
Pulmonary Disposition of Gallium-67 in Patients with *Pneumocystis* Pneumonia: An Analysis Using Bronchoalveolar Lavage

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Gallium-67 localizes to the cellular fraction of bronchoalveolar lavage (BAL) fluid in patients with sarcoidosis, idiopathic pulmonary fibrosis, as well as normal subjects. To further study ^{67}Ga disposition in BAL fluid, 11 patients with *Pneumocystis carinii* pneumonia (PCP) and 8 patients with a variety of other lung diseases, underwent BAL 24 hr after ^{67}Ga injection. Compared to the non-PCP patients, PCP patients had high uptake gallium scans at 24 and 72 hr, and showed significantly increased radioactivity in both unfractionated lavage and in the acellular, supernatant fraction of BAL. The mean ratio of total supernatant/cell pellet radioactivity was also higher in patients with PCP (1.23 ± 0.27 versus 0.24 ± 0.05 , $p < 0.01$). Supernatant radioactivity correlated with the presence of neutrophil alveolitis, but not with BAL transferrin concentrations. We conclude that neutrophil alveolitis in PCP promotes ^{67}Ga accumulation in the acellular fraction of BAL fluid. However, the high uptake ^{67}Ga scans observed in PCP patients without neutrophil alveolitis suggest that the mechanism of pulmonary uptake of ^{67}Ga is not fully elucidated by BAL fluid analysis alone.

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The mechanism of pulmonary uptake of ^{67}Ga in a variety of lung diseases is unknown. Several studies have investigated pulmonary uptake of ^{67}Ga using bronchoalveolar lavage (BAL) fluid. In both normal subjects and patients with sarcoidosis or idiopathic pulmonary fibrosis, ^{67}Ga localizes to the cellular fraction of BAL fluid with relatively little radioactivity detected in the acellular supernatant (1-3).

Pneumocystis carinii pneumonia (PCP) is associated with pulmonary uptake of ^{67}Ga (4,5). The infection has also been shown to cause an increase in the permeability of the alveolo-capillary membrane (6-8). We performed BAL after ^{67}Ga injection in patients with PCP to evaluate the hypothesis that pulmonary accumulation of ^{67}Ga

might be due to increased membrane permeability to transferrin, the gallium-binding protein in plasma. If this was so, we further hypothesized that the acellular supernatant fraction would contain more ^{67}Ga radioactivity than the cell fraction of lavage fluid and correlate with BAL transferrin concentration.

METHODS

The study included 19 male patients who were undergoing diagnostic bronchoscopy because of pulmonary symptoms, including dyspnea and cough. All 19 were considered at risk for AIDS by their primary physicians. The mean age of the group was 40 yr (range 33 to 56 yr); all patients were cigarette smokers. Of the 19 patients, 11 proved to have PCP. Diagnoses in the other eight patients included *M. tuberculosis* (1 patient), *M. avium* complex (2 patients), noninfectious granulomatous disease (1 patient) and nonspecific interstitial pneumonitis (4 patients). Chest roentgenograms showed bilateral interstitial infiltrates in 12 patients and focal infiltrates in 2 patients. Five patients had normal chest roentgenograms.

After giving informed consent to participate in the study, all patients were injected intravenously with 5 mCi of ^{67}Ga -citrate. External scans were obtained at 24 and 72 hr after injection. Scans were read in a blinded fashion by a physician from the department of nuclear medicine and were defined as positive and consistent with PCP if lung uptake was equal to or greater than that of the liver at 72 hr (Grades 3 and 4, respectively) (9). Scans were considered negative if no pulmonary uptake was seen or if minimal lung uptake was present without a photopenic cardiac defect (Grade 1). Scans were considered nonspecific if lung uptake less than liver was noted along with a photopenic cardiac defect (Grade 2, Fig. 1).

Bronchoalveolar lavage was performed with the bronchoscope wedged gently into either a right middle lobe or lingular subsegmental bronchus. Normal saline was instilled in 35 ml aliquots to a total of 140 ml, and each aliquot was immediately aspirated manually into a syringe; mean recovery was 55% (range 32% to 75%). The collected lavage material was pooled for each patient and strained through a single layer of surgical gauze. A 1-ml aliquot was removed for cell counting. Cells were counted in a hemocytometer to obtain total cell counts. Differential cell counts were obtained by counting at least 300 cells on a Wright-stained cytocentrifuged preparation.

A 1-ml aliquot of lavage fluid was set aside and the remaining BAL was centrifuged at 500 g for 10 min. The supernatant and

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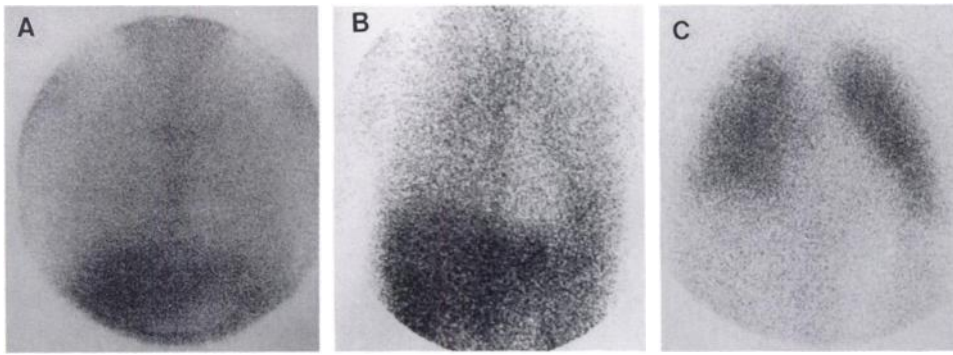


FIGURE 1. Representative gallium pulmonary images, anterior views at 72 hr. (A) Mild gallium labeling without a photopenic cardiac defect (Grade 1); (B) pulmonary uptake is less than in the liver and a photopenic cardiac defect is present (Grade 2); (C) intense pulmonary uptake, greater than in the liver (Grade 4). Positive scans are defined as pulmonary uptake equal to (Grade 3) or greater than liver.

cell fractions were separated and the cell pellet was resuspended in 10 ml of Hank's Balanced Salt Solution. The 1 ml of unseparated lavage as well as 1 ml each of the supernatant and cell fractions were counted for radioactivity in a single channel sodium iodide gamma counter. The ratio of supernatant/cell fraction counts was based on the radioactivity in the entire supernatant and cell fractions of the lavage fluid, as calculated from the counts in the 1 ml aliquots.

The remaining BAL supernatant and a specimen of serum obtained at the time of bronchoscopy were stored at -70°C for subsequent protein analysis. After thawing, BAL supernatant was concentrated 30- to 40-fold through an Amicon Y-10 membrane (Amicon Co., Danvers, MA). The concentrated BAL specimens and thawed serum were assayed for transferrin by single radial immunodiffusion (Behring Diagnostics, LaJolla, CA).

Data were expressed as mean \pm s.e.m. Group means were compared using the unpaired Student's t-test. Linear regression equations were calculated by the method of least squares. Statistical significance was defined as $p < 0.05$.

RESULTS

Ten of eleven patients with PCP had positive gallium scans after 72 hr, defined as lung uptake equal to or greater than liver uptake. Of these, two had lung uptake equal to liver, and eight had lung uptake greater than liver. None of the eight patients without PCP had a positive gallium scan: three patients had no pulmonary uptake, three had slight gallium uptake, and two others had mild uptake that produced a photopenic cardiac defect but was less than liver uptake. No differences were observed between scans at 24 and 72 hr.

Total BAL cell recovery did not differ significantly between patients with and without PCP ($27.8 \times 10^6 \pm 3.9 \times 10^6$ cells versus $46.4 \times 10^6 \pm 12.8 \times 10^6$ cells, $p = 0.13$). The BAL cell differential also showed no significant differences in the percentages of macrophages and lymphocytes between patients with and without PCP (macrophages, $70.6\% \pm 10.2\%$ versus $81.9\% \pm 14.9\%$, $p = 0.07$; lymphocytes $18.0\% \pm 10.1\%$ versus $16.9\% \pm 13.9\%$, $p = \text{ns}$). However, the percentage of BAL neutrophils was significantly higher in patients with PCP ($11.5\% \pm 7.9\%$) than in patients without PCP ($1.0\% \pm 1.6\%$, $p < 0.002$).

Mean BAL transferrin, expressed as the ratio of BAL/serum transferrin concentrations, was not significantly

different between patients with and without PCP ($6.27 \times 10^{-3} \pm 0.55 \times 10^{-3}$ versus $5.31 \times 10^{-3} \pm 0.87 \times 10^{-3}$, $p = \text{ns}$).

Patients with PCP had significantly more radioactivity in unfractionated BAL fluid than patients without PCP (7635 ± 5124 cpm/ml versus 2647 ± 1309 cpm/ml, $p < 0.02$), as well as an increased ratio of total BAL supernatant counts/total cell pellet counts (1.23 ± 0.27 versus 0.24 ± 0.05 , $p < 0.01$, Fig. 2).

For the patients with PCP, a significant linear correlation was found between BAL percent neutrophil counts and radioactivity in unfractionated BAL fluid ($r = 0.821$, $p = 0.002$), between BAL total neutrophil counts and transferrin in unfractionated BAL fluid ($r = 0.798$, $p = 0.003$), and between BAL percent neutrophil counts and radioactivity in the BAL supernatant fraction ($r = 0.808$, $p < 0.003$, Fig. 3). A significant linear regression was also calculated between BAL total neutrophil counts and radioactivity in the BAL cell fraction ($r = 0.776$, $p = 0.005$). No correlations were found between other BAL cell types and BAL radioactivity.

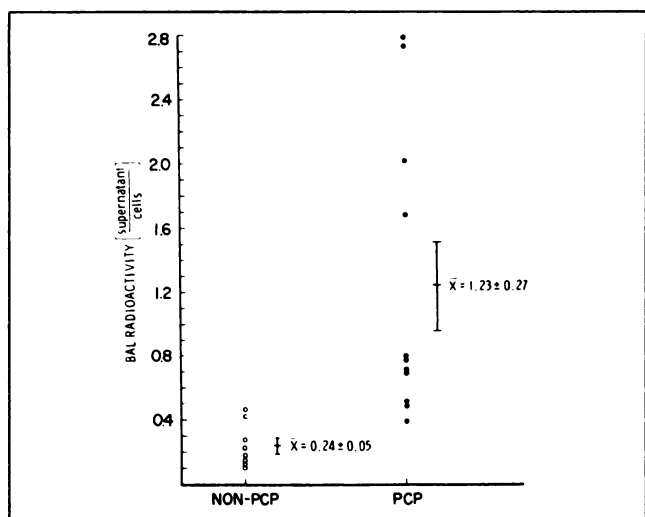


FIGURE 2. Comparison of the mean ratios of BAL total supernatant radioactivity/total cell pellet radioactivity, for non-PCP patients (open circles) and PCP patients (closed circles). The ratio for PCP patients is significantly greater at the $p < 0.01$ level.

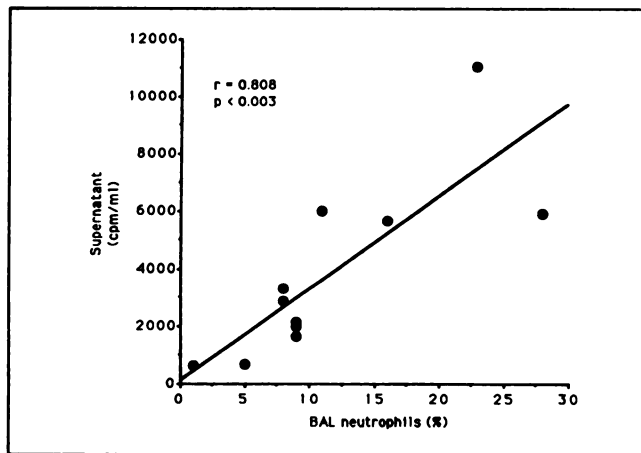


FIGURE 3. Correlation between BAL percent neutrophil counts and radioactivity in the BAL supernatant fraction.

No correlation was found between BAL percent or absolute neutrophil counts and the degree of gallium uptake seen on external scans.

DISCUSSION

Prior investigations have evaluated the disposition of ^{67}Ga in BAL fluid. In normal subjects without evidence of ^{67}Ga on external gamma scanning, Braude and co-workers reported finding ^{67}Ga radioactivity in BAL 72 hr after intravenous injection (1). The majority of the radioactivity, greater than 85%, was localized to the cellular fraction of the BAL. Similar findings have been reported in patients with positive scans as well. Hunninghake et al. noted that greater than 99% of ^{67}Ga radioactivity, 72 hr after intravenous injection, was also associated with the cellular fraction of the BAL from patients with active sarcoidosis and idiopathic pulmonary fibrosis (2). In addition, Trauth and colleagues found ^{67}Ga radioactivity exclusively in the cell-containing fraction of BAL from patients with sarcoidosis (3). In the present study, the eight patients without PCP had a variety of lung diseases associated with low or minimal pulmonary uptake of ^{67}Ga on external scanning. Consistent with the earlier studies in normal subjects with negative scans, and as reported in patients with positive scans, our patients without PCP had ^{67}Ga radioactivity largely confined to the cellular fraction of BAL.

In patients with *pneumocystis* infection, by contrast, ^{67}Ga radioactivity was found predominantly in the acellular, supernatant fraction of the lavage fluid. Technical aspects of our study, particularly the 24-hr interval from intravenous injection of ^{67}Ga to the performance of BAL, may have contributed to these findings. Presumably, ^{67}Ga is transferred from a circulating gallium-transferrin complex in plasma to a cellular location in the airspaces (10); 24 hr may not be sufficient time for this transfer to be complete. However, the patients without PCP in our study did have a variety of other lung pathology, including granulomatous inflammation, mycobacterial disease, and

nonspecific inflammatory changes. In all of these cases the BAL fluid, obtained 24 hr after injection, demonstrated cellular localization of ^{67}Ga .

Relatively increased radioactivity in the acellular supernatant of BAL from patients with PCP may be due to increases leakiness of the alveolo-capillary membrane (11, 12). Increased permeability of the membrane has been noted in PCP and could permit excess amounts of transferrin, the gallium-carrier protein in blood, to enter the airspaces (6-8). The movement of the gallium-transferrin complex from blood into the airspaces could possibly exceed the uptake of gallium by airspace cells, and thus result in relatively increased radioactivity in the lavage fluid supernatant. We found, however, no significant difference between BAL transferrin concentrations in patients with and without PCP. Although this finding argues against increased alveolo-capillary membrane permeability as the mechanism of ^{67}Ga accumulation in the BAL supernatant, measurement of BAL protein concentrations alone may not be a reliable index of membrane permeability. BAL transferrin concentration represents protein present in the alveoli prior to ^{67}Ga injection as well as that which accumulates after ^{67}Ga injection. Thus, BAL protein concentration, at a single point in time, may not reflect acute protein flux across the alveolo-capillary membrane.

We observed a correlation between radioactivity in the BAL supernatant and the percentage of lavage fluid neutrophils. A number of prior studies have reported an association between elevated BAL neutrophil counts, signifying airspace inflammation, and the severity of PCP (13,14). During inflammation, disruption of neutrophils is known to cause leakage of intracellular contents, including secondary granules that are rich in lactoferrin (11). Lactoferrin is also exocytosed by activated neutrophils (15). Since lactoferrin is a gallium-binding protein, with even greater affinity for gallium than transferrin (10), its extracellular release may account for the ^{67}Ga radioactivity in the BAL supernatant from patients with PCP and neutrophil alveolitis. In support of this mechanism, Tsan et al. demonstrated increased gallium accumulation in the acellular supernatant of experimental abscesses in rabbits (16). These investigators attributed this finding to gallium-binding by the spilled intracellular contents of neutrophils.

The findings of the present study are limited. Although evidence of pulmonary uptake of gallium on external scanning was associated with higher mean counts of radioactivity in BAL overall, there was a wide range in lavage fluid counts, with overlapping values for positive and negative scans. It is possible that radioactivity in BAL is an epiphenomenon that reflects spillover from gallium uptake in the lung interstitium. In patients with PCP, we found that neutrophil alveolitis accounts for the increased radioactivity counted in all components of lavage fluid. However, neutrophil alveolitis was not found in all patients with PCP. Those without neutrophil alveolitis had lower

counts of radioactivity in lavage fluid and a relatively decreased ratio of supernatant/cell fraction radioactivity. Yet, PCP patients with and without neutrophil alveolitis had similar, high uptake gallium scans. Thus, BAL neutrophils may not be essential to the mechanism of gallium uptake in PCP, as detected by external scanning.

In conclusion, ^{67}Ga in BAL fluid from patients with PCP was found in the acellular supernatant, as well as the cell fraction of lavage fluid. The mechanism of ^{67}Ga accumulation does not appear to be simply increased alveolo-capillary membrane permeability, as BAL transferrin concentrations did not differ between patients with and without PCP. Supernatant accumulation of ^{67}Ga correlated best with the presence of a neutrophil alveolitis in patients with PCP. Neutrophil alveolitis, however, was not found in all patients with PCP and positive gallium scans. Thus, the mechanism of pulmonary gallium uptake in PCP, as detected by external gamma scanning, will require further elucidation.

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