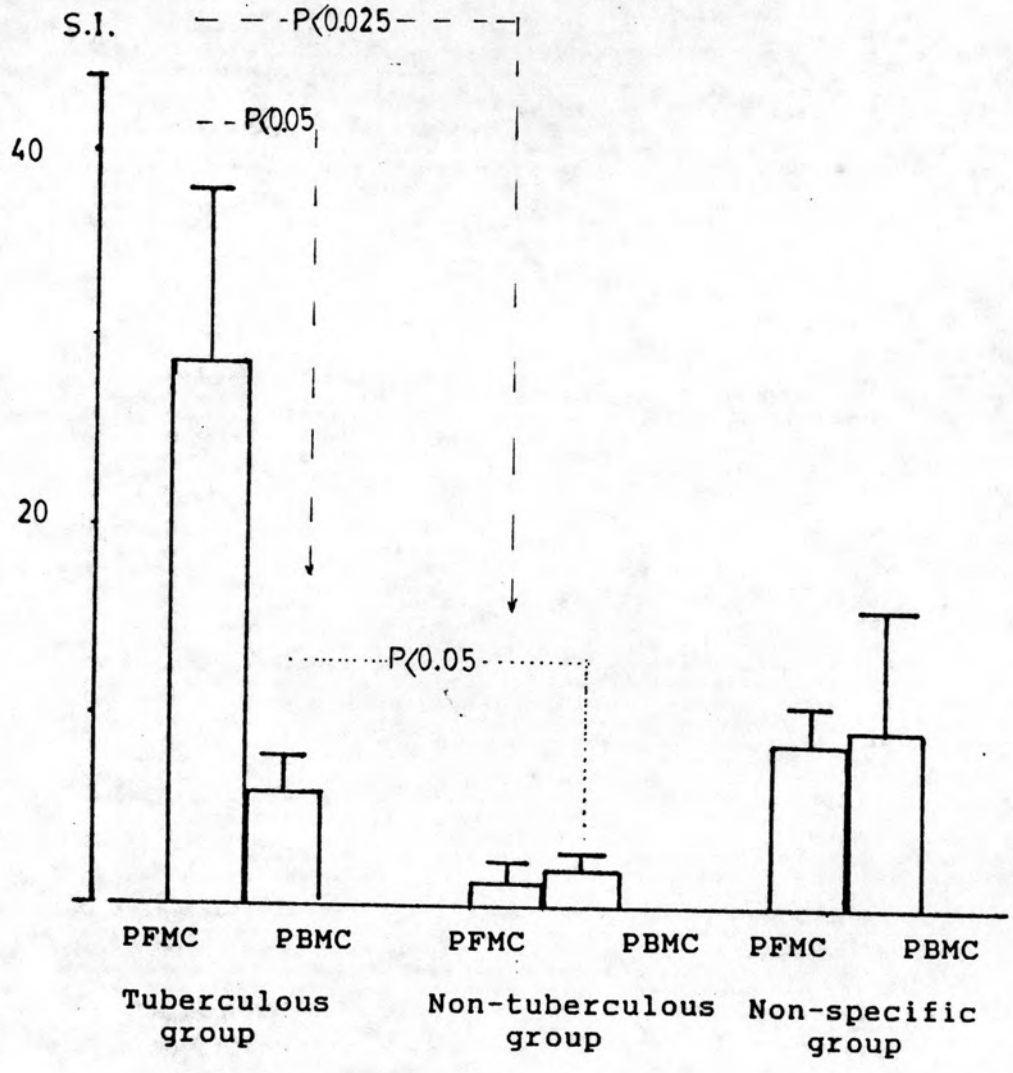


Figure 27. Stimulation Index (S.I.) of PPD (10 Ug./ml.) -stimulated lymphocyte transformation test.

of the non-tuberculous group. The Poorer PPD response of PFMC and PBMC from the non-tuberculous group was not due to the intrinsic defect of their lymphocytes, since they responded equally well to PHA stimulation as the PFMC and the PBMC from the tuberculous and the non-specific groups (Figures 28&29).

D. PPD stimulated leukocyte migration inhibition factor test (LIF test).

The migration indices (M.I.) of PPD stimulated PFMC modified direct LIF test of PFMC from the tuberculous and the non-specific groups were significantly less than those of the non-tuberculous group (Figure 30). The M.I. of PPD-stimulated direct LIF test of tuberculous periperal blood lymphocytes was also lower than those of the non-tuberculous group. However, the M.I. of PFMC LIF test of tuberculous and non-tuberculous groups were not significantly different from their PBMC cuonterparts.

E. Corelation between clinical diagnosis of tuberculous efffusion and in vitro antigen specific function tests.

For PPD stimulated proliferative response, the criteria for positive test are the Δ cpm. of over 2000 cpm. or S.I. of more than 2 times. By using this criteria of positivity and by using the clinical diagnosis of tuberculous and non-tuberculous effusions (malignant) as

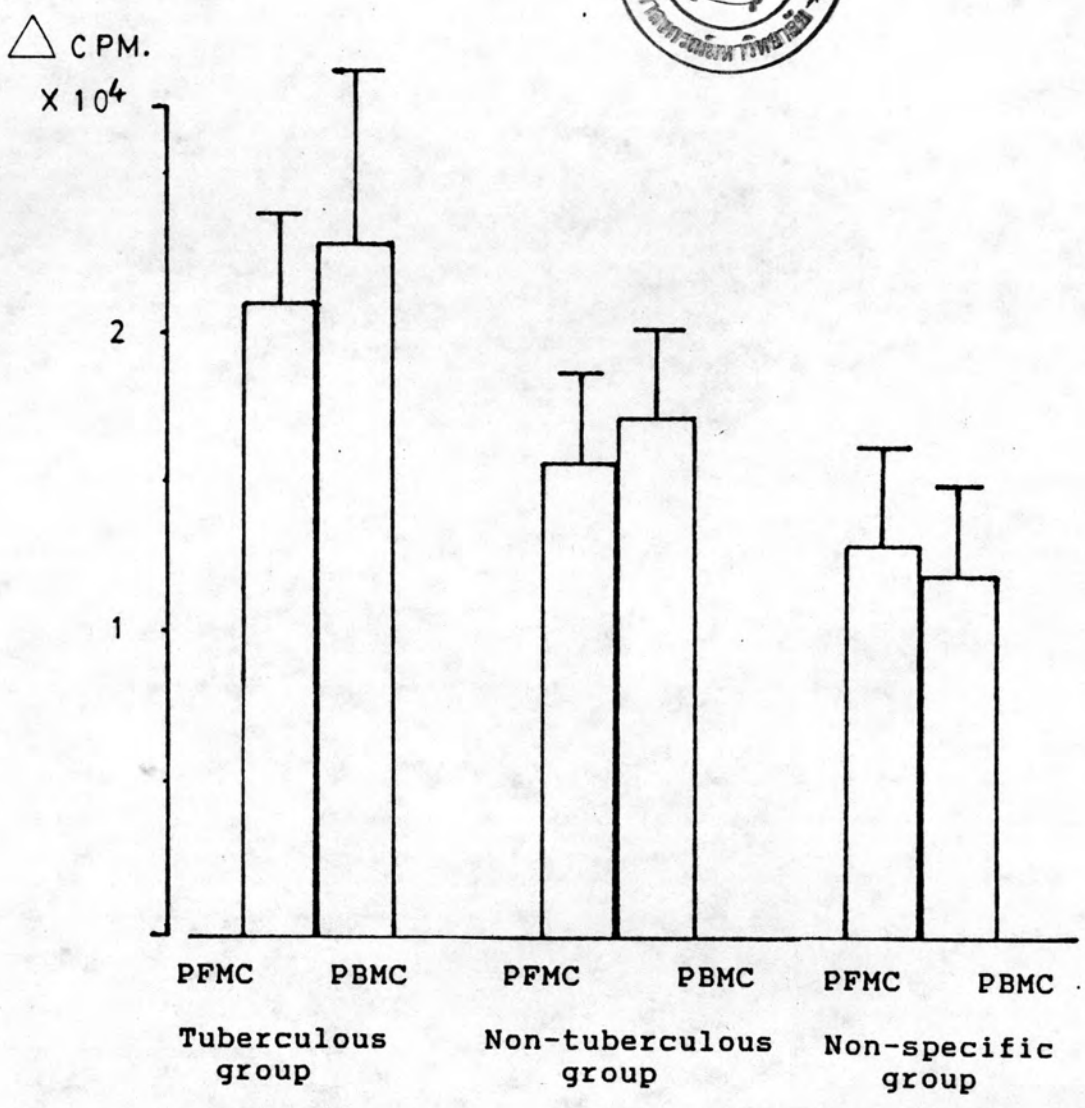


Figure 28. △ cpm. of PHA stimulated lymphocyte transformation test.

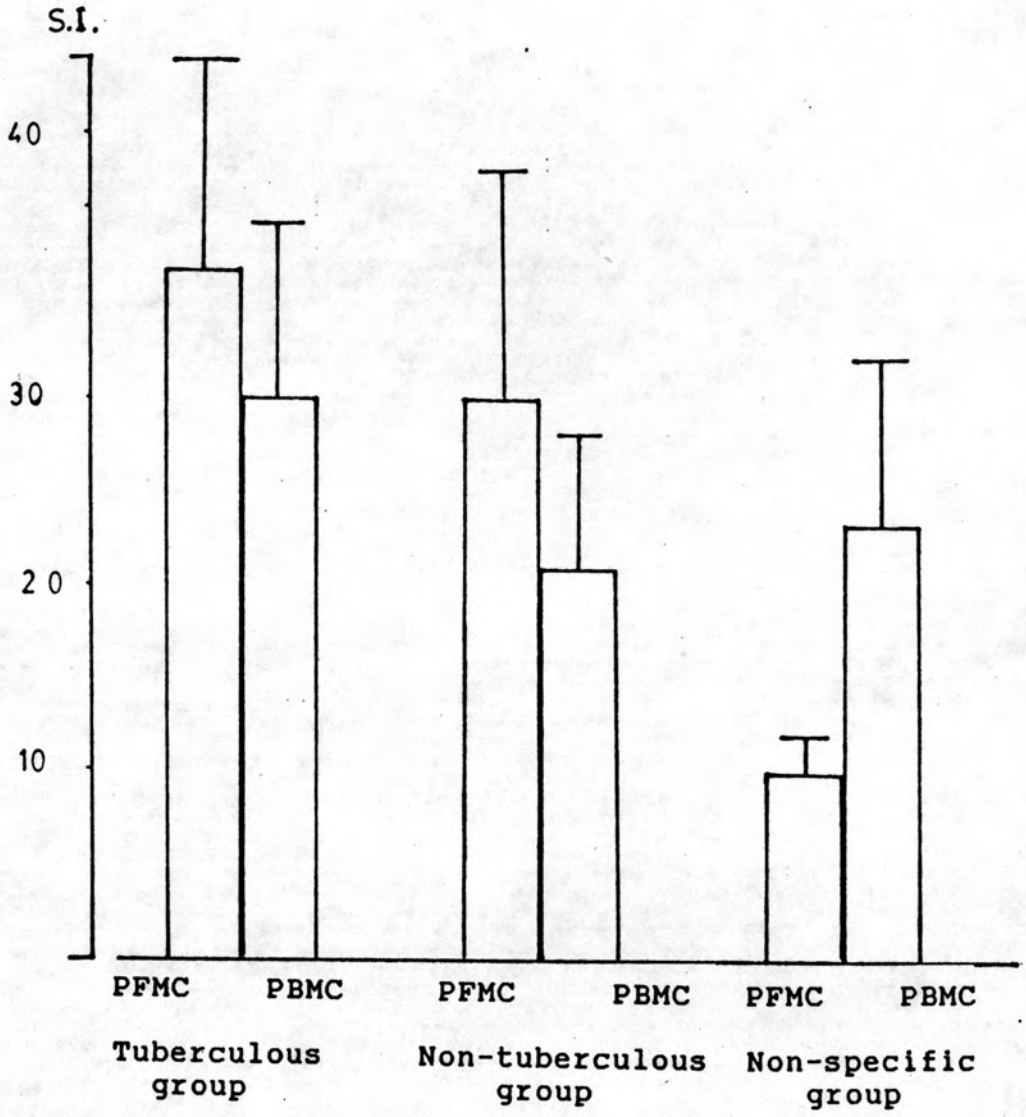


Figure 29. Stimulation Index (S.I.) of PHA stimulated lymphocyte transformation test.

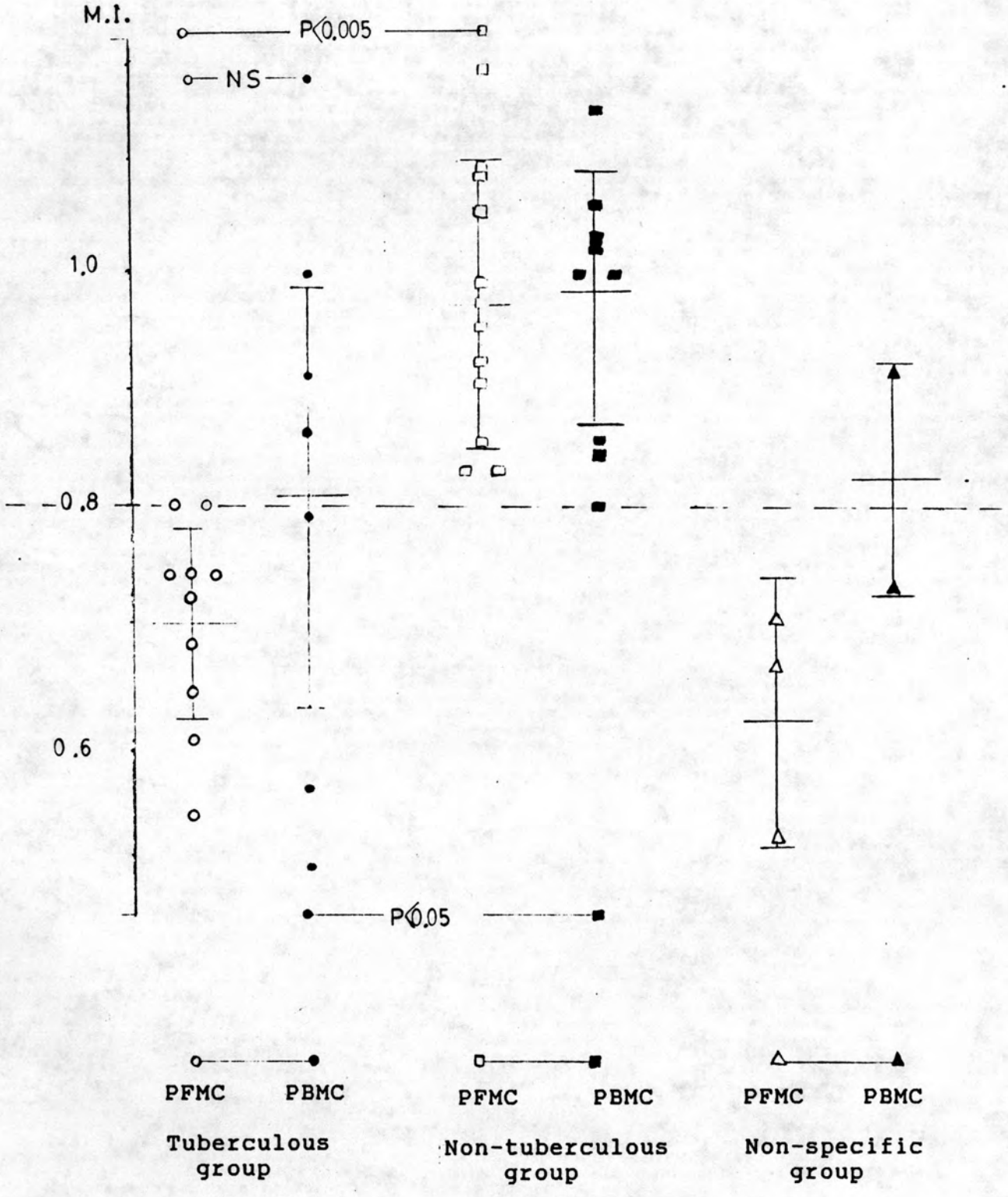


Figure 30. PPD-stimulated LIF test of PFMC and PBMC.

gold standards, the sensitivity and specificity of PPD-stimulated PFMC and PBMC proliferative responses could be calculated. Good corelation between clinical diagnosis and PPD-stimulated PFMC proliferation was observed (Table 8-a; $P<0.005$). On the other hand, PPD stimulated PBMC proliferation gave low sensitivity and specificity (Table 8-b; $P>0.05$). Similar results were obtained with PPD-stimulated LIF test, using M.I. of less than 0.8 as the criteria for positivity (Table 9-a & 9-b). These results indicate that antigen-specific functional assays of pleural fluid mononuclear cells correlate very well with our diagnostic criteria of tuberculous effusion.

F. Corelation between tuberculin skin test and in vitro antigen specific functional tests.

When the results of PPD-stimulated blast transformation of PFMC and PBMC of all patients (3 groups) were analysed against their tuberculin test results, it was found that $\triangle$ cpm. of the ones with positive tuberculin test ($\geq$ 10 mm.) were significanyly higher than those with negative tuberculin test (<10 mm.)(Figure 31; $P<0.05$). However, only the results with PBMC were found to correlate with tuberculin reactivity by regression analysis (Figure 32-b; $r=0.6$ whereas $r=0.3$ for PFMC, Figure 32-a). In contrast to the PPD-stimulated blast transformation test, no correlation was found when PPD-stimulated LIF test was analysed against

Table 8. Correlation between clinical diagnosis of tuberculous effusion and PPD-stimulated lymphocyte transformation test of PFMC (a) and PBMC (b).

(8-a) PFMC.

PPD-stimulated proliferation	Clinical diagnosis	
	TB	Non-TB
+	10	1
-	0	11

P<0.005

Sensitivity = 100 % positive predictive value = 90.9 %

Specificity = 91.7 % Negative predictive value = 100 %

Accuracy = 95.5 %

(8-b) PBMC

PPD-stimulated Proliferation	Clinical diagnosis	
	TB	Non-TB
+	6	2
-	3	8

P>0.05

Sensitivity = 66.7 % Positive predictive value = 75 %

Specificity = 80 % Negative Predictive Value = 73 %

Accuracy = 73.7 %

Table 9. Correlation between clinical diagnosis of tuberculous effusion and PPD-stimulated LIF test of PFMC (a) and PBMC (b).

(9-a) PFMC.

PPD-stimulated proliferation	Clinical diagnosis	
	TB	Non-TB
+	8	0
-	2	12

P<0.005

Sensitivity = 80 % Positive predictive value = 100 %

Specificity = 100 % Negative predictive value = 86 %

Accuracy = 90.9 %

(9-b) PBMC

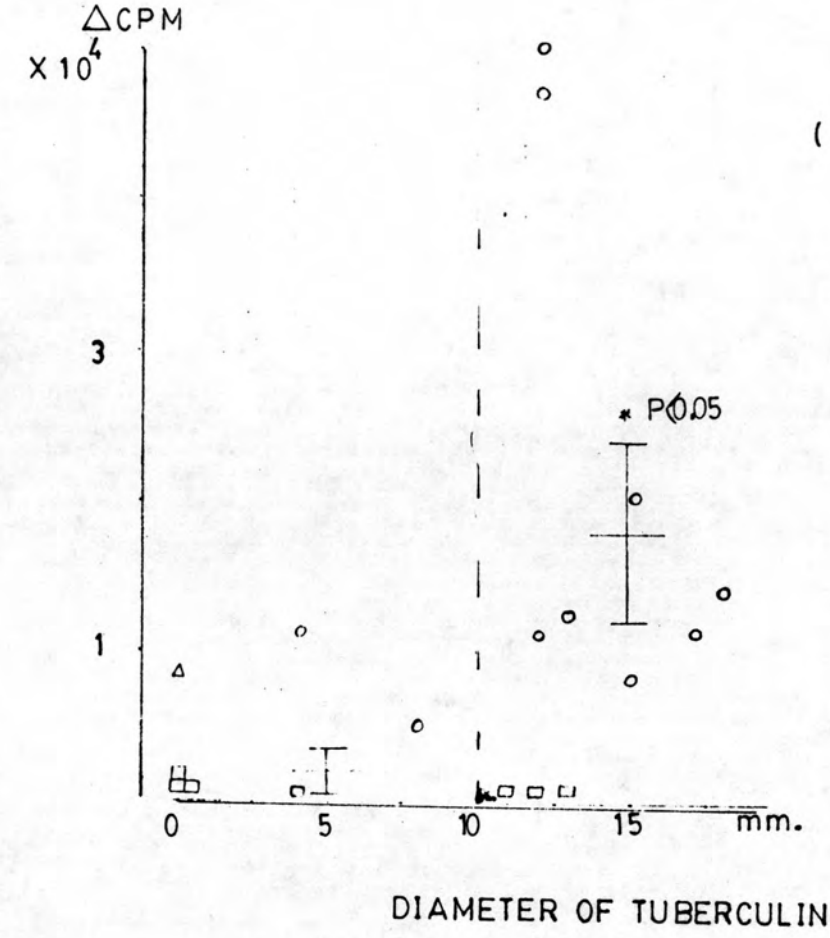
PPD-stimulated Proliferation	Clinical diagnosis	
	TB	Non-TB
+	3	0
-	3	10

P>0.05

Sensitivity = 50 % Positive predictive value = 100 %

Specificity = 100 % Negative Predictive Value = 77 %

Accuracy = 81.3 %



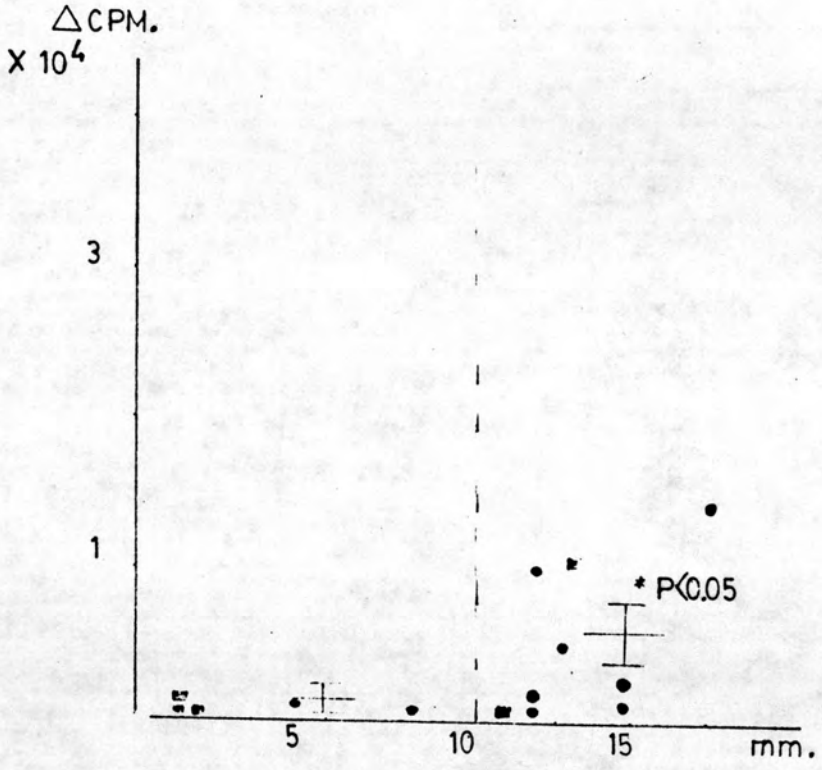
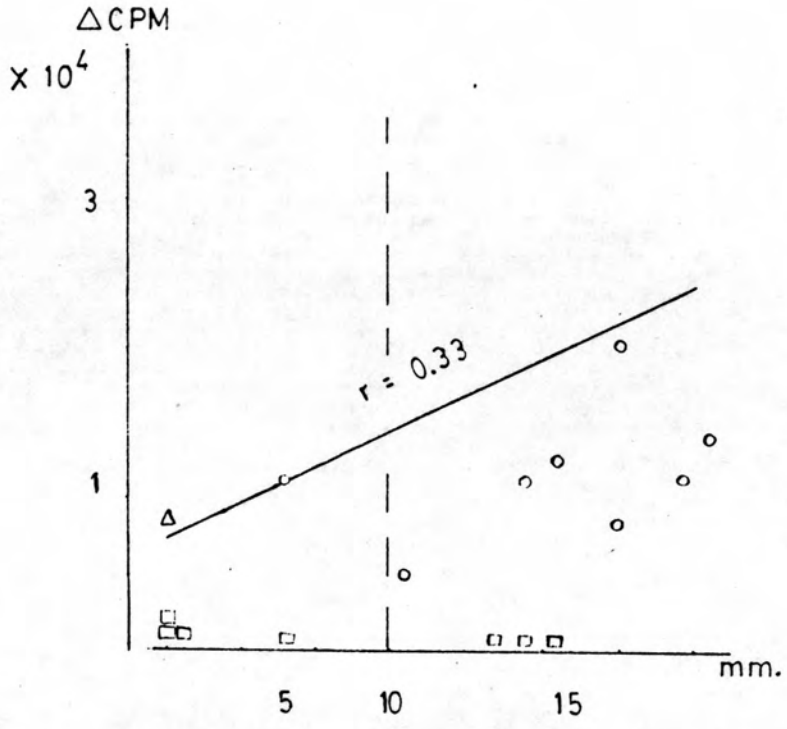
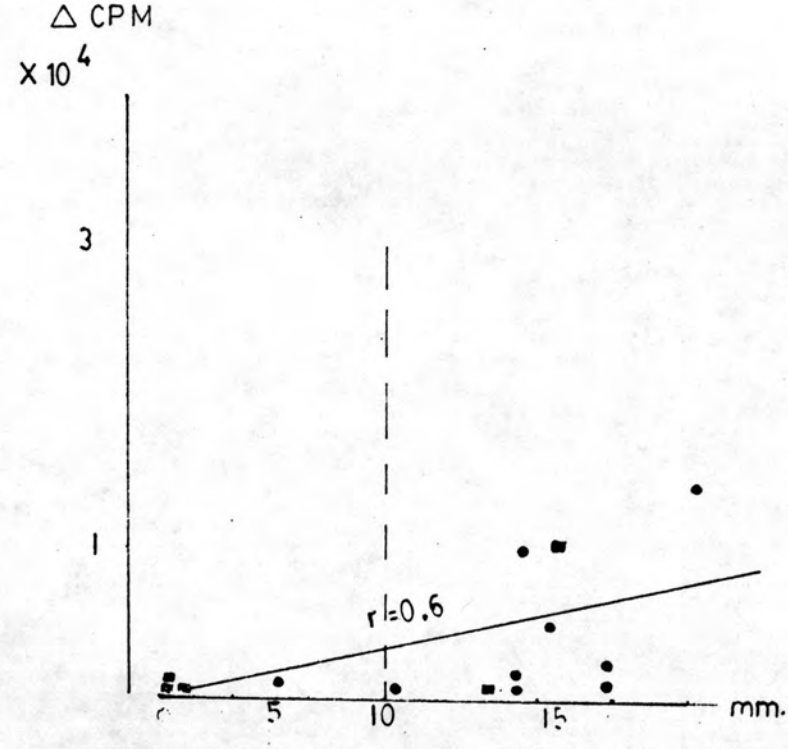


Figure 31. Results of PPD stimulated PFMC (a) and PBMC (b) proliferation according to the tuberculin skin test positivity.



(32-a) PFMC



(32-b) PBMC

Figure 32. Regreesion analysis of PPD stimulated PFMC (a) and PBMC (b) blast transformation and the reactivity of tuberculin skin test.

tuberculin reactivity , either with PFMC or with PBMC (Figure 33-34).

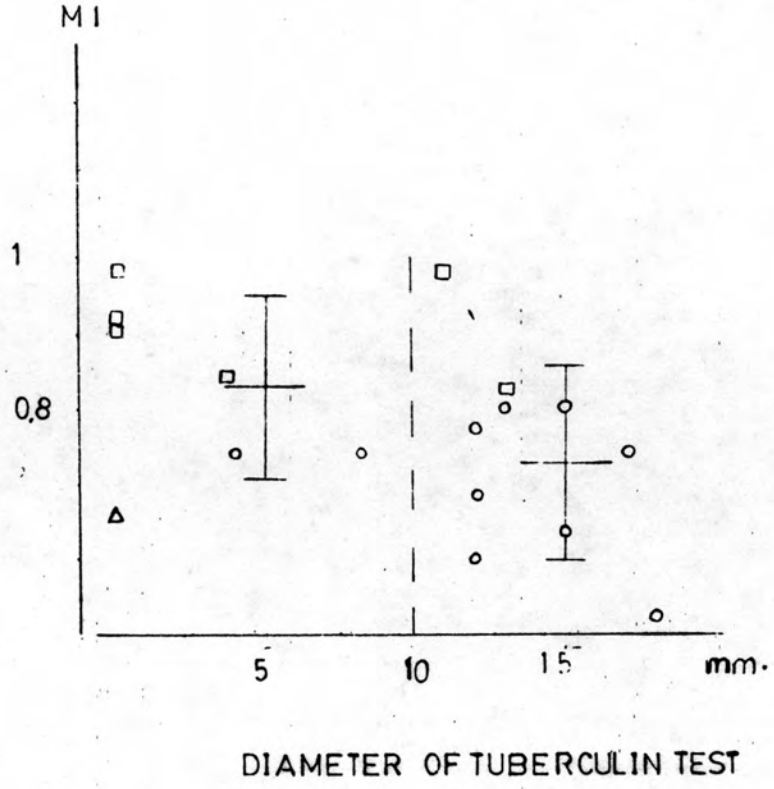
G. Free LIF like activity in pleural fluids.

1. Pilot study.

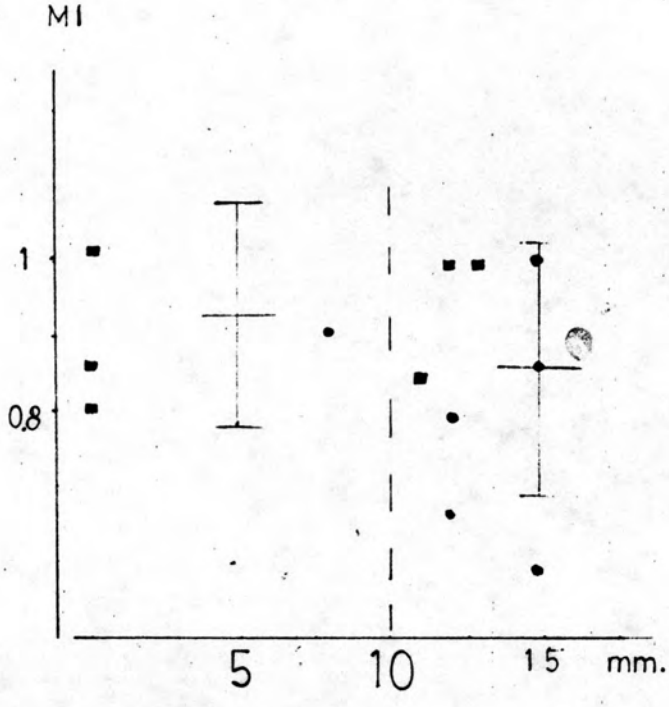
Two tuberculous pleural fluid samples were diluted in two fold serial dilutions with TC 199 medium and heat inactivated at 56 C for 30 mins. These heat inactivated pleural fluids were tested for the ability to inhibit the migration of pooled purified normal human PMN cells in agarose medium compared with the unheated pleural fluids. The results obtained from one patient was shown in Figure 35. Decreasing migratory activity was observed with decreasing dilutions of pleural fluid. Both heat-labile and heat-stable LIF-like factors were contained in the tuberculous pleural fluids. Therefore, all pleural fluid samples to be assayed for LIF like activity were heat inactivated before use.

2. Free LIF-like activity in pleural fluids.

All of the heat-inactivated tuberculous pleural fluids could inhibit the migration of pooled purified normal human PMN cells in agarose medium (Table 10). Free LIF-like activity was observed in 6 of 9 cases of carcinomatous pleural effusion. The negative cases were

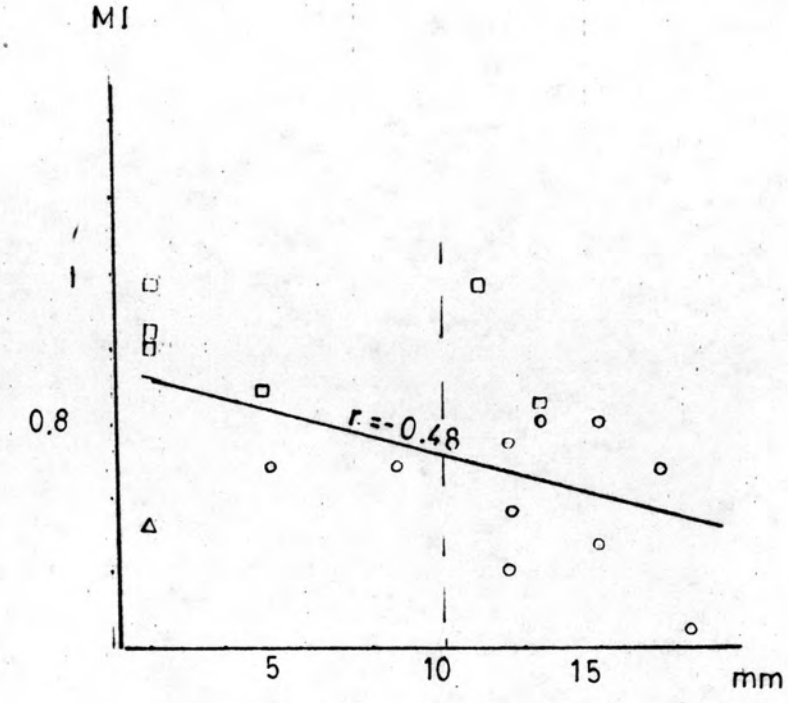


(33-a) PFMC

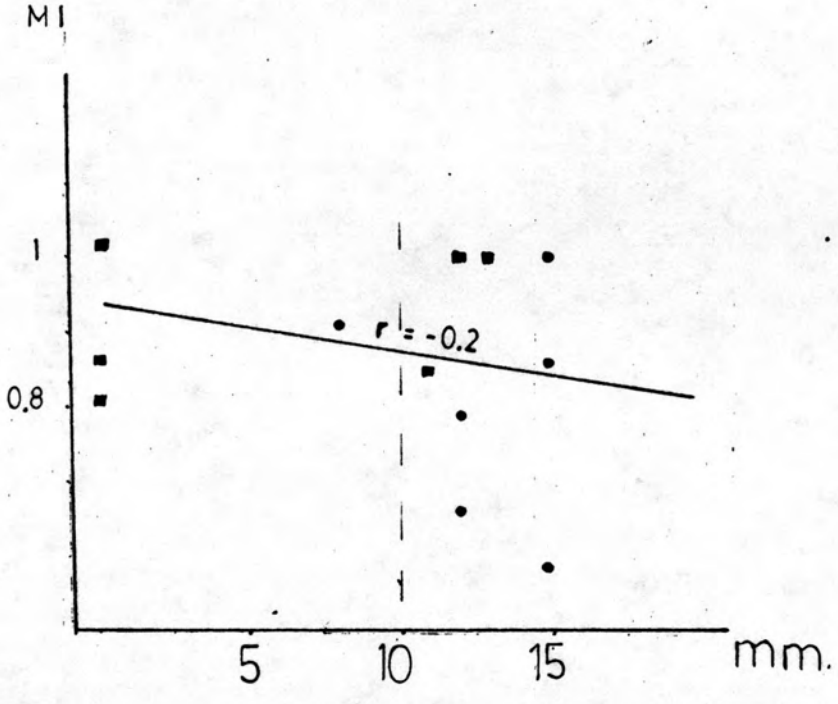


(33-b) PBMC

Figure 33. Lack of correlation of PFMC (a) or (b) with tuberculin test positivity.



(34-a) PFMC



(34-b) PBMC

Figure 34. Lack of PFMC (a) or (b) with tuberculin test results

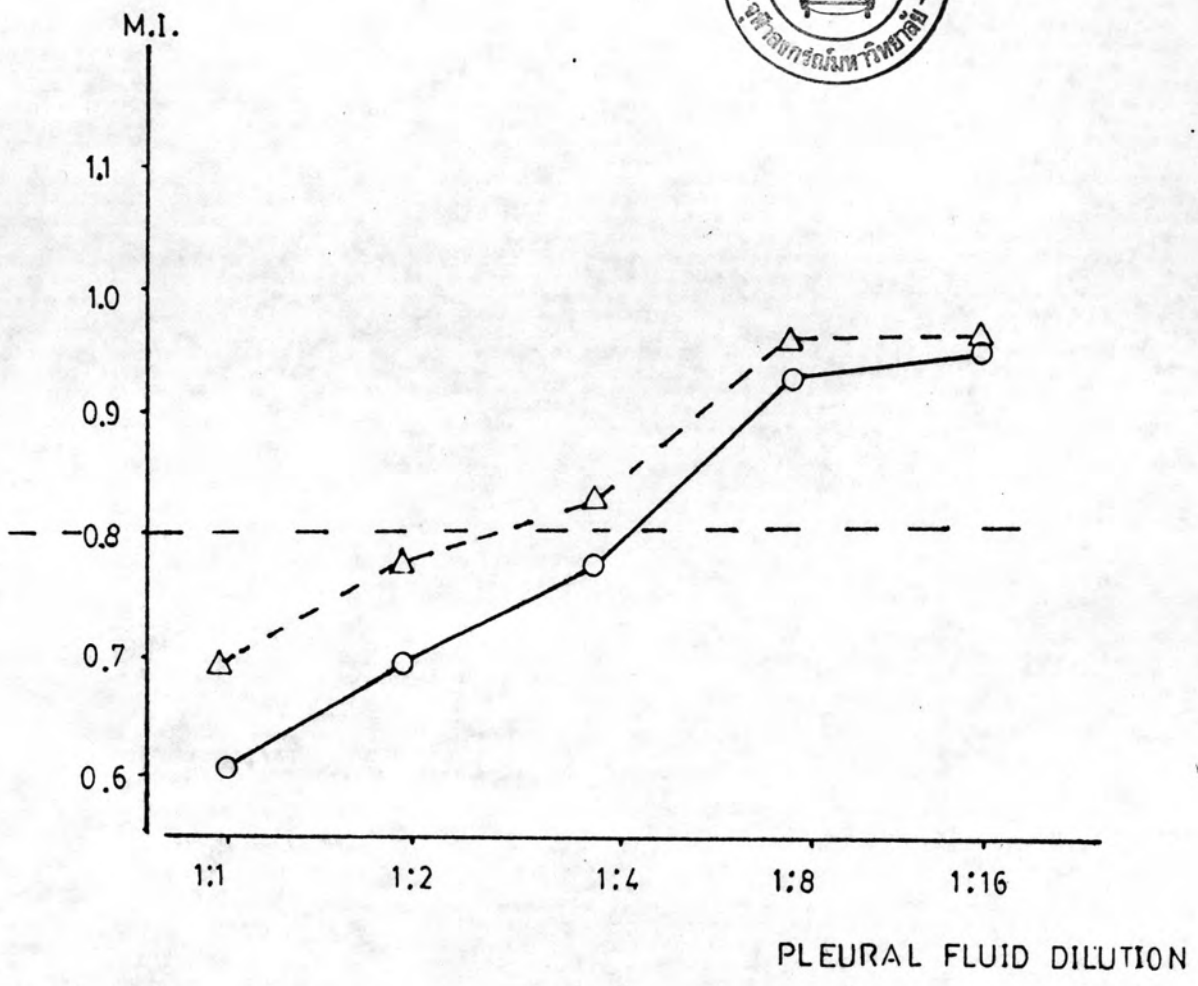


Figure 35. Migrating inhibitory activity of serially diluted unheated pleural fluid from a patient with tuberculous effusion compared with the heat inactivated pleural fluid.

Table 10. Free LIF-like activity in various types of pleural effusion.

Types of effusion	Number of positive cases per number examined	Percent positive
Tuberculous effusion	9/9	100 %
Carcinomatous effusion	6/9	67 %
Non-inflammatory pleural effusion	0/1	0 %

one patient with stage III lymphoma with pleural effusion, and 2 elderly men with adenocarcinoma and undifferentiated carcinoma. The patient with pleural effusion secondary to congestive heart failure contained no free LIF-like activity in his pleural fluid.