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In vivo platelet and T-lymphocyte activities during pulmonary tuberculosis

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In vivo *platelet and T-lymphocyte activities during pulmonary tuberculosis. Y. Büyükaşık, B. Soylu, A.R. Soylu, O.İ. Özcebe, S. Canbakan, İ.C. Haznedaroğlu, Ş. Kirazlı, Y. Başer, S.V. Dündar. ©ERS Journals Ltd 1998.*

ABSTRACT: Platelets have been suggested to play a role in the inflammatory response, including defence against bacteria. The aims of this study were to determine *in vivo* platelet activity during the clinical course of pulmonary tuberculosis and to investigate whether or not there is a correlation between the magnitude of platelet activation and the extent of the pulmonary disease. T-lymphocyte activity was also analysed in the patients. Platelet factor-4 (PF4) and soluble interleukin-2 receptor-alpha (sIL-2Rα) concentrations were used as markers of platelet and T-lymphocyte activation, respectively.

Twenty-five patients with pulmonary tuberculosis were studied. Fifteen healthy subjects served as a control group.

The levels of both sIL-2Rα (3,000±1,948 pg·mL^{-1}) and PF4 (103.1±6.7 IU·mL^{-1}) were significantly higher in the patients with tuberculosis than in the control group (984±360 pg·mL^{-1} and 78.2±23.9 IU·mL^{-1}, respectively) (Mann–Whitney U-test, p<0.001 for both comparisons). The plasma PF4 levels were found to be well correlated with the extent of pulmonary lesions on chest radiography (the Spearman's bivariate correlation analysis, r=0.65, p<0.001). However, sIL-2Rα concentrations did not correlate with the extent of disease.

In conclusion, it has been suggested that platelet and T-lymphocyte activation occurs during pulmonary tuberculosis. The good correlation between platelet activation and the extent of pulmonary tuberculosis might be ascribed to a pathophysiological role of platelets in pulmonary tuberculosis.
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Platelets are considered to be pulmonary immune cells, because they possess many of the classical features of immune cells and participate in the pathogenesis of some pulmonary diseases [1–3]. These cells have been suggested to play a role in the evolution of inflammatory response against mycobacteria [4]. Microvascular thrombosis around tuberculous foci, in which platelets are possibly involved, may contribute to the prevention of dissemination of pulmonary mycobacterial infection [5]. However, very few clinical investigations have studied platelet activity in tuberculosis [4, 6]. To the authors' knowledge, clinical studies investigating *in vivo* platelet activity during pulmonary tuberculosis have not been published.

Platelet factor-4 (PF4) is a platelet-derived pro-inflammatory cytokine which is stored in the α-granules and released when platelets are activated. It has been demonstrated that plasma concentrations of this molecule reflect *in vivo* platelet activity well [7, 8]. T-lymphocytes secrete interleukin (IL)-2 and express a high affinity receptor for this molecule on their surface. The soluble IL-2 receptor-α (sIL-2Rα), released from cell membranes, is a soluble fraction of this receptor. sIL-2Rα can be used as a marker of T-lymphocyte activation [9–14].

The purposes of this study were: 1) to evaluate *in vivo* platelet and T-lymphocyte activities during pulmonary tuberculosis by measuring plasma PF4 and soluble sIL-2Rα levels, respectively, and 2) to investigate the relationship between the extent of pulmonary infection and platelet and T-lymphocyte activities.

Patients and methods

Twenty-five patients with active pulmonary tuberculosis and 15 healthy control subjects were enrolled in this study. The ratio of males to females was 22/3 and their ages ranged 18–72 yrs, with a mean of 34 yrs. The control subjects were statistically similar regarding sex (male/female ratio 13/2, Chi-squared test p>0.05) and age (range 24–66 yrs, mean 32 yrs, Mann–Whitney U-test, p>0.05). No patient had any additional systemic illness or immunodeficiency. No patients or control subjects consumed salicylates or any other drug that might interfere with platelet functions within 2 weeks of blood sampling. Sputum samples of all patients were positive for acid-fast bacteria and the samples of 20 patients grew *Mycobacterium tuberculosis*. The failure to demonstrate *M. tuberculosis* in five samples could be due to inadequate culturing techniques.

Atypical mycobacterial infection could not be present in any of these five cases, since none of them had any underlying lung disease or immunodeficiency and all were cured with conventional antituberculosis treatment.

Complete blood counts, the erythrocyte sedimentation rate, serum C-reactive protein (CRP) concentration and a tuberculin test of each patient were obtained at the presentation. The extent of pulmonary lesions was determined in each patient as previously described in the literature [15].

Briefly the classification of parenchymal lesions on chest radiography in the patients with pulmonary tuberculosis is as follows: 1) Minimal: the sum of parenchymal areas involved is less than one-fifth of the total lung area. The lesions are of mild or moderate density. No cavities. 2) Moderate: mild or moderate-density lesions involving less than one-half of the total lung area, or high-density lesions involving less than one-third of the total lung area. The total area of cavities is <4 cm. 3) Extensive: the lesions are more extensive than in group 2. The total area of cavities is >4 cm.

Before any treatment was started, blood samples of the patients and the control subjects were drawn into 1:9 citrate-anticoagulated vacuum tubes and into platelet inhibitor-supplemented tubes (Diatube® H; Diagnostica Stago, Asniéres, France), which were placed in crushed ice and centrifuged at 4°C for PF4 determinations, without tourniquet application. The samples were immediately centrifuged at 2,000×*g* for 15 min and the plasma stored at -70°C until needed for PF4 and sIL-2Rα assays. A sandwich-type enzyme immunoassay technique was used for both PF4 (Asserachrome® PF4; Diagnostica Stago) and sIL-2Rα (Quantikine® Human IL-2 sRα Immunoassay; R&D Systems, Minneapolis, MN, USA) determinations. The plasma samples of all patients and control cases were tested simultaneously by the same kits, within 3 months of plasma isolation. A semiquantitive method was used to assay serum CRP concentrations. The tuberculin test was performed by the Mantoux's method, *i.e.* 0.1 mL of 5 U·mL^{-1} tuberculin solution was injected into the volar side of the forearm intradermally *via* a 26-gauge disposable needle. The diameter of the induration was measured 72 h later.

Statistics

The platelet, sIL-2Rα, and PF4 levels are given as mean ±SD. The differences between PF4, sIL-2Rα and platelet levels of the patients and controls were tested with the

Table 1. – Laboratory data of the patients with pulmonary tuberculosis

Patient no.	CRP	PPD mm	AFB/ culture	sIL-2Rα pg·mL^{-1}	PF4 IU·mL^{-1}	Extent of disease	Chest radiograph
1	3	0	+/+	2328	103	1	RUZ infiltration, mild density
2	4	17	+/+	2708	100	1	RLoZ infiltration, mild density
3	1	10	+/+	1952	96	1	RUZ infiltration, moderate density
4	4	10	+/+	1960	106	1	LUZ infiltration, mild-moderate densities
5	0	19	+/-	1108	90.5	1	LUZ infiltration, mild-moderate densities
6	0	12	+/-	4748	97.5	1	RUZ infiltration, moderate density
7	0	12	+/+	1956	92	1	RUZ infiltration, mild density
8	3	6	+/+	4516	103	1	RUZ infiltration with fibrosis, mild-moderate densities
9	0	15	+/+	1028	96.5	2	RMZ infiltration with a cavity of 3 cm diameter, moderate density
10	4	15	+/+	2088	104.5	2	RUZ and RMZ infiltrations containing three cavities of 1-cm diameter, moderate density
11	0	12	+/-	1408	96.5	2	RUZ infiltration with a cavity of 2 cm diameter, mild density
12	4	20	+/-	2768	105.5	2	LMZ infiltration and a cavity of 1 cm diameter in RMZ, mild-moderate densities
13	4	10	+/+	2304	106	2	RUZ and RMZ infiltrations containing three cavities of 1-cm diameter, moderate density
14	0	13	+/+	2244	96.5	2	LMZ infiltration and a cavity of 2 cm diameter, mild density
15	2	7	+/+	4384	100	2	RUZ infiltration with a cavity of 3 cm diameter, high density
16	4	15	+/-	3280	106.5	2	Bilateral UZ infiltrations, mild density
17	2	15	+/+	2196	106.5	2	LMZ infiltration and a cavity of 3 cm diameter, mild-moderate densities
18	1	17	+/+	4892	103	2	RUZ infiltration with a cavity of 3 cm diameter, moderate density
19	2	0	+/+	4512	108	3	Complete infiltration of left lung and RUZ infiltration, moderate density
20	3	8	+/+	2368	110	3	Complete infiltration of right lung, moderate density
21	3	12	+/+	1796	112	3	Bilateral UZ and MZ infiltrations, a cavity of 4 cm diameter in LUZ, moderate density
22	3	12	+/+	2320	112.5	3	Bilateral UZ and LMZ infiltrations, a cavity of 5 cm diameter, moderate density
23	1	21	+/+	2908	97.5	3	RUZ and RMZ infiltrations with a cavity of 7 cm diameter, moderate density
24	4	17	+/+	2556	115	3	Complete infiltration of left lung and RMZ infiltration, moderate density
25	3	12	+/+	10,680	112.5	3	Bilateral complete infiltrations, moderate density

CRP: C-reactive protein; PPD: tuberculin test; AFB: acid-fast bacteria; sIL-2Rα: soluble interleukin-2 receptor-alpha; PF4: platelet factor-4; U: upper; M: middle; Lo: lower; R: right; L: left; Z: zone.

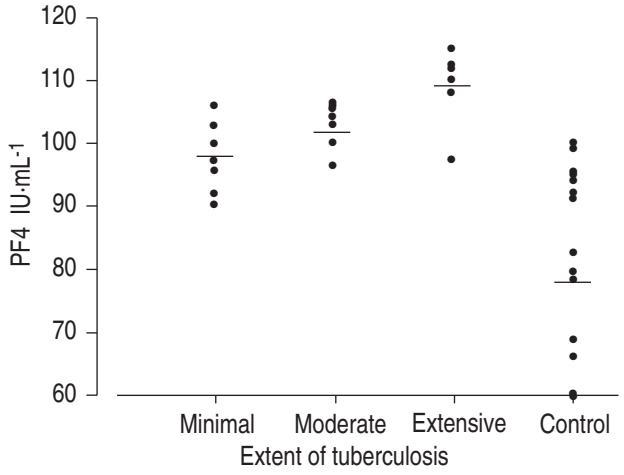

Fig. 1. – Scatter-plot graph of the correlation between plasma platelet factor-4 (PF4) concentration and the extent of pulmonary tuberculosis (small horizontal lines represent means). Note that PF4 levels are elevated in every group of patients, more prominently as the extent of tuberculosis increases. Some values are not seen owing to overlying data points.

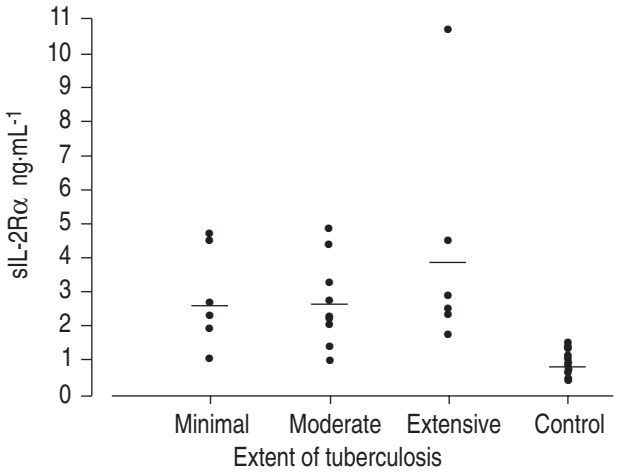

Fig. 2. – Scatter-plot graph of the correlation between plasma soluble interleukin-2 receptor-alpha (sIL-2Rα) concentration and the extent of pulmonary tuberculosis (small horizontal lines represent means). sIL-2Rα values are elevated in every group of patients compared with the control subjects. Some values are not seen owing to overlying data points.

Mann–Whitney U-test. The correlations between plasma PF4 and sIL-2Rα levels, and various molecular and clinical markers related to tuberculosis, including the extent of pulmonary disease, were investigated with Pearson's or Spearman's bivariate correlation analyses, depending on whether both of the variables were numerical or not, respectively. A p-value <0.05 was considered to indicate statistical significance.

Results

Important laboratory values of each patient are summarized in the table 1. Eight patients had minimal, 10 had moderate and seven had extensive disease on chest radiography. The patients had significantly higher plasma PF4 (103.1±6.7 IU·mL^{-1}) and sIL-2Rα (3,000±1,948 pg·mL^{-1}) concentrations than the control group (78.2±23.9 IU·mL^{-1} and 984±360 pg·mL^{-1}, respectively) (p<0.001 for both comparisons). The patients also had higher platelet counts than the control subjects ($4.2\times10^{11}\pm1.3\times10^{11}$ *versus* $2.39\times10^{11}\pm 0.66\times10^{11}$, p<0.001). There was a significant positive correlation between plasma PF4 values and the extent of disease on the chest radiograph (r= 0.65, p<0.001) (fig. 1 and table 2). There was also a significant positive correlation between plasma CRP and PF4 concentrations (r=0.68, p< 0.001). However, significant correlations were not observed between the extent of disease and sIL-2Rα (r=0.2, p= 0.34) or CRP (r=0.16, p=0.43) concentrations. Although higher sIL-2Rα levels were found in the patients with ex-tensive disease than in the remaining patients, this difference was not statistically significant (3,877±3,119 pg·mL^{-1} *versus* 2,659±1,218 pg·mL^{-1}, p=0.27). sIL-2Rα levels were significantly increased in every extent of disease compar-ed with the control group (p<0.001 for all comparisons) (fig. 2). The plasma sIL-2Rα values did not correlate with the tuberculin reaction (r=-0.2, p=0.33).

Discussion

Platelets are anucleate blood cells, which are primarily involved in blood clotting. However, they also have many of the features of classical inflammatory cells, *i.e.* chemotaxis, phagocytosis, complement activation, vascular tone alteration, enhancement of vascular permeability and ability to release potent inflammatory mediators such as IL-1, platelet activating factor, PF4 and platelet-derived growth factor [1, 16, 17]. The inflammatory and haemostatic functions of platelets are not separable, *i.e*. they are accomplished together.

It has been suggested that severe tuberculosis is often complicated by deep venous thrombosis (DVT). The incidence of DVT was found to be 3–10% in this population [18]. Two-thirds of all such cases are thought to be clinically silent [19, 20]. It was proposed that elevated plasma fibrinogen concentration, impaired fibrinolysis, decreased plasma antithrombin-III concentration and reactive thrombocytosis were responsible for the development of DVT during the clinical course of severe tuberculosis [18]. The role of platelet activation in the thrombotic predilection in severe tuberculosis has not been properly investigated previously. The positive correlation between the extent of

Table 2. – Important statistical correlations between various clinical and laboratory markers of patients with tuberculosis

	PF4 extent	sIL-2Rα extent	CRP extent	PF4 CRP	sIL-2Rα ESR	sIL-2Rα Hb	sIL-2Rα platelets	sIL-2Rα PPD
r	0.65	0.2	0.16	0.68	0.52	-0.71	0.67	-0.2
p	<0.001	0.34	0.43	<0.001	0.007	<0.001	0.006	0.33

PF4: platelet factor-4; extent: extent of pulmonary tuberculosis; sIL-2Rα: soluble interleukin-2 receptor-alpha; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; Hb: haemoglobin; PPD: tuberculin test.

pulmonary tuberculosis and platelet activation raises the possibility of contributions of the platelets in this process.

The vessels around tuberculous cavities have end arteritis obliterans and multiple microthromboses [5]. Occlusion of a blood vessel in the site of inflammation by platelet aggregates has the useful effect of entrapping leukocytes and preventing the spread of antigen through the circulation [17, 21, 22]. Thus, a possible role of the platelets in tuberculosis may be related to the development of microthromboses around the foci of infection and consequently the prevention of dissemination of the infection. One might expect that the greater the extent of infection, the more extensive the thrombotic involvement and, thus, the higher PF4 level. In this study, the presence of elevated plasma PF4 concentrations that are correlated well with the extent of the pulmonary tuberculosis on the chest radiograph supports this hypothesis. Platelets secrete their granule contents and aggregate following contact with various bacterial pathogens, which then become sequestered in clumps of platelets [1, 23–25]. Therefore, another explanation for the good correlation between the extent of pulmonary tuberculosis and platelet activation may be attributed to the bacterial load.

The association between thrombocytosis and tuberculosis has been reported previously [6]. This phenomenon and the platelet activation possibly play similar roles in the pathogenesis of tuberculosis. The present results support the relationship between thrombocytosis and pulmonary tuberculosis. The high platelet count should be considered as supportive data for pulmonary tuberculosis in the differential diagnosis of pulmonary infections.

A significant positive correlation between CRP and PF4 levels was also found. Modified CRP has been demonstrated to activate platelets *in vitro* [4, 26]. This molecule, which also reflects the acute phase response, may contribute to the platelet activation during tuberculosis [4, 6]. However, the absence of a correlation between the extent of tuberculosis and serum CRP concentration suggests that the good statistical relationship between the extent of disease and platelet activity could not be simply mediated by the acute phase response.

The plasma sIL-2Rα concentrations were also found to be elevated in the patients with pulmonary tuberculosis compared with the control subjects. However, sIL-2Rα concentrations were not correlated well with the extent of pulmonary disease. Elevated levels of this molecule were observed at all stages of pulmonary tuberculosis, including minimal changes on the chest radiograph. Although higher levels were observed in the patients with advanced disease, this difference was not significant. Elevated sIL-2Rα values in the patients with pulmonary tuberculosis were also observed in several previous studies [10–14]. Some authors discovered a correlation between the extent of tuberculosis and the level of this molecule [10–12]. However, contrary results have also been reported [14]. Takahashi *et al.* [27] reported no difference between ser-um sIL-2Rα levels of the tuberculosis patients with minimal changes on chest radiography and control subjects. Although these results seem to be controversial, they can be simply related to the differences in the numbers of patients studied and to the criteria used for staging of pulmonary tuberculosis. In the present study, sIL-2Rα concentrations of the patients did not correlate with the tuberculin reaction, suggesting that Mantoux's test may not always correctly represent cellular immunity, which is in agreement with Takahashi *et al.* [27].

In conclusion, activation of platelets and T-lymphocytes occur during pulmonary tuberculosis. The platelet activity is correlated well with the extent of disease. Therefore, platelet activation may contribute to the pathophysiology of pulmonary tuberculosis and to the thrombotic tendency reported in patients with severe forms of this disease. The functions of platelets in the pathophysiology of pulmonary tuberculosis is a subject that deserves further investigation.

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