Toxic-Oil Syndrome*

Gallium-67 Scanning and Bronchoalveolar Lavage Studies in Patients with Abnormal Lung Function

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The toxic-oil syndrome (TOS) is a multisystem disorder whose etiology and pathogenesis are as yet unknown. Lung alterations persist in a significant number of TOS patients due to the underlying vascular lesion. Computer-assisted "Ga scanning and bronchoalveolar lavage (BAL) studies were performed in 14 TOS patients with sustained abnormal diffusing capacity for carbon monoxide (Dco). No significant difference was observed between the "Ga uptake index of the TOS and control populations. Likewise, there was no significant difference in the number of effector cells recovered from the lungs of TOS patients and controls by bronchoalveolar lavage. However, a rise in IgA and IgG concentrations (p<0.002) and a fall in α, antitrypsin (p<0.05) and transferrin (p<0.01) were observed in the TOS group. Phospholipid and lecithin concentrations in the lavage fluid were similar for patients and controls. The alveolar macrophage function assayed in three TOS patients was normal. These observations raise new questions about the outcome of lung pathology in TOS and warrant further follow-up studies of the lung abnormalities observed.

The appearance in Central and Northwestern Spain in May, 1981 of a new syndrome of an epidemic nature which accounted for high morbidity and mortality, presented a challenge to the Spanish health authorities. The WHO Working Group linked the new illness to an as yet unidentified toxic substance present in denatured rapeseed oil, referring to it as the toxic-oil syndrome (TOS).*

During the acute stage, diagnosis was mainly based on epidemiologic data and a clinical picture characterized by noncardiogenic pulmonary edema which occasionally evolved to become a respiratory distress syndrome. Steroid therapy proved effective in the management of this lung disorder in the acute stage.

Because the precise etiology of TOS is as yet unknown, its pathogenesis remains unclear, no specific treatment regimens have proved effective in the chronic stage, and the clinical course is dissimilar, further follow-up studies of the TOS population are warranted in order to elucidate the natural history of this new and unique disease.

In general, the evolution of TOS was characterized by the presence of multisystem manifestations, involving the neuromuscular, cardiorespiratory and mucocutaneous systems preferentially and, occasionally, specifically.** A nonnecrotizing, nongranulomatous oblitative vasculitis mainly involving the intima of vessels of every size and type was a common histologic finding. To date, pulmonary interstitial fibrosis has not been observed.† A significant number of patients developed mild-to-moderate pulmonary hypertension of a benign course.

In order to ascertain the presence of possible changes at the alveolar-Interstitial level as a factor associated with the vascular lesion, "Ga scanning and bronchoalveolar lavage (BAL) studies were performed in a group of TOS patients with persistently abnormal diffusing capacity for carbon monoxide (Dco) two years following the onset of TOS.

Subjects and Methods

Patient Population

Fourteen patients (five men and nine women) with a mean age of 30.2 ± 8 yr (mean ± SD), diagnosed between May and September, 1981, as having TOS, were selected for study due to persistently abnormal Dco lung function test results (Table I). Serum analytic and biochemical parameters were normal. No patient had a previous lung disorder and only two had mild neuromuscular involvement. No patient had received drug therapy six months prior to the study. Six patients were cigarette smokers (10-20 cigarettes/day during the last five years).

Control Population

Gallium-67 Scanning: The control population consisted of 13 subjects (seven men and six women) with a mean age of 28 ± 11 yr (mean ± SD). Scanning was indicated because of suspected osteoarthritis in lower limbs or as a follow-up procedure to discard the presence of infectious activity six months after completion of treatment. Eight subjects were smokers. No subject presented evidence of cardiorespiratory, neoplastic, hepatic or inflammatory disease. No subject met the criteria for TOS.*

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Bronchoalveolar Lavage (BAL): The control population comprised 20 subjects (15 men and 5 women) with a mean age of 42 ± 10 yr (mean ± SD). They had been referred for fiberoptic bronchoscopic examination for diagnostic purposes. BAL was performed in those who showed no evidence of chronic obstructive lung disease (COLD), pathologic signs on chest roentgenograms, hemoptysis or infection. All subjects had normal lung function and gasometric parameters. No subject had previously received steroids or anti-neoplastic medications. Informed consent was obtained. Eleven were moderate smokers. No subject fulfilled the criteria for TOS.10

Gallium-67 Scanning Technique and Evaluation: Scanning was performed 48 h after intravenous administration of 2.5 mCi 67Ga citrate. Anterior and posterior static scans were obtained using a gamma camera (Anger type) with medium energy collimator and three analyzers (40 percent window on the 93 KEV photopeak; 24 percent window on the 184 KEV photopeak; 32 percent window on the 296 KEV photopeak). The gamma camera was linked to a computer (Gamma II) which permitted simultaneous recording of images for subsequent processing. Measurement time was 240 seconds/frame for reliable count recording, necessary for statistical evaluation and computer reading. An estimate of the degree of 67Ga uptake by the lungs was made using a quantitative procedure.11 To minimize the effects of body morphology (patients' size, sex) and cardiac artefacts, only the posterior 67Ga scans were used in determining the degree of lung uptake. Both lungs were outlined on the posterior view of the gallium scan excluding the hilar and mediastinal structures. A portion of the total liver area was defined as a reference region of interest (ROI) (maximum uptake). The absolute mean 67Ga uptake intensity was obtained by assigning a value to each lung in relation to the activity (counts) and its corresponding area (pixels). The same computation methods were used for the liver scan.

The relative mean 67Ga uptake intensity was obtained from the following formula:

\[ \text{RM}^67\text{Ga} = \frac{A_i}{A_{\text{AMI}_i}} + \frac{A_j}{A_{\text{AMI}_j}} \]

where \( \text{RM}^67\text{Ga} \) is the relative mean 67Ga uptake intensity; \( A_i \) and \( A_j \) are the absolute mean intensities for the left lung, right lung and liver, respectively; \( A_{\text{AMI}_i} \) and \( A_{\text{AMI}_j} \) are the relative areas for the left and right lung, respectively, obtained from the corresponding pixels and expressed in percentage. For convenience, we assigned to \( \text{RM}^67\text{Ga} \) the value of the uptake index (UI67Ga), graded on a scale of 0 to 100 (UI67Ga value may be greater than 100 when lung is greater than liver activity). Background activity was not considered.

Bronchoalveolar Lavage Technique and Evaluation: Fiberoptic bronchoscopic examination was performed in all patients within one week of the 67Ga scanning. Following the technique previously described by other authors,12 the tip of a fiberoptic bronchoscope was wedged into a segmental bronchus of the right middle lobe. A total of 150 ml of 0.9 percent sterile saline solution (3 × 50 aliquots) at 30°C was instilled through the bronchoscope. After each instillation, the fluid was aspirated with the use of 60-100 mm Hg negative pressure from a suction apparatus (Ohio intermittent suction unit) and collected in a sterile specimen trap (Lukens). Following the lavage, the fluid was immediately strained through sterile gauze to remove the mucoid component. The volume of fluid was then measured. The cells were separated from the lavage fluid by centrifugation (at 1,500 rpm, at 500 g, 34°C for 15 minutes) and resuspended in Hanks' solution (without Ca ++ or Mg ++ ) with 10 percent inactivated fetal calf serum. The total number of cells was quantitated in a Neubauer cell. Cell viability was determined by trypan blue staining. After a portion of the sample had been cytocentrifuged (Cytopsin, Shandon Southern Instruments) and Wright-Giemsa and Pan-acidine-stained, a differential cell count was made on a total of 200 cells. The T-lymphocytes present in the lavage fluid were identified by their ability to rosette with neuraminidase-treated sheep red blood cells and in the presence of 25 percent fetal calf serum absorbed by sheep erythrocytes. Alveolar macrophage function and properties were studied in three patients. We evaluated chemotactic response with Zymosan-activated serum and E coli endotoxins, phagocytic ability according to the method of Lherer13 and adherence properties using the Territo and Cline method.14

The supernatant fluid was decanted and total protein concentration determined by turbidimetry (sodiumchloride acid technique) expressed in mg/100 ml as all other parameters. Quantitation of immunoglobulins (IgG, IgA and IgM), albumin, \( \alpha_1 \)-antitrypsin, \( \alpha_2 \)-macroglobulin, \( \alpha_2 \)-glycoprotein, haptoglobin and transferrin were made by the single radial immunodiffusion15 precipitation in low level antibody impregnated agar plates (LC Partigen, Behring Institute Lab), lysozyme by the lysoplate technique (Kallestad Lab), total phospholipid using Takayama's colorimetric enzymatic technique16 and lecithin by Diehrich's enzymatic technique.17 All the foregoing parameters were likewise determined in sera of both groups obtained after lavage. The values for serum assays were within normal for both groups. Following individual correction, immunoglobulin and protein values were expressed in relation to the amount of albumin present.

Pulmonary Function Tests: Computer-assisted spirometry was done with a model HP-982 5 A (Hewlett Packard). Forced vital capacity (FVC) was expressed as percent predicted value. Diffusing capacity for carbon monoxide was obtained by the single breath test (Jaeger System). Transfer factor for carbon monoxide (Dco) in ml min⁻¹ mm Hg⁻¹ and KCO in mm min⁻¹ mm Hg⁻¹⁻¹ were expressed as percent predicted value.18 All values were estimated under BTPS conditions. The alveolar-arterial \( O_2 \) difference ([Paw]-[O2]] in mm Hg was obtained from the equation for ideal alveolar gas (Table 1).

Statistical Method: Comparison of the different variables studied for control and patient population was made by using Student's 't' test, significant at p <0.05.

RESULTS

Gallium-67 scanning failed to demonstrate a significant difference between patients and control subjects. The 67Ga index for the TOS patients was 45.19 ± 8.70 (mean ± SD) and 43.38 ± 6.85 (mean ± SD) for the controls. BAL assay showed that total cells recovered and number of cells per ml of aspirated fluid had very similar values for both groups (Table 2). Likewise, there was no significant difference in the cell differential for the TOS patients and the control subjects (Table

Table 1—Physiologic Characteristics in Patients with Toxic-Oil Syndrome (TOS)*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TOS (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO2</td>
<td>83.66 ± 1.70</td>
</tr>
<tr>
<td>P(A-a)O2</td>
<td>14.47 ± 1.93</td>
</tr>
<tr>
<td>FVC</td>
<td>100.85 ± 4.45</td>
</tr>
<tr>
<td>FEV1</td>
<td>96.78 ± 7.75</td>
</tr>
<tr>
<td>V50</td>
<td>79.13 ± 5.45</td>
</tr>
<tr>
<td>Dco</td>
<td>58.57 ± 3.36</td>
</tr>
<tr>
<td>KCO</td>
<td>50.01 ± 4.44</td>
</tr>
</tbody>
</table>

Abbreviations: PaO2 = partial pressure of O2 in arterial blood (mm Hg); P(A-a)O2 = alveolar-arterial O2 difference (mm Hg); FVC = forced vital capacity (% pred); FEV1 = forced expiratory volume in one second (% pred); V50 = maximum expiratory flow rate at 50% of FVC; Dco = single-breath diffusion capacity (% pred); KCO = coefficient of diffusion (% pred).

*Values are mean ± SEM.
Table 2—Clinical Characteristics of Lavage Fluid from TOS Patients and Control Subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>No. (yr)</th>
<th>Sex</th>
<th>Smoking History</th>
<th>% Fluid Recovered (× 10⁶)</th>
<th>Cells/ml Recovered (× 10⁶)</th>
<th>Protein</th>
<th>Albumin</th>
<th>Alb/Prot</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOS</td>
<td>14</td>
<td>30</td>
<td>5/9</td>
<td>53.1</td>
<td>35.5</td>
<td>16.5</td>
<td>7.5</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>(7)*</td>
<td></td>
<td></td>
<td>(15.4)</td>
<td>(35.5)</td>
<td>(3.8)</td>
<td>(1.8)</td>
<td>(0.09)</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>44</td>
<td>15/5</td>
<td>49.9</td>
<td>33.7</td>
<td>15.3</td>
<td>8.7</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>(11)</td>
<td></td>
<td></td>
<td>(22.9)</td>
<td>(232.9)</td>
<td>(5.1)</td>
<td>(5.4)</td>
<td>(0.11)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses are SD.

3). In two patients (both smokers) a slight increase of polymorphonuclear components (15 and 17 percent, respectively) was observed. Trypan blue staining showed cell viability of 86-97 percent for the TOS group. Analysis of the lymphocyte population showed 3.1 ± 5.6 percent T-lymphocytes of total cells recovered (35.9 ± 17.8 percent of total lymphocytes), 1.2 ± 1.0 percent being activated T-lymphocytes.

Studies of alveolar macrophage function and properties performed in three TOS patients were compatible with mean normal values (phagocytosis 66.3 percent, Candida killing activity 94.0 percent, chemotaxis 11.0 cells/field and adherence to glass 90.3 percent).

Determination of component concentration analyzed in the supernatant fluid showed similar values for total protein and albumin (Table 2) for both patient and control population. Comparison of mean immunoglobulin values of both groups showed a significant increase of mean IgG and IgA concentrations in the TOS group (p<0.01). Of the remaining parameters evaluated, only α1-antitrypsin and transferrin decreased significantly in the TOS group compared with controls (p<0.05) (Table 4).

DISCUSSION

Follow-up studies of TOS patients revealed two forms of clinical and pathologic lung involvement, the first characterized by gas exchange abnormalities, and the second a ventilatory disorder caused by neurovascular compromise of the chest wall.

Early follow-up of TOS patients with noninvasive markers of lung function (FVC, P[A-a]O₂, Dco) revealed a gradual progression toward normal values. However, present patient follow-up has revealed persistently reduced percentile values of Dco and KCO.

Table 3—Cellular Composition of BAL Fluid of Normal Subjects and Patients with TOS*

<table>
<thead>
<tr>
<th>Subject</th>
<th>Macrophages</th>
<th>Lymphocytes</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% × 10⁶</td>
<td>% × 10⁶</td>
<td>% × 10⁶</td>
<td>% × 10⁶</td>
</tr>
<tr>
<td>TOS</td>
<td>90.2</td>
<td>402.0</td>
<td>6.5</td>
<td>27.2</td>
</tr>
<tr>
<td></td>
<td>(n = 14) (7.9)*</td>
<td>(396.0)</td>
<td>(6.9)</td>
<td>(36.0)</td>
</tr>
<tr>
<td>Control</td>
<td>94.4</td>
<td>360.9</td>
<td>6.5</td>
<td>25.7</td>
</tr>
<tr>
<td></td>
<td>(n = 20) (5.9)</td>
<td>(226.0)</td>
<td>(3.6)</td>
<td>(17.4)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses are SD.

For this reason and because Dco has been shown to correlate well with ⁹⁹mTc scanning,⁹ these lung function tests may be good noninvasive indicators of vascular changes in TOS.

Attention has been focused on the immune alterations observed during the early stage of TOS. During the acute stage, peripheral eosinophilia were observed in all patients and an increased serum IgE and other immunoglobulins in more than one third of the patients.⁵ BAL studies during this stage showed a predominance of eosinophils, an elevated IgG, IgA, α1-antitrypsin and lysozyme concentrations. Similarly, α1-macroglobulins were a constant finding suggesting an important transudation from plasma vascular bed as a result of the vascular changes.

Although eosinophilia during the acute stage of TOS may have influenced vascular lesion through hypercoagulation, platelet aggregation, thrombosis and endothelial lesions, there has been no evidence of a specific and constant alteration of immune response. Moreover, there was no correlation between the TOS manifestations and/or clinical course and the immunologic alterations observed. These foregoing alterations have led to the consideration of the immunologic alterations in TOS as either a direct effect of the toxic

Table 4—Quantitation of Macromolecules in Lavage Effluent from Healthy and TOS*

<table>
<thead>
<tr>
<th>Components</th>
<th>Control (n = 20)</th>
<th>TOS (n = 14)</th>
<th>Unpaired t test (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG/albumin</td>
<td>0.253 ± 0.169</td>
<td>0.421 ± 0.107</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>IgA/albumin</td>
<td>0.059 ± 0.037</td>
<td>0.100 ± 0.028</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>α1-antitrypsin/albumin</td>
<td>0.079 ± 0.046</td>
<td>0.050 ± 0.008</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>α1-macroglobulin/albumin</td>
<td>0.014 ± 0.014</td>
<td>0.111 ± 0.009</td>
<td>NS</td>
</tr>
<tr>
<td>Haptoglobin/albumin</td>
<td>0.871 ± 0.150</td>
<td>0.050 ± 0.014</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Transferrin/albumin</td>
<td>0.073 ± 0.022</td>
<td>0.006 ± 0.026</td>
<td>NS</td>
</tr>
<tr>
<td>Lysozyme/albumin</td>
<td>0.073 ± 0.022</td>
<td>0.006 ± 0.026</td>
<td>NS</td>
</tr>
<tr>
<td>α1-acid glycoprotein/</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>albumin</td>
<td>0.008 ± 0.014</td>
<td>0.005 ± 0.010</td>
<td>NS</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>3.395 ± 0.847</td>
<td>3.685 ± 0.870</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>2.286 ± 0.545</td>
<td>2.347 ± 1.046</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphatidylcholine/</td>
<td>0.675 ± 0.074</td>
<td>0.609 ± 0.171</td>
<td>NS</td>
</tr>
<tr>
<td>phospholipids</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The data are presented as a ratio to the respective albumin level in the same sample (mg/100 ml).

*Numbers are mean ± SD; tNot quantifiable.

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Toxic-Oil Syndrome (De la Cruz et al)
agent or as a nonspecific response to tissue damage and not as a mediation in the pathogenic mechanism of the disease. Moreover, these alterations were not observed in serum studies performed posteriorly.\textsuperscript{20,21}

In the present study, the results obtained from \textsuperscript{67}Ga scanning and BAL, techniques which are sensitive to alveolitis,\textsuperscript{22} suggest the absence of inflammatory cell activity at the alveolar-interstitial level two yr after the onset of TOS, a finding which is in agreement with the lung studies performed to date.

Similarly, the normal values obtained after evaluation of the alveolar surfactant components make alterations of the functions of pulmonary tenso-active structure highly unlikely. However, the observed increase in IgG and IgA concentrations and the fall in \(\alpha_1\)-antitrypsin and transferrin in the lavage fluid warrant special attention.

These immunoglobulins of the lower respiratory tract, quantified in numerous pulmonary disorders,\textsuperscript{23-24} have been accepted to be increased through two possible ways: 1) transudation through the blood-air interface, and 2) local synthesis of lymphocytes (plasma cells) localized intrinsically in the lung or free lymphocytes in airway lumen.\textsuperscript{23-24} However, although some investigators consider that most of the IgG found in the BAL fluid originates from serum,\textsuperscript{20} the prevalence of one or the other route in healthy subjects has as yet not been elucidated.

In TOS patients, the absence of an increase in the number of lymphocytes in the BAL fluid and the observed vascular lesion which could mediate in active or passive transport of immunoglobulins, particularly IgG,\textsuperscript{20} suggest the participation of the transudation pathway. Similarly, an increased IgA concentration encountered in the lavage fluid of these patients might be accounted for by the selective changes in capillary wall permeability and the subsequent flow of IgA from the intravascular pool. However, no definite conclusions can be drawn in this regard because of the scant knowledge available to date with respect to the breakdown kinetics of immunoglobulins and lung clearance.

A significant drop in \(\alpha_1\)-antitrypsin concentration in the lavage fluid and normal values in serum of the TOS group could be attributed to an alteration in local production and consumption or, more likely, to an altered regulation due to transport block from the vascular compartment. Further follow-up of these patients is warranted because of a possible evolution towards alterations of lung interstitial components from imbalance of local protease-antiprotease mechanisms.

Finally, the observed decrease in transferrin concentrations in the lavage fluid of TOS patients which may affect its bacteriostatic role in the lower respiratory tract, supports the hypothesis of a "specific" transport deficit from the vascular component,\textsuperscript{20} although at present, an alteration of local synthesis or release (alveolar macrophages) has not been completely discarded.

These findings raise further questions as to the natural course and outcome of TOS. Careful follow-up of the TOS population and further investigations for useful prognostic markers are clearly warranted.

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