Thoracoscopic talc poudrage decreases T-lymphocytes in the peripheral blood

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\textbf{Summary}
\textbf{Background:} Thoracoscopic talc poudrage induces peripheral blood granulocytosis and lymphopenia. The aim of this study is to investigate the type of lymphopenia in patients undergoing thoracoscopic talc poudrage.

\textbf{Methods:} We have measured peripheral blood lymphocyte subsets in 11 patients undergoing thoracoscopic talc poudrage, before (baseline), at 24 and 48 h after the procedure. Lymphocyte numbers were analysed by flow cytometry for the evaluation of the CD3+, CD4+, CD8+ cells (total T-lymphocytes, helper T-lymphocytes, cytotoxic T-lymphocytes, respectively), the CD19+ cells (B-lymphocytes), and the CD16+, CD56+, and CD57+ cells (NK-cells). No anti-inflammatory medication was permitted before, during or after the procedure.

\textbf{Results:} Absolute peripheral blood lymphocyte count significantly decreased following thoracoscopic talc poudrage compared to baseline values ($p = 0.007$). Similarly, peripheral blood CD3+, CD4+ and CD8+ lymphocyte counts significantly decreased compared to baseline ($p = 0.005$, $0.02$ and $0.03$, respectively) with a more prominent reduction of CD3/CD45RO memory cells. No significant difference was found in the absolute number of CD19+, CD16+, CD56+, and CD57+ cells before and after thoracoscopic talc poudrage.

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Conclusion: Patients undergoing thoracoscopic talc poudrage display peripheral blood T-lymphopenia following the procedure.
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Introduction

The pleural space is a cavity of potential immunological and/or inflammatory process, since the pleura is a highly permeable layer closely related to local microcirculation. Although, it is generally accepted that any kind of aggression, such as infectious, inflammatory or malignant disease may affect the pleural space, little effort has been made to understand the immunological and molecular mechanisms underlying pleural disorders. The pleural mesothelium responds to any aggression by actively recruiting inflammatory phagocytic cells and allowing the movement of proteins from the vascular compartment into the pleural space. Acute inflammatory process is associated with the development of an inflammatory cell rich pleural effusion. A migration of neutrophils followed by mononuclear phagocytes and lymphocytes is a characteristic feature of this inflammation. These inflammatory cells move from the vascular compartment into the pleural space.

Talc is actually the agent of choice for chemical pleurodesis, with an efficacy rate of more than 90%. It may be administered as a powder by insufflations during thoracoscopy, inducing fibrosis and inflammation in the pleural cavity. Talc pleurodesis is generally well tolerated. However, reports have stated minor side effects such as, pleuritic pain during pleurodesis, nausea and fever after the procedure. Fever after thoracoscopic talc poudrage occurs in at least 20% of the patients who have been included in prospective studies. We have recently published a study showing that intra-pleural insufflation of talc during thoracoscopy induces fever and systemic inflammatory reaction. Inflammation was significant 24 h after talc poudrage with a significant increase of C-reactive protein (CRP) in the serum, white blood cells and neutrophil counts in the peripheral blood of the patients. In parallel, we noted a significant decrease in patient peripheral blood lymphocyte counts.

However, discussion of the choice of sclerosing agent is hampered by the lack of knowledge of the inflammatory process. It is assumed that sclerosing agents may induce an inflammatory response leading to a rapid adhesion of the parietal and visceral pleura. In the literature, only scarce information is available on the inflammatory mechanism itself. Since we observed several humoral immune modifications in the peripheral blood related to talc poudrage, we initiated a study to further investigate the alterations in the number and function of lymphocytes. The aim of our study is to assess peripheral blood lymphocyte subsets in order to investigate the lymphopenia induced by thoracoscopic talc poudrage. The clinical relevance of our report is to bring a piece in the understanding of the systemic inflammatory reaction due to talc pleurodesis. To our knowledge, there is no published report studying specifically the talc-induced peripheral blood lymphopenia in patients undergoing thoracoscopic talc poudrage.

Methods

Data collection

From May 2004 to December 2004, blood samples from 11 consecutive patients undergoing thoracoscopic talc poudrage were analyzed after consent. Patient characteristics are shown in Table 1. Blood samples are anyway drawn before thoracoscopy (standard pre-procedure control) and after the intervention to follow the patient’s clinical status. Though, peripheral blood lymphocytes and their subpopulations were evaluated before thoracoscopy (baseline) and 24 h (day 1) and 48 h (day 2) after thoracoscopy. We also evaluated the same way blood eosinophils. Following the procedure, patients had a baseline treatment for the pain with fentanyl citrate patch 25 mg every 72 h. In case of persistent pain, patients received supplement of parenteral narcotics (morphine) on request. No anti-inflammatory or antipyretic medications were permitted before, during, or after the procedure.

Thoracoscopy

Thoracoscopy has been approved by the Internal Review Board (IRB) of the Heraklion University Hospital, as a standard procedure in the diagnosis and therapy (talc pleurodesis) of pleural effusions. Though, no further IRB was necessary to perform this procedure, as it is done for routine clinical practice (diagnosis and therapy of pleural effusions). Patients were admitted the same morning of thoracoscopy and were discharged on the day of chest tube removal. During the procedure, blood pressure, oxygen saturation, and electrocardiogram, were monitored. Oxygen supplementation was provided if needed. In case of pain during thoracoscoppy, patients received supplement of parenteral narcotics (morphine) on request. No general anesthesia was used at any case.

Thoracoscopy was performed in the lateral decubitus position using single port of entry, under local anesthesia with 1% lignocaine. Patients were administered morphine 0.5 mg as needed. A 7 mm trocar was inserted into the fifth or sixth intercostal space along the mid-axillary line. After evacuation of the pleural fluid, a 0° optical telescope was inserted and connected to a light source. The entire pleural cavity was then inspected, at times requiring supplemental air insufflation.

Sterile asbestos-free talc (Steritalc®, Novatech, La Ciotat France) was insufflated into the pleural space of patients for pleurodesis (4 g of talc for malignant pleural effusion and 2 g for pneumothorax). At the end of the procedure, a chest tube (24–28 French gauge) was inserted and connected to underwater seal with a negative suction of 20 cm H₂O for at least 2 days. Chest tube was removed when less than 100 ml...
of pleural fluid was drained over a 24 h period in patients who underwent pleurodesis.

Enumeration of blood cells

Venous blood samples were drawn directly into sterile tubes containing EDTA-K3 (0.17 mol/l) as anticoagulant. Blood cell enumeration and white blood cell differential counts (lymphocytes and eosinophils) were performed in a Coulter STKS hemocytometer (Coulter Electronics, UK). Values were expressed in absolute numbers of cells per microliter (μl) of blood. All samples were studied within 1–2 h following blood collection without storage. The same blood samples were subsequently used for analysis of lymphocyte subsets. Percentages of lymphocyte subsets were determined on the basis of cell surface marker analysis, carried out by the Epics ELITE model of flow cytometer (CoulterElectronics, UK) in the gate of cell with low forward and low side-scatter properties where more than 98% lymphocytes are included (Fig. 1). In each case, 5 × 10⁵ positive-gated events were counted.

Statistical analysis

We used a statistical software package (StatView™, version 4.5; Abacus Concepts Inc., Berkeley CA, USA) for the statistical evaluation of our data. The mean values and standard deviation (SD) were calculated for continuous data. Continuous data were compared using the Student t-test. Analysis of variance (ANOVA) was used to evaluate differences in repeated measurements. A value of p < 0.05 was considered to be statistically significant.

Results

Peripheral blood lymphocytes

Peripheral blood lymphocyte counts at baseline were (mean ± SD/μl blood) 1963 ± 375 (range: 1349–2548). Lymphocytes decreased on Day 1 at 1467 ± 332 (range: 1010–1970) and on Day 2 at 1612 ± 428 (range: 858–2213). The overall decrease of lymphocyte counts was statistically significant (p = 0.007) (Fig. 2). Lymphocyte reduction was more pronounced on Day 1 (p = 0.009) than on Day 2 (p = 0.01) compared to baseline values.

Peripheral blood eosinophil counts at baseline were (mean ± SD/μl blood) 449 ± 374 (range: 81–1722). Eosinophils decreased on Day 1 at 116 ± 143 (range: 0–414) and on Day 2 at 482 ± 218 (range: 191–768). The overall decrease of eosinophil numbers was statistically significant (p = 0.005) (Fig. 3). Eosinophil reduction was seen only on Day 1 in comparison to baseline values (p = 0.005).

T-cell lymphocyte subsets

T-cell subpopulations defined by the CD3, CD4, and CD8 cell surface markers, were all significantly decreased in our patients at day 1 and 2 compared to baseline (p = 0.005, 0.022, 0.03, respectively) (Table 2 and Figs. 4–6). The CD4+/CD8+ cell ratio did not change significantly (p = 0.24), suggesting a parallel diminution of both CD4+ and CD8+ (PBS, pH 7.4), resuspended in paraformaldehyde solution, and studied immediately. The following mouse anti-human MoAbs were used: FITC- or PE-conjugated anti-CD3 (UCHT1), anti-CD4 (13B8.2), anti-CD8 (B9.11), anti-CD19 (J4.119), anti-CD16 (3G8), anti-CD57 (NCI), anti-CD56 (N901), anti-CD45RA (J.33), and anti-CD45RO (UCHL1). Two-color flow cytometry was used for the detection of CD56/CD57, CD3/CD45RA, CD3/CD45RO double positive cells, representing the NK-cells, the naïve and memory T-lymphocytes, respectively. All antibodies were purchased from Beckman Coulter.

Flow-cytometric analysis

Cells were evaluated for membrane fluorescence using an Epics ELITE model of flow cytometer (CoulterElectronics, UK) in the gate of cell with low forward and low side-scatter properties where more than 98% lymphocytes are included (Fig. 1). In each case, 5 × 10⁵ positive-gated events were counted.
T-cell subpopulations (Table 2). To probe further the mechanisms underlying T-cell lymphopenia in the patients, we evaluated the CD3/CD45RA and CD3/CD45RO cell subsets representing the naive and memory cells, respectively (Table 3). Although the overall decrease of both naive and memory cells was not significant, we observed a significant decrease from baseline on days 1 and 2 (D-1) and day 2 (D-2) compared to baseline (D-0). The background fluorescence obtained from the isotypic control is also shown.

The CD57 cell phenotype

The CD57 cell phenotype of peripheral blood lymphocytes is illustrated in Table 4. No significant differences were found
in the mean number of CD57+ cells or the CD56+/CD57+ cells between day 1, 2 and baseline. Since many CD57+ cells are in fact T lymphocytes, it is probable that their diminution in our patients is a consequence of the aforementioned decrease of the CD3+ T-cell populations. No significant correlation was demonstrated between the numbers of circulating neutrophils and the numbers of CD57+ cells in the subjects studied.

B-cell and NK-cell populations

Patient B-cell and NK-cell populations are presented in Table 4. The numbers of peripheral blood B-cells, defined by the expression of CD19 cell surface antigen, did not differ statistically between baseline, day 1 or 2. Also, the mean numbers of NK cells defined by the expression of CD16 and/or CD56 cell surface markers were not significantly different between baseline, day 1 or 2. Both CD16+ and CD56+ cell counts were not correlated with the numbers of circulating neutrophils.

**Discussion**

We have previously shown that patients undergoing talc poudrage display peripheral blood lymphopenia following the procedure. In the current report we investigated whether this lymphopenia concerns T- and/or B- and NK lymphocyte subsets. To the best of our knowledge this is the first report investigating the effect of thoracoscopic talc pleurodesis in lymphocyte subsets.

The helper to suppressor T-cell ratio in the peripheral blood of our patients at baseline (Table 2) was similar to that previously reported for healthy subjects. Other authors have already reported similar results in patients with malignancies including breast cancer and melanoma. A possible explanation is that patients with localized carcinoma display total T-cell subpopulations similar to those of healthy subjects. Indeed, our 10 patients with carcinomatous pleurisy had limited disease and were all in good shape (performance score of Eastern Cooperative Oncology Group (ECOG): 0 or 1), with life expectancy for at least 6 months, to undergo thoracoscopic talc pleurodesis. In contrast, patients with extensive metastatic disease and low performance status have been reported to display CD4+/CD8+ ratio <1 as in immunocompromized subjects.

Our patients had decreased T-lymphocyte numbers in peripheral blood 24 h after thoracoscopic talc poudrage (Table 2). The lymphocytes probably decreased due to enhanced extravasation in the pleura. In favor of this hypothesis is the prominent decrease of the memory CD3+/CD45RO lymphocyte subsets in the patients. An accumulation of lymphocytes, especially memory T-lymphocytes, in pleural fluid has been reported to occur in intrapleural inflammatory processes such as neoplastic and tuberculous effusions. This transient T-lymphocyte extravasation may result in transient lymphopenia. This phenomenon is probably followed by spontaneous expansion of the remaining T-cells in the periphery to restore the original T-cell pool size and maintain homeostasis. Consistent with this hypothesis was the partial recovery of the T-lymphocytes in patients’ peripheral blood 48 h after the talc poudrage.
In our patients, the acute development of fever and neutrophilia, which subsided after 24 h advocates for a systemic acute inflammatory response. Such reactions are normally accompanied by endogenous steroid production as a compensatory mechanism. Elevated levels of circulating glucocorticoids result in temporary lymphopenia due to redistribution of peripheral blood lymphocytes to other lymphoid compartments and depletion of recirculating lymphocytes. Usually, the lymphopenia resolves after 24 h, whereas T-cells are selectively depleted with a predilection for the memory T-cell subset.

Eosinophils are also profoundly decreased 4–6 h after corticosteroid administration through an identical mechanism involving migration of eosinophils to other tissues. Consistent with all of the above, our study population displayed a parallel fluctuation of both lymphocyte and eosinophil count, while NK cells were the only lymphocyte subsets that remained unaffected (Table 4), as expected in innate immune system activation. Additionally, the slight but not significant decrease of B-cells along with the persistent reduction of T-memory cells after 48 h further concurs for the aforementioned mechanism (Tables 3 and 4).

Interleukin-8 (IL-8) is a cytokine with pleiotropic effects which is secreted during acute systemic inflammation, when it stimulates the synthesis of acute-phase proteins. In addition, IL-8 is a member of the CXC chemokine family and can evoke T-lymphocyte migration into the pleural space. Although pleural fluid lymphocyte subsets were not assessed in the current study, it is tempting to speculate that production of IL-8 from stimulated mesothelial cells after thoracoscopic talc poudrage induced the recruitment of peripheral blood T-cells in the pleural space, resulting in the observed T-cell decrease in the periphery. Data on the phenotype of pleural lymphocytes have been reported in pleural immune reactions in case of malignant and tuberculous pleural effusion. However, data are lacking concerning the type and role of these cells in the process of pleurodesis by a sclerosant agent.

We concluded that patients undergoing thoracoscopic talc poudrage display peripheral blood T-lymphopenia following the procedure, probably due to enhanced extravasation in the pleura.

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