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TITLE: Interleukin-8 activates coagulation and correlates with survival after talc pleurodesis.

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RUNNING TITLE: TALC PLEURODESIS, COAGULATION AND SURVIVAL

ABSTRACT

Objectives: Our aim was to investigate if IL-8 activates the systemic coagulation after talc pleurodesis in malignant pleural effusion (MPE), and whether levels of IL-8 in plasma are related to early dead after talc pleurodesis.

Methods: IL-8 and tumor necrosis factor-α (TNF-α) in pleural fluid and plasma from 231 patients with MPE were measured before pleurodesis (baseline), and 3, 24, 48 and 72 hours after talc. Also, whole blood from 31 healthy volunteers was incubated with IL-8, TNF-α, and thromboplastin for 3-hours *in vitro*, and thrombin-antithrombin (TAT) levels were measured afterwards. The same *in vitro* stimulation of blood samples was repeated using different doses of calibrated talc (Steritalc®, Novatech, France).

Results: Nine, twelve and seventeen patients died within the first 7, 10 and 15 days (respectively) after talc pleurodesis. IL-8 was elevated in plasma of 102 patients (44%) within the first 48 hours, and thrombotic events were observed in six of those patients; both IL-8 and TAT were elevated at several time-points in them. Survival correlated inversely with IL-8 at 24 and 48 hours in plasma, and a significant correlation was also found between IL-8 and TAT. A positive dose-dependent correlation ($p<0.01$) with TAT production was observed when blood was stimulated with IL-8 *in vitro*. However, we did not observe any significant response to stimulation with talc, as compared with control blood samples.

Conclusions: IL-8 is involved in the activation of coagulation that may occur after talc pleurodesis, and might also be implicated in early death of patients with malignant pleural effusion.

Key words: cytokines, inflammation, malignant pleural effusion, talc, thrombin-antithrombin complex.

INTRODUCTION

There is a growing concern regarding occurrence of systemic side effects after intrapleural application of talc for pleurodesis[1-5], and it seems that some of those adverse effects are related to release of pro-inflammatory mediators in the bloodstream[6-8].

A clear relation has been established between inflammatory events and the development of thrombovascular disease in several clinical settings[9], and multiple genetic and environmental factors are involved in this relationship[10]. Thus, inflammation plays a key role in venous thromboembolism (VTE), and patients with VTE show higher plasma levels of interleukin-8 (IL-8) than those without VTE[11-13].

The cytokine IL-8 is a member of the C-X-C family, and participates in the inflammatory response[14]. It has been reported that IL-8 production directly correlates with thrombin-antithrombin complex (TAT)[15].

Tumor necrosis factor alpha (TNF-α) is a pleiotropic cytokine that exerts a large variety of biological effects on many cell types. It has been shown that it is involved in the pathogenesis of the sepsis syndrome[16] and, according to some studies, it contributes to the procoagulant effect by enhancing the expression of tissue factor and inhibiting the fibrinolytic response[17].

Malignant pleural effusions (MPE) are a common complication in many neoplastic diseases. The most effective agent to control MPE is talc[18] but there are concerns about its safety. In the last 25 years, there have been several series reporting cases of acute respiratory distress syndrome after intrapleural talc administration[19-23]. Furthermore, one of the most critical adverse side effects of talc would be related to the incidence of early death following talc

pleurodesis. We hypothesised that it could be due to the changes in plasma levels of the inflammatory and coagulation factors produced as a response to an irritant agent, and that pleural talc instillation provoke a systemic dissemination of pro-inflammatory factors that could alter the coagulation/fibrinolysis balance.

The aim of this study was to elucidate the link between inflammation and coagulation after talc pleurodesis for malignant pleural effusions, and to clarify whether thrombotic events activate the inflammatory process or whether, as seems more likely, it is inflammatory mediators that trigger the coagulation cascade via cytokine production. Moreover, we wanted to investigate if early death of patients undergoing talc pleurodesis for MPE was associated to increased levels of inflammation and coagulation markers in plasma.

In the first part of the study, we investigated the link between inflammation and coagulation pathways after talc pleurodesis for MPE. We wanted to clarify if early death of patients undergoing talc pleurodesis for MPE was associated to increased levels of inflammation and coagulation markers in plasma. In the second part, we analyzed whether thrombotic events activate the inflammatory process or whether, as seems more likely, it is inflammatory mediators that trigger the coagulation cascade via cytokine production.

In order to obtain evidence for this hypothesis, we measured serial levels of IL-8, TNF-α and thrombin-antithrombin complex (TAT) pre and post-talc pleurodesis, both in pleural fluid and plasma. On the other hand, we stimulated *in vitro* whole blood samples from healthy volunteers with IL-8 and TNF-α cytokines, and also with thromboplastin and talc. Then we measured levels of both inflammation and coagulation mediators.

The conversion of prothrombin into active thrombin is a key event within the coagulation cascade; thrombin is inhibited by antithrombin III resulting in an inactive thrombin-antithrombin III complex (TAT)[24]. Therefore, TAT indicates the activation of the coagulation cascade.

PATIENTS AND METHODS

Study population:

From July 1993 to September 2007, 231 consecutive patients with MPE (125 women and 106 men, mean age: 60 years, range: 16 - 91) were submitted to thoracoscopic talc poudrage, always following the same technique for thoracoscopy. Four grams of asbestos-free sterile talc (Steritalc®, Novatech, France) with undetectable levels of endotoxin as determined with the *Limulus* amebocyte lysate assay (Toxate, Sigma) were used. The talc particles had a mean size of 24.5 μm. All the patients were followed-up until death.

For the *in vitro* study, a second group of 31 volunteers were recruited (19 women and 12 men), with ages ranging from 27 to 50 years (mean 30). Individuals with chronic disease or any kind of clinically detectable inflammation at recruitment time were excluded from the study. Both patients and healthy donors agreed to participate and gave written informed consent. The study was approved by the local Ethics Committee at our Institution.

Blood collection:

Peripheral venous blood was taken from MPE patients at baseline, and 3, 24, 48 and 72 hours after talc application. Each sample was immediately centrifuged and the supernatants were aliquoted, and stored at -80º C for further determinations.

From each healthy volunteer, we collected 5ml of fresh whole blood in EDTA K_3 tubes (Vacuette®, Bio-one, Greiner, Austria) which was immediately aliquoted into polypropylene tubes for stimulation assay.

Cytokine stimulation:

Whole blood, from healthy donors, was stimulated in parallel with 62.5 and 250 pg/ml of human IL-8 (R&D Systems Minneapolis, MN) or 62.5 and 250 pg/ml of human TNF-α (R&D Systems Minneapolis, MN). Non-stimulated samples were used as negative controls. The samples were incubated in a humidified atmosphere of 5% CO_2 at 37º C for 3 hours. After incubation the samples were centrifuged for 5 min at 1620g, and the plasma obtained was aliquoted and frozen at -80ºC for further determinations.

We performed a dose-response study by repeating the cytokine stimulation with different doses of both IL-8 and TNF-α as described above, at concentrations ranging from 31.2 to 1000 pg/ml. The doses of cytokines were chosen within the detection limits of the ELISA (R&D Systems).

Talc stimulation

Whole blood from healthy donors was stimulated with 50, 100, 200, 500, 800 and 1600 µg/ml of calibrated talc (Steritalc®, Novatech, France). Non-stimulated samples were used as negative controls, and all the samples were processed as explained above.

Induction of coagulation:

Human placental thromboplastin containing calcium (which is used to activate the coagulation process *in vitro*), (Dade Behring, Marburg, Germany) was added to 1ml of whole blood and incubated for 3h as explained above. The doses of thromboplastin assayed ranged from 10 to 200 IU.

Inflammation and coagulation mediator determinations:

IL-8 and TNF-α were measured by ELISA (Quantikine R&D Systems Minneapolis, MN), according to the manufacturer's recommendations. The minimum detectable dose of IL-8 ranged from 1.5-7.5 pg/ml and 0.5-5.5 pg/ml for TNF-α. TAT levels were determined by ELISA with the Enzygnost TAT® kit (Dade Behring, Marburg Germany) according to the manufacturer's recommendations.

Statistical analysis:

The SPSS software (version 15.0; SPSS, Inc, Chicago, IL) was used. The relation between survival of patients and markers was studied using both linear and non-linear regression models. Differences in mediators between patients with early death and those with longer survival were studied using T-test, after equal variance analysis. Non parametric Mann-Whitney U-test was used when distribution of the data did not meet the equal variance criteria. Data was expressed as mean±SEM (standard error of the mean).

RESULTS

Early death after talc pleurodesis.

Nine, twelve and seventeen patients died within the first 7, 10 and 15 days, respectively. *Sudden death occurred in four patients,* and acute pulmonary embolism was clinically suspected in all of them. Presence of massive pulmonary embolism was proven in the only one who was submitted to autopsy. Also, thrombotic events were observed in six of the patients with early death (all of them with increased levels of IL-8 in plasma). Other associated causes of early death were: Heart failure in seven patients (three of them associated to malignant pericardial tamponade), advanced neoplastic disease with bilateral pulmonary involvement in four patients, severe hyponatremia in one and unexplained coma in one. No acute respiratory distress was observed after talc application in our present series.

Increased IL-8 plasma levels after talc pleurodesis.

IL-8 dramatically increased in pleural fluid after talc application, and about the same occurred with TNF-α (*see Table 1*). Elevated levels of IL-8 (but not of TNF-α) were found in plasma of 102 patients (44%) within the first 48 hours after intrapleural application of talc (*Table 2*). Although the overall differences in IL-8 were no significant –mostly due to dispersion in different types of tumors, there was a clear trend to an increase of IL-8 at 24 hours in plasma of patients with metastatic lung and breast cancer when compared with baseline levels (84 vs. 52, and 109 vs. 54 pg/mL, respectively). This was also the case with tumors of renal origin (312 vs. 108 pg/ml), but the weight of the

remaining groups of tumors provoked the lack of statistical difference in the overall series.

An inverse correlation between IL-8 levels and patient survival was observed when we analyzed the production of this pro-inflammatory cytokine in plasma samples 24 and 48 hours after talc pleurodesis (*Figure 1A and B*). Furthermore, we found significant differences between IL-8 plasma levels in patients who died within the first 15 days after talc pleurodesis, as compared with those who lived longer. The mean cytokine production at 3 hours following pleurodesis was 80±34 (SEM) and 55±12 pg/ml in plasma samples of patients who died earlier and those who died after 15 days respectively (no significant difference). At 24 hours, the IL-8 levels were 516±219 and 50±7 pg/ml respectively ($p<0.001$), (*Figure 2*).

The *baseline values* of IL-8 in plasma were different in patients with or without early death, although the differences did not reach statistical significance: 147±61 pg/mL in patients dying within the first 15 days, as compared to 100±28 in those with longer survival. ROC analysis showed sensitivity=1 and specificity=0.90 for death within the first seven days when the cut-off for IL-8 in plasma was 92.5 pg/mL at 48h time-point (area under the curve = 0.937).

We did not find any significant differences when we compared the TNF-α plasma levels with the survival of patients submitted to talc pleurodesis (Data not shown).

Thrombin-antithrombin complex (TAT) was elevated in plasma of all of the patients who died suddenly or had thrombotic events registered. A significant correlation ($p<0.02$) was found between IL-8 and levels of TAT in

plasma of our patients at several time-points. Also, levels of TAT correlated inversely with survival at 24, 48 and 72 hours following talc pleurodesis.

TAT production is related to IL-8 cytokine in vitro stimulation.

Whole blood from healthy volunteers was stimulated with 62.2 and 250 pg/ml of both IL-8 and TNF-α for 3 hours, and TAT levels were then measured. As shown in *Figure 3*, there was a clear relationship between IL-8 doses and TAT levels ($p<0.001$), as compared to non-treated samples (controls). This response was higher with the 250-pg/ml dose. Surprisingly, we did not find any significant increase in TAT production when TNF-α was added to the blood samples, at either 62.5 or 250 (pg/ml) doses.

TAT production is activated in a dose-dependent fashion.

In order to establish if TAT levels were dose-dependent, we repeated the experiment incubating the whole blood samples with both IL-8 and TNF-α. The doses ranged from 31.2, 62.5, 125, 250, 500 to 1000 (pg/ml). A clear dose-dependent response was found in TAT production when IL-8 was added (*Figure 4A*). On the other hand, there was no statistical significance when different doses of TNF-α were assayed (*Figure 4B*). Non-treated samples did not show any significant TAT production.

EDTA tubes did not alter the activation of coagulation cascade as determined by TAT production.

It is known that EDTA binds calcium ions, thus blocking the coagulation cascade. To verify that this anticoagulant would not interfere in TAT production,

or in the activation of the coagulation cascade, the experiment was repeated adding Calcium as Calcium Chloride Solution for the *in vitro* coagulation test (0.025 mol/L, Dade Behring Marburg, Germany). After incubation, TAT production was measured, but no significant differences were found between samples stimulated with or without Calcium (Figure 5). Once again, samples incubated with TNF-α did not respond to the treatment compared to control samples.

Thomboplastin did not activate cytokine in vitro production in whole blood samples.

To investigate if the inflammatory pathway may be activated by the coagulation cascade, whole blood was incubated with different doses of thromboplastin (Dade Behring Marburg, Germany) as explained above. Both IL-8 and TNF-α production were then measured by ELISA-test, and no significant differences between treated and control samples were observed (data not shown).

Talc did not show a significant cytokine or TAT production in whole blood samples after in vitro stimulation.

In order to elucidate if talc would provoke direct production of cytokine and/or TAT, we incubated whole blood samples with 50, 100, 200, 500, 800 and 1600 µg/ml of calibrated talc (Steritalc®, Novatech, France), and non-stimulated samples were used as negative controls. No significant differences were found between treated samples and controls, both in IL-8 and TAT production in any of the doses of talc applied *in vitro* (see Discussion).

DISCUSSION

For more than 20 years, our Group has been involved in investigating the mechanisms of pleural inflammation, and we have reported a close relationship between inflammation and the coagulation-fibrinolysis balance in the pleural space of patients submitted to talc pleurodesis[25]. Specifically, we found dramatically increased levels of TAT in the pleural fluid of patients with MPE who had a successful pleurodesis[26], but the possible simultaneous activation of coagulation in the systemic circulation could be a life-threatening side effect. Therefore, we have been long concerned about this particular problem, especially as we have had several cases of massive pulmonary embolism following pleurodesis[27].

The specific aims of our study were to evaluate the relation between the inflammatory process and the activation of the coagulation cascade, and to test whether the inflammatory process is a cause or a result of thrombotic events. Also we wanted to find out if there was any relationship between early deaths after talc pleurodesis and plasma levels of pro-inflammatory markers.

IL-8 and activation of coagulation cascade in the systemic circulation:

Our findings support the idea that IL-8 is involved in the activation of coagulation that may occur after talc pleurodesis: We found that IL-8 was elevated *in plasma* of 102 of our patients submitted to talc pleurodesis (44%) within the first 48 hours, and thrombotic events were observed in six of them. In addition, both IL-8 and TAT were elevated in plasma of those patients at several time-points following talc pleurodesis, and a negative correlation was found between plasma levels of IL-8 and survival.

A significant body of evidence has now emerged to support the hypothesis that pro-inflammatory cytokines, that could be released in response to a variety of stimuli –including thoracoscopy without talc application (Froudarakis et al)[7]- could also play a key role in coagulation activation. An increase in inflammatory mediators such as IL-6, IL-8, MCP-1 and TNF-α has been found in patients with venous thromboembolism[12,13], and just as pro-inflammatory mediators they may regulate coagulation activation; some products of the clotting cascade may also affect the inflammation process[28]. It has been proposed that coagulation factors, like tissue factor (TF), FVII, FX and thrombin play an important role in inflammation[29]. However, other studies have found no evidence of the role of cytokines in determining the risk of venous thromboembolism[30], and it remains uncertain whether inflammation is a cause or a result of thrombosis. A possible explanation for the discrepancies in the reported results could be due to differences in the design of the studies. The studies mentioned above measured the cytokine levels in patients with confirmed venous thrombosis (VT), or prior to endotoxin stimulation, but all of them were conducted *in vivo,* while our study was of whole blood stimulated *in vitro*. The response found in the *in vivo* studies could be due to the capacity of the cytokines to interact with each other. However, the increased TAT levels that we found after IL-8 stimulation *in vitro* suggest a direct implication of this cytokine in coagulation activation. Moreover, the correlation was dose-dependent. In the light of our results and due to the multifunctional characteristics of this mediator, IL-8 appears to be a key link in the cross-talk between inflammation and coagulation pathways, at least regarding talc pleurodesis. Indeed, we found a clear response of TAT production in plasma

samples when whole blood was stimulated with IL-8 (*Figure 4A*). The increase in TAT levels supports the hypothesis that coagulation is somehow activated by this cytokine, although the molecular mechanism still remains unclear. Interestingly, a study by van Aken et al made similar findings[11]. This group reported that patients with venous thrombosis showed higher IL-8 plasma levels, and concluded that this cytokine is a risk factor for thrombotic disease. TNF-α has been implicated in host defence, inflammatory response and pathophysiological processes. A role has been reported for TNF-α in the activation of both coagulation and fibrinolysis[16], but according to our results this cytokine was not able to produce TAT when it was added to whole blood *in vitro*. This negative response was clear up to a dose of 1000 pg/ml. Therefore, it does not appear that TNF-α participates in the coagulation pathway, at least via TAT production. However, it remains unclear whether TNF-α could be involved in both inflammation and coagulation pathways by activating other factors that might indirectly activate clotting through other cytokines or mediators, or requiring the active participation of the vascular endothelial cells, that were obviously absent in our *in vitro* experiments.

Early death after talc pleurodesis:

Nine, twelve and seventeen patients died within the first 7, 10 and 15 days, respectively, following intrapleural application of talc, and sudden death occurred in four of them. Autopsy was performed in one, and massive pulmonary embolism was found. Since he had no cases of acute respiratory distress in this series, we speculated if there were more unknown thrombotic events than expected in those patients with short survival.

It is well established that venous thromboembolism is a common complication in patients with cancer[31,32]. The interactions between inflammation and coagulation-fibrinolysis balance in the pleural space described by us and others[25,26,33] –taken together with the finding of elevated levels of IL-8 *in plasma* of up to 44% of our patients in the present study- would suggest that, after application of the pleurodesis agent, IL-8 could disseminate from the inflamed pleural space into the bloodstream, then activating the systemic coagulation. The association found by us between high levels of IL-8 in plasma and short survival (*see figs. 1A and 1B, and Table 2*) is striking in our study, and would support this hypothesis.

It would be interesting to know if our above results would also apply to other sclerosants that are frequently used for pleurodesis. We have undertaken a multicenter study on pleurodesis for malignant pleural effusions, comparing results of pleurodesis and levels of cytokines in pleural fluid and plasma when either talc, doxycycline or tetracycline were used[34], and our preliminary results indicate that both doxycycline and tetracycline –but especially tetracycline- might provoke increased levels of IL-8 in plasma following the pleurodesis procedure.

Our study has a few limitations: If IL-8 disseminates from the pleural space into the bloodstream after the acute inflammation produced by talc instillation in the pleural cavity, a good correlation between levels in pleural fluid and peripheral venous blood samples would be expected, but this was not the case in our series. *De novo* synthesis of IL-8 in blood following some –unexplained so far- stimuli coming from the pleural space would be another plausible alternative. Further studies involving mRNA investigation for IL-8 and

other mediators in peripheral blood samples would be necessary to elucidate this point. Our *in vitro* study incubating whole blood separately with IL-8 and TNF-α was aimed primarily to elucidate if IL-8 was able to stimulate *per se* the production of thrombin-antithrombin complex (TAT), and we succeeded in that. However, the lack of response to TNF-α stimulation was unexpected to us, and unexplained so far. As we have pointed out above, the absence of *vascular structures* (and the possible interaction with endothelial cells) in our *in vitro* experiment could account for this lack of response. This would probably also be the explanation for the lack of significant differences found when whole blood was stimulated with talc.

In conclusion, the main clinical implication of our study would be that high plasma IL-8 levels might be a predictor for possible future thrombotic events and/or early death in patients with MPE who are submitted to pleurodesis. Although it reached no statistical significance, our finding that IL-8 levels *at baseline* were higher in patients with early death (*see Table 2*) would support this assertion. Further studies elucidating which mediators regulate the link between inflammatory events and coagulation would have great interest in future target-therapies for patients with advanced cancer.

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FIGURE LEGENDS

Figure 1: **Survival and plasma levels of IL-8 from patients with MPE submitted to talc pleurodesis.** The X-axis represents the IL-8 plasma levels (pg/ml), 24 (A) and 48 hours (B) after talc pleurodesis and the Y-axis indicates the survival of patients in months (log scale).

Figure 2: **IL-8 levels in plasma of patients with/without early death (≤ 15 days) after talc pleurodesis**.

Figure 3: **Plasma levels of TAT complex**. The vertical axis represents the TAT plasma levels when whole blood was stimulated with 31.2 and 62.5 pg/ml of both IL-8 and TNF-alpha cytokines.

Figure 4: **Whole blood stimulated with different doses of IL-8 and TNF-α.** (A) TAT levels correlate to IL-8 stimulation at different doses ($p<0.001$). (B) TAT levels did not show any significant difference between the stimulated samples and control.

Figure 5: **Whole blood samples stimulated with or without Calcium ion (Ca).** There were no significant differences in the TAT levels when cytokine IL-8 and TNF-α were added with Calcium ion.

FIGURES

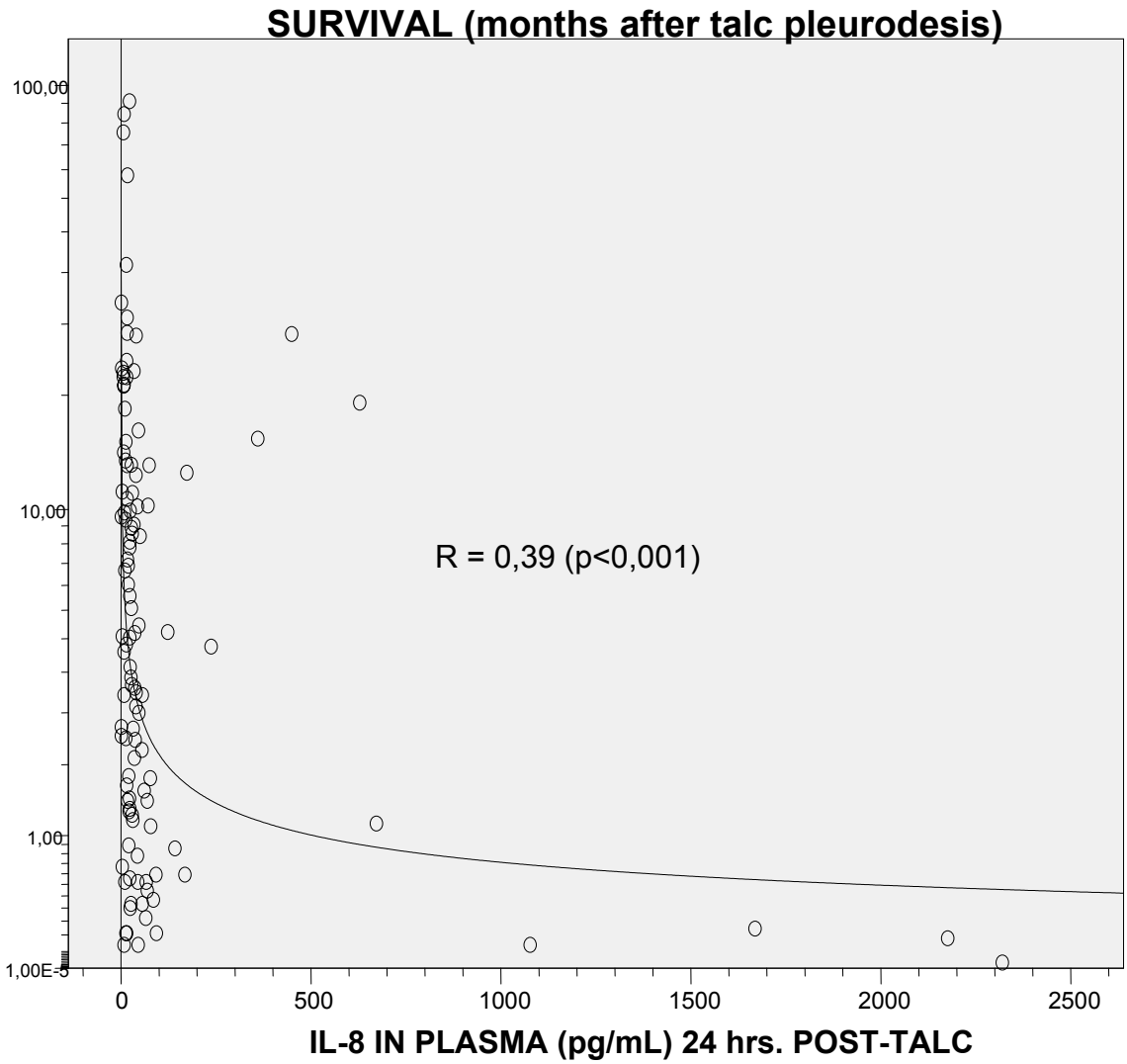

Figure 1A

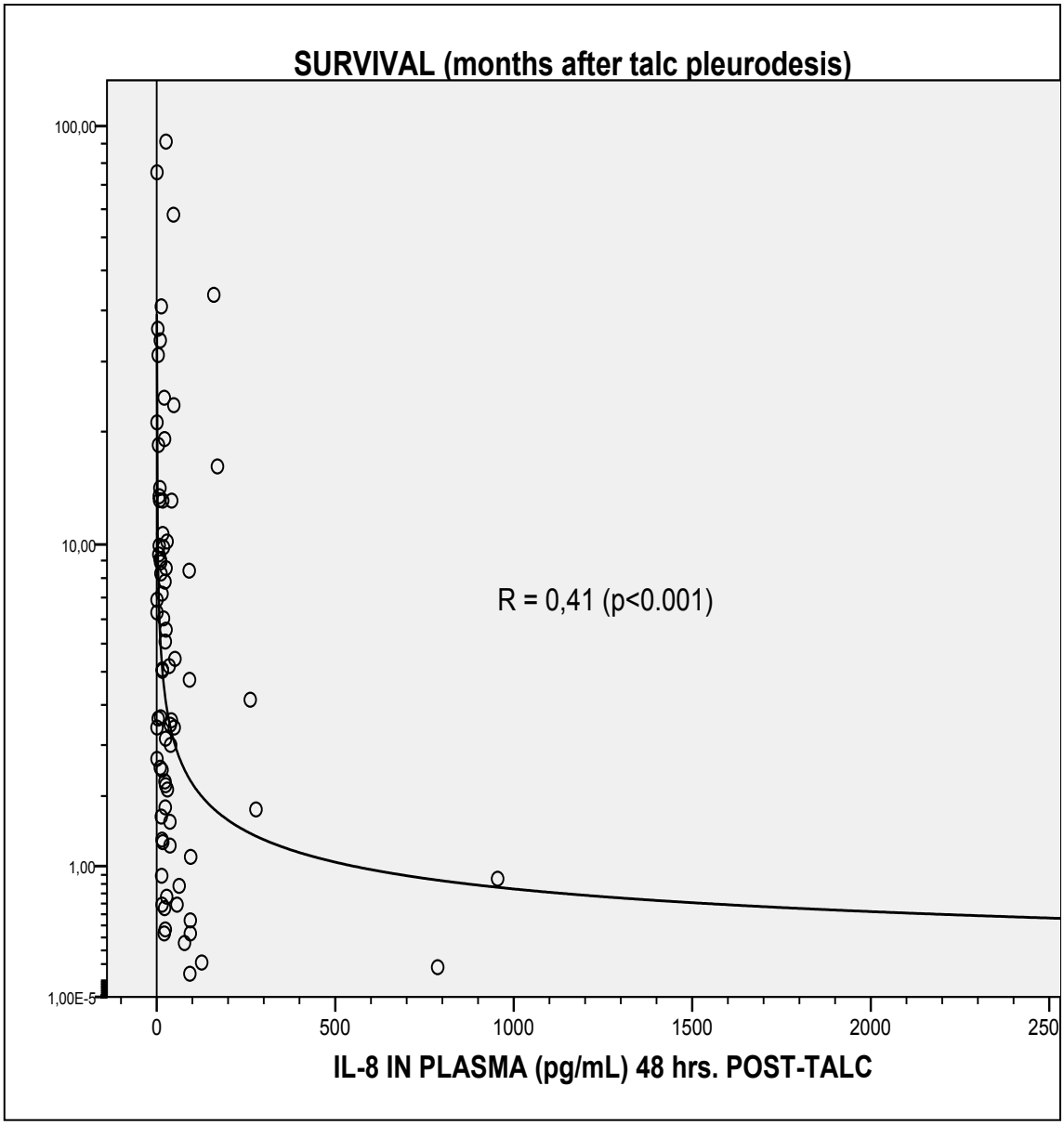
SURVIVAL (months after talc pleurodesis)
100,00
10,00
1,00
1,00E-5
R = 0,41 (p<0.001)
0
500
1000
1500
2000
250
IL-8 IN PLASMA (pg/mL) 48 hrs. POST-TALC

Figure 1B

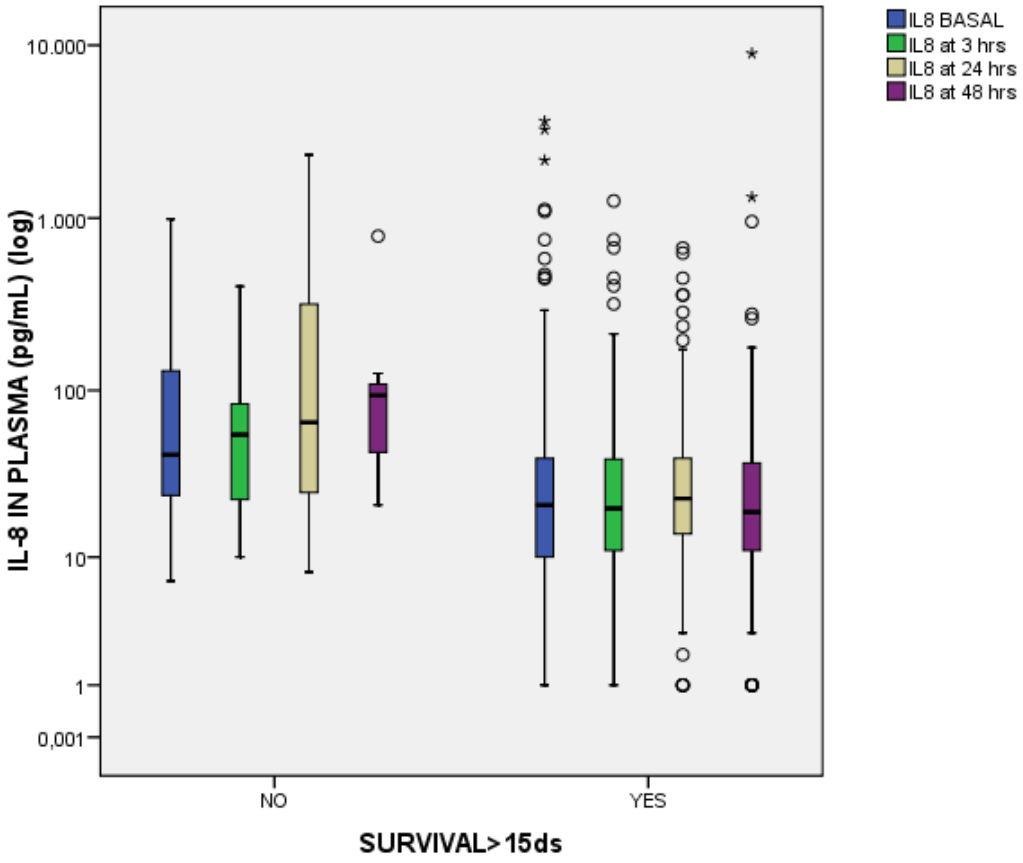
IL8 BASAL
IL8 at 3 hrs
IL8 at 24 hrs
IL8 at 48 hrs
10.000
1.000
100
10
1
0,001
IL-8 IN PLASMA (pg/mL) (log)
NO
YES
SURVIVAL>15ds

Figure 2.

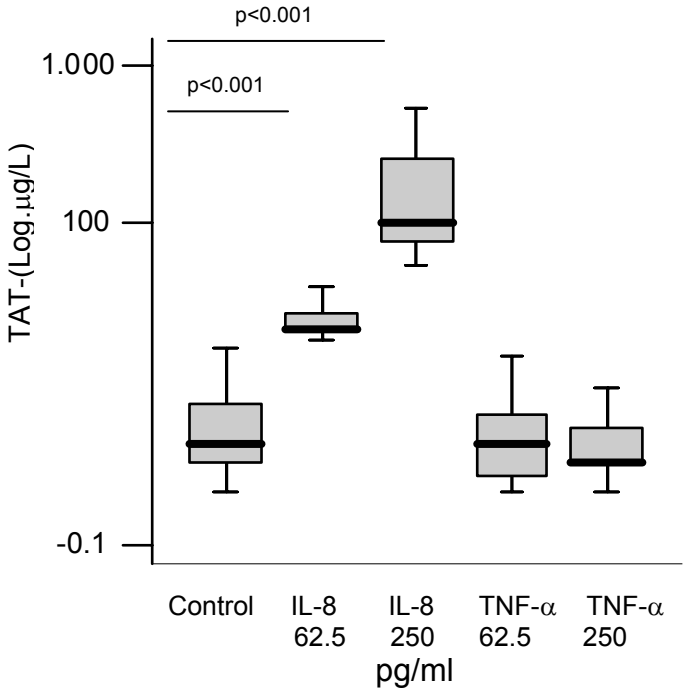
p<0.001
p<0.001
1.000
100
-0.1
TAT-(Log.µg/L)
Control
IL-8 62.5
IL-8 250
TNF-α 62.5
TNF-α 250
pg/ml

Figure 3

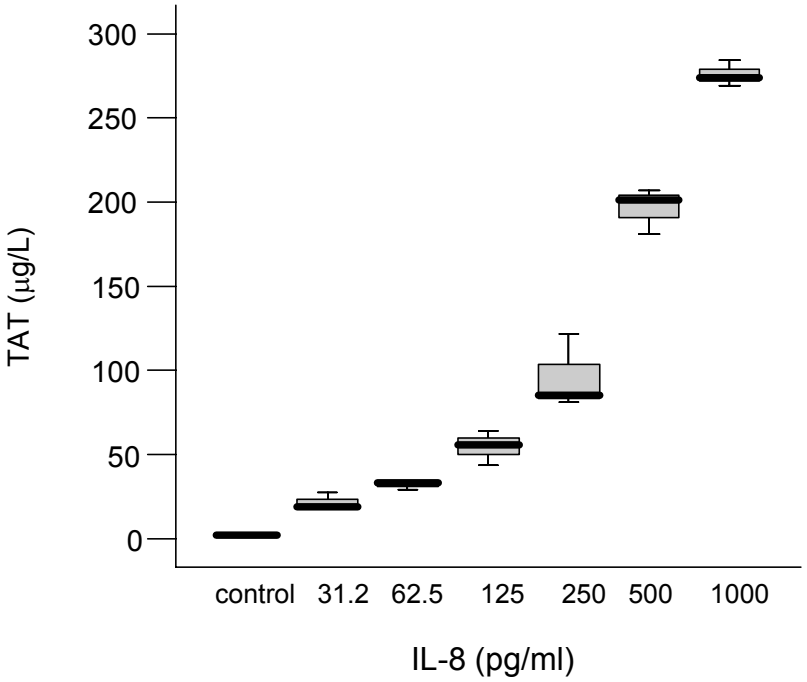

Figure 4A.

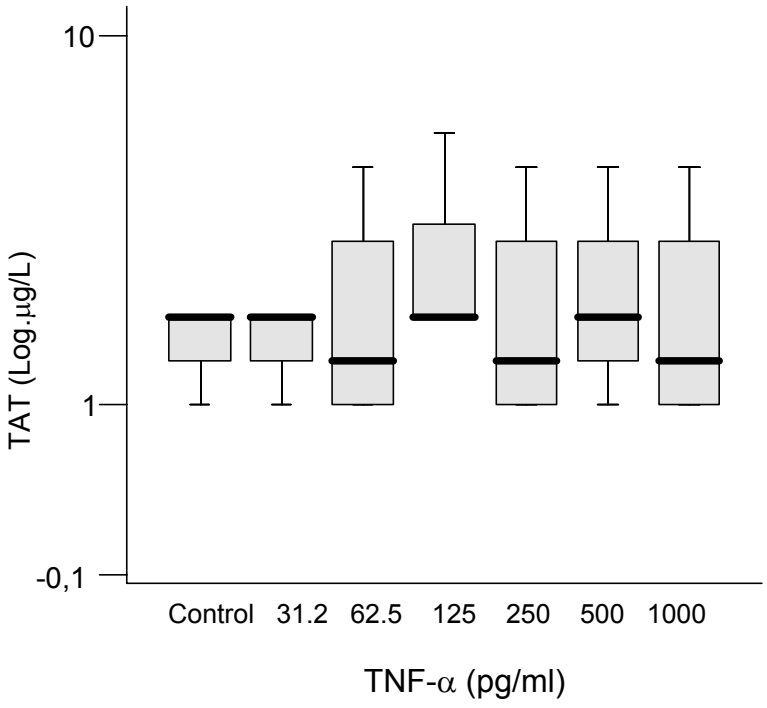

Figure 4B.

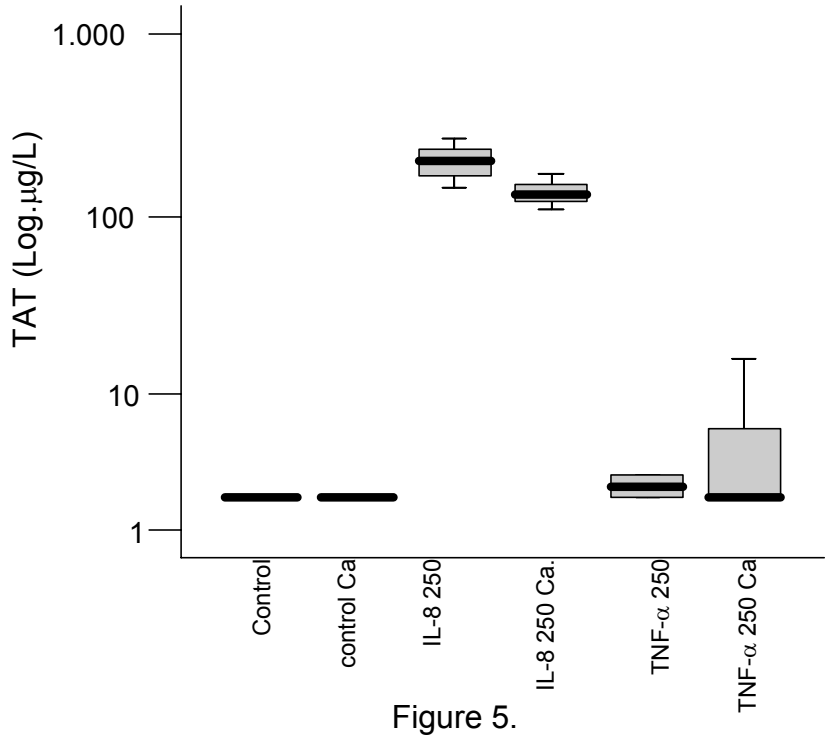
1.000
100
10
1
TAT (Log.µg/L)
Control
control Ca
IL-8 250
IL-8 250 Ca.
TNF-α 250
TNF-α 250 Ca

Figure 5.

Origin of tumors	Median Survival (months)	IL-8 (pg/mL) in Pleural fluid		TNF-α (pg/mL) in Pleural fluid		TAT (ug/L) in Pleural fluid	
		Baseline	24 hrs	Baseline	24 hrs	Baseline	24 hrs
Lung (52)	2.7	1,851±440	14,035±6,605	52±12	74±16	7,414±1,565	27,447±10,567
Breast (51)	6.9	913±203	16,654±5,218	54±18	58±18	7,472±3,322	19,930±3,194
Mesothelioma (37)	9.3	1,476±367	19,169±8,486	231±106	61±12	1,648±466	11,735±1,824
Lymphoma (15)	1.6	230±156	7,460±3,942	268±241	150±121	2,361±1,034	18,462±8,418
Colon (13)	3.2	1,094±453	7,560±1,086	114±84	54±20	2,340±874	20,106±5,046
Kidney (11)	2.1	617±137	6,755±1,428	104±88	68±23	5,732±1,885	69,448±57,273
Ovary (11)	7.9	1,128±597	9,431±4,181	39±19	55±17	10,957±4,862	27,920±7,257
Stomach (7)	1.5	918±306	23,495±9,592	30±11	82±37	4,568±1,888	31,298±4,886
Sarcoma (4)	0.8	537±205	16,645±7,248	59±13	33±13	13,730±4,137	21,403±9,592
Others (8)	1.6	1,141±529	15,378±6,602	26±5	33±14	2,437±804	25,463±11,526
Unknown (22)	4.2	4,063±2,620	13,865±4,036	75±28	80±26	4,622±1,287	22,017±3,127
TOTAL (231)	**4.2**	**1,461±292**	**14,415±2,356 (p<0.001)**	**9±1**	**68±10 (p<0.001)**	**5,665±879**	**23,442±3,569 (p<0.001)**

Table 1. Median survival, IL-8, TNF-α and Thrombin-antithrombin complex (TAT) in pleural fluid just before and 24 hours after talc pleurodesis in 231 malignant pleural effusions. Data is expressed as mean±SEM. (Clearly significant differences between baseline values and those obtained at 24 hours following talc application).

Origin of tumors	Median Survival (months)	IL-8 (pg/mL) in Plasma		TNF-α (pg/mL) in Plasma		TAT (ug/L) in Plasma	
		Baseline	**24 hrs**	**Baseline**	**24 hrs**	**Baseline**	**24 hrs**
Lung (52)	2.7	52±12	84±27	6±2	5±1	517±174	367±101
Breast (51)	6.9	54±18	109±60	3±0.9	4±0.9	463±96	450±129
Mesothelioma (37)	9.3	231±106	56±17	4±0.8	7.5±2	1,053±616	220±75
Lymphoma (15)	1.6	268±241	231±195	4±1	4.5±2	405±110	519±185
Colon (13)	3.2	114±84	35±7	1.4±0.3	3±1.7	525±189	659±207
Kidney (11)	2.1	104±88	312±231	3±1	26±22	242±125	259±144
Ovary (11)	7.9	39±19	36±9	3±0.7	2.4±0.9	258±163	364±281
Stomach (7)	1.5	30±11	31±8	4±1.5	4.7±2	1,313±690	587±244
Sarcoma (4)	0.8	59±13	35±18	5±3.7	2±1	729±277	518±182
Others (8)	1.6	26±5	30±6	6±3.7	3±1.4	346±228	505±316
Unknown (22)	4.2	75±28	22±3	11±6	6±2.8	1,109±853	298±121
Total (231)	**4.2**	**101±25**	**87±21 (N.S.)**	**4.8±0.8**	**5.9±1 (N.S.)**	**632±133**	**389±47 (p<0.05)**

Table 2. Median survival, IL-8, TNF-α and TAT in plasma just before and 24 hours after talc pleurodesis in 231 malignant pleural effusions. Data is expressed as mean±SEM. (N.S= non-significant differences between baseline values and those obtained at 24 hours).