Indium-111-Labeled Polyclonal Human Immunoglobulin: Identifying Focal Infection in Patients Positive for Human Immunodeficiency Virus

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Pooled human immunoglobulin labeled with indium-111 (\(^{111}\text{In}-\text{HlgG}\)) was used to identify the presence and extent of infection in patients positive for human immunodeficiency virus (HIV), presenting with either symptoms and/or signs of acute chest infection or with pyrexia without localizing signs or symptoms. Fifty-five studies were performed in 51 patients with suspected chest infection or pyrexia without localizing signs. Of these, \(^{111}\text{In}-\text{HlgG}\) identified intrapulmonary accumulation in 17 patients with \textit{Pneumocystis carinii} pneumonia, eight with bacterial pneumonia, five with cytomegalovirus pneumonia, three with pulmonary \textit{Mycobacterium avium intracellulare} infection and one with a fungal pneumonia. There was no intrapulmonary accumulation of \(^{111}\text{In}-\text{HlgG}\) in five patients with bronchopulmonary Kaposi's sarcoma and in three patients with intrathoracic lymphoma. Quantification of lung/heart activity was significantly increased \((p < 0.05)\) in patients with active chest infection compared with those with intrapulmonary tumor or no active lung pathology. Indium-111-HlgG scintigraphy also localized at 14 sites of extrapulmonary infection, including six patients with colitis. There were no false-negative studies but false-positive uptake was seen in four studies. These results confirm that \(^{111}\text{In}-\text{HlgG}\) correctly identifies the presence and extent of infection in patients positive for HIV antibody.

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The diagnosis of infection in patients with human immunodeficiency virus (HIV) infection and those with acquired immunodeficiency syndrome (AIDS) is frequently hampered by the nonspecific nature of presenting symptoms and signs. Conventional imaging techniques such as chest radiographs and x-ray-computed tomography (CT), although sensitive, may fail to demonstrate abnormalities in the presence of active infection \((1-3)\). Conventional imaging may not distinguish changes due to infection from those secondary to Kaposi's sarcoma.

Functional imaging with \(^{67}\text{Ga}\)-citrate has proven useful in the investigation of HIV-positive patients with focal and diffuse infections. The technique is at its most useful in distinguishing \textit{Pneumocystis carinii} pneumonia (PCP) from bronchopulmonary Kaposi's sarcoma \((4,5)\). Intrapulmonary accumulation of \(^{67}\text{Ga}\)-citrate is not specific for PCP and uptake is also seen in several other pathologies including lymphoma \((6)\). The presence of physiological bowel uptake, which may be increased in patients with AIDS, further reduces the utility of \(^{67}\text{Ga}\)-citrate in identifying intraabdominal sepsis or colonic infection \((7)\).

Pooled human immunoglobulin (HlgG) labeled with \(^{111}\text{In}\) or \(^{99m}\text{Tc}\) has been shown to have a high sensitivity and specificity in localizing infection in immune competent patients \((8-10)\). Like \(^{67}\text{Ga}\)-citrate it has the advantage of simple intravenous injection, in contrast to labeled autologous white cells in which extensive handling of blood products is required. Unlike \(^{67}\text{Ga}\)-citrate there is only minimal physiological bowel uptake of \(^{111}\text{In}-\text{HlgG}\). In steroid-immunosuppressed rats with PCP imaging with \(^{111}\text{In}-\text{HlgG}\) demonstrated higher sensitivity than imaging with \(^{67}\text{Ga}\)-citrate \((11)\).

The aim of this study was to determine the efficacy of \(^{111}\text{In}-\text{HlgG}\) in identifying the presence and extent of infection in HIV-positive patients presenting with either symptoms and/or signs of acute chest infection or pyrexia without localizing symptoms or signs.

\begin{center}
\textbf{METHODS AND MATERIALS}
\end{center}

We prospectively studied consecutive HIV-positive patients presenting with suspected infection. Patients were included in the trial if they: (1) had signs or symptoms suggestive of chest infection in which initial investigations, including chest radiology,
blood chemistry, full blood count, blood and sputum cultures, had
failed to provide a diagnosis; and (2) had pyrexia for at least four
days without localizing signs or symptoms, and simple investiga-
tions such as radiology, ultrasound, blood chemistry, full blood
count and culture of blood, sputum, urine or feces were nondiag-
nostic.

All patients gave informed written consent for participation in
the study, and the protocol was approved by the Clinical Investi-
gation Committee of the Middlesex Hospital and the Adminis-
tration of Radioactive Substances Advisory Committee (AR-
SAC). Fifty-five studies were performed in 51 patients (50 males);
mean age was 32 (range 18 - 57) yr. Two patients had a second
study performed after intervals of 2 and 5 mo, and one patient had
three studies over a 7-mo period. These repeat studies were car-
ried out because the patients presented with new symptoms or a
recurrence of old symptoms. Thirty-eight studies were performed
for suspected chest infection and 17 for pyrexia without localizing
features.

Radiopharmaceutical

Pooled nonspecific human polyclonal immunoglobulin G (San-
doglobulin, Sandoz AG, Nurnberg, Germany), which was nega-
tive for HIV and Hepatitis B surface antigen was conjugated with
diethylenetriamine pentacetic acid (DTPA). The conjugated solu-
tion was sterilized by gel filtration and aliquots of 0.5 ml and
stored at –20°C in sterile glass vials until used. For each study,
a vial was allowed to thaw and conjugated with 111In chloride
(Mallinckrodt Medical, Petten, The Netherlands) (12). A dose of
0.25 – 0.5 mg of human immunoglobulin labeled with 1 mCi (37
MBq) of 111In in a volume of 0.3 – 0.5 ml was used for each study
and injected intravenously into the antecubital vein of the non-
dominant arm.

Imaging Protocol

Using an IGE 400AC Starcam gamma camera and computer
(IGE International, Radlett, Berkshire UK) anterior and posterior
planar images of the abdomen, pelvis and chest were obtained at
4, 24 and 48 hr after injection of 111In-HIgG. The camera was fitted
with a medium-energy parallel-hole collimator. Images were col-
lected in digital form into a 128 X 128 matrix. Two photopeaks,
173 and 247 keV, each with 20% windows, were used. Each image
was acquired for 600k counts and formatted onto x-ray film for
reporting. Each image was obtained in about 4 – 6 min.

Qualitative Analysis of Studies

The 4, 24 and 48-hr images from each study were reported
independently by two observers who were blind to patient clinical
diagnosis and laboratory results. A study was considered positive
if both observers agreed there was diffuse or focal accumulation
of 111In-HIgG in the lungs equal or greater than surrounding bone
marrow or surrounding soft tissue activity in neck or shoulder
(intrapulmonary). If bone marrow activity was absent, compar-
ison was made to soft tissue alone. A study was also considered
positive if both observers agreed there was focal accumulation at
any site in the chest outside the lungs, abdomen or pelvis greater
than surrounding soft tissue (extrapulmonary). If there was dis-
agreement between the two observers, the study was considered
negative.

Results of 111In-HIgG scans were compared in all studies with
the final diagnosis obtained by microbiological and histological
investigations of blood, urine and sputum, including expressed
sputums, bronchoalveolar lavage fluid and feces. Results of each
study also were compared to information obtained by other in-
vestigations, including upper gastrointestinal endoscopy, colonos-
copy, open biopsy and CT-guided percutaneous biopsy. All pa-
tients had undergone chest x-ray in the 48 hr before or after ini-
iation of the 111In-HIgG study.

Only histological or microbiological data were used for com-
parison with 111In-HIgG images.

Quantitative Analysis

Instead of a subjective grading of lung uptake, a quantitative
analysis of intrapulmonary accumulation of 111In-HIgG was cal-
culated using the 48-hr images in all studies. This was done using
the geometric mean of lung activity obtained by using a 10 X 10
pixel (31.2 mm X 31.2 mm) region of interest (ROI), placed in the
upper, middle (avoiding the hilum) and lower thirds of the right
lung on both the anterior and posterior images of the chest (Fig.
1). The left lung was not used to quantify lung activity as it was
impossible to draw ROIs in the lower third of the lung that did not
include activity in the heart. The mean-lung activity was normal-
ized to blood-pool activity by dividing the geometric mean of lung
activity by the geometric mean of activity in the left ventricle
(determined by using a 10 X 10 pixel ROI drawn over the heart in both
anterior and posterior images).

The resulting lung-to-heart ratio was calculated for the different
groups of studies; those in patients with PCP, those with pulmo-
nary infection due to other causes, those with intrapulmonary
tumor and studies of patients with no acute chest disease. The
mean lung-to-heart ratio at 48 hr in those patients with PCP and
those with infection due to other causes was compared with the
mean lung-to-heart ratio of the normal studies using an unpaired
Student t-test.

RESULTS

Intrapulmonary Disease

Seventeen cases of PCP were confirmed, in which all
patients had abnormal pulmonary uptake of 111In-HIgG
(Fig. 2, Table 1). Positive pulmonary uptake of 111In-HIgG
was also found in 20 cases of pulmonary infection due to other
causes, including bacterial infection (eight studies), pulmonary
Mycobacterium avium intracellulare (MAI) (six studies)
cytomegalovirus (five studies) and fungal infection
(one study). Images taken 48 hr postinjection provided the
most positive images of infection, although 24-hr images
were scored positive in all studies. Images at 4 hr postin-
jection were not helpful.
Eight studies, including five in patients with bronchopulmonary Kaposi’s sarcoma and three in patients with extensive intrapulmonary B-cell lymphoma, had no coexistent pulmonary infection. In all studies there was no elevated uptake of $^{111}$In-HlgG in the lungs (Fig. 3). Of the ten patients in whom no intrapulmonary disease was confirmed, only one patient, who had endstage renal failure, had diffuse pulmonary accumulation of $^{111}$In-HlgG.

In the 17 cases of PCP, there was evidence of diffuse pulmonary accumulation of $^{111}$In-HlgG in 14 cases, as opposed to evidence on x-ray of diffuse changes in only six of 14 cases, focal abnormalities in two of the cases, and normal readings in the remaining six. In the other three of 17 cases, both $^{111}$In-HlgG and chest x-ray revealed focal abnormalities. In four cases of pulmonary infection due to other causes, $^{111}$In-HlgG showed diffuse intrapulmonary uptake while chest x-rays were normal. In these four cases the organisms of infection were MAI (two cases), Haemophilus influenzae (one case), and cytomegalovirus (one case).

**Quantitative Analysis**

Quantitative analysis of lung/heart activity confirms the qualitative data and demonstrates a significantly increased lung-to-heart ratio of $^{111}$In-HlgG at 48 hr postinjection in patients with chest infection due to both PCP and other causes, compared with patients in whom there was no intrapulmonary disease (Table 2). There was no significant difference in the lung-to-heart ratio of $^{111}$In-HlgG in patients with intrapulmonary neoplasia such as Kaposi’s sarcoma and B-cell lymphoma and in patients with no intrapulmonary disease.

**Extrapulmonary Disease**

Uptake of $^{111}$In-HlgG outside the lungs (extrapulmonary) was seen in 13 cases (Table 3). Colitis/proctitis was correctly identified in all six cases in which it was confirmed histologically. One patient had bacterial pericarditis, one had sinusitis and one had an unsuspected iliorectal abscess; all were correctly identified by $^{111}$In-HlgG.

**TABLE 1**

Intrapulmonary Accumulation of $^{111}$In-HlgG in 55 Studies

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>$^{111}$In-HlgG study result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrapulmonary disease</td>
<td></td>
</tr>
<tr>
<td>Pneumocystis carinii pneumonia</td>
<td>17</td>
</tr>
<tr>
<td>Bacterial pneumonia*</td>
<td>8</td>
</tr>
<tr>
<td>Mycobacterium avium intracellulare</td>
<td>6</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>5</td>
</tr>
<tr>
<td>Fungal pneumonia (Aspergillus sp)</td>
<td>1</td>
</tr>
<tr>
<td>Bronchopulmonary Kaposi’s sarcoma</td>
<td>0</td>
</tr>
<tr>
<td>Intrapulmonary lymphoma sarcoma</td>
<td>0</td>
</tr>
<tr>
<td>No pulmonary disease</td>
<td>1</td>
</tr>
</tbody>
</table>

*Causative agents were Haemophilus influenzae (n = 4), Streptococcus pneumoniae (n = 3) and Cryptococcus neoformans (n = 1).*
In one patient with colitis and in the patient with ischiorectal abscess, coexistent pulmonary infection was present and correctly identified.

In four cases there was positive accumulation of $^{111}$In-HIgG in the absence of infection. In the patient with end-stage renal disease who had diffuse lung accumulation of $^{111}$In-HIgG, diffuse abdominal accumulation of $^{111}$In-HIgG also occurred. In two patients with B-cell lymphoma, there was colonic accumulation of $^{111}$In-HIgG but no histological evidence of colon involvement by either infection or tumor. In the fourth case, $^{111}$In-HIgG accumulated at the site of a sterile hematoma secondary to a stab wound.

**DISCUSSION**

Results demonstrate that $^{111}$In-HIgG can identify a wide range of infection in HIV-positive patients, including bacteria, fungi and viruses. The sensitivity of $^{111}$In-HIgG was high (100%) in the chest with no false-negative studies. It is known that $^{67}$Ga-citrate has a sensitivity greater than 90% in localizing chest infection in HIV-positive patients (4, 5, 13) and these results are therefore comparable. As previously reported in a small number of HIV-negative patients and in immunosuppressed rats, the predominant pattern of $^{111}$In-HIgG seen with PCP was diffuse intrapulmonary activity (DIPA) (8, 11). This distribution, seen in 14 of 17 patients with PCP is similar to that reported using $^{67}$Ga-citrate in this disease (4, 5).

The use of $^{111}$In as the radiolabel allows longer imaging times than other radiolabels such as $^{99m}$Tc. This may explain why the 100% sensitivity for identifying pulmonary infection obtained in this series was higher than the 33% obtained in a previous study that used $^{99m}$Tc to label HIgG (14). It is known that $^{111}$In-HIgG will accumulate in sites of infection in HIV-negative patients but not in immunocompromised patients (15) and imaging is required beyond the 24-hr limit set by the short physical half-life of $^{99m}$Tc. This is probably because significant blood-pool activity, obscuring any infection is still present in the chest at 24 hr postinjection but clears by 48 hr. This was confirmed in our study

<table>
<thead>
<tr>
<th>TABLE 2</th>
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<tbody>
<tr>
<td>Lung-to-Heart Ratios of $^{111}$In-HIgG at 48 Hours Postinjection</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient group</th>
<th>n</th>
<th>Lung-to-heart ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pneumocystis carinii pneumonia</em></td>
<td>17</td>
<td>0.66 (0.05)*</td>
</tr>
<tr>
<td>All other causes of pulmonary infection</td>
<td>20</td>
<td>0.59 (0.09)*</td>
</tr>
<tr>
<td>No pulmonary disease</td>
<td>10</td>
<td>0.51 (0.04)</td>
</tr>
<tr>
<td>Pulmonary tumor</td>
<td>8</td>
<td>0.51 (0.07)*</td>
</tr>
</tbody>
</table>

Data displayed as mean (± s.d.). Using student's t-test difference in lung/heart activity in these groups compared with those studies in which no intrapulmonary disease was present was * significant (p < 0.05) or † not significant.

<table>
<thead>
<tr>
<th>TABLE 3</th>
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<tbody>
<tr>
<td>Extrapulmonary Accumulation of $^{111}$In-HIgG in 55 Studies</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>$^{111}$In-HIgG study result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extrapulmonary disease</td>
<td>Positive</td>
</tr>
<tr>
<td>Colitis/procititis</td>
<td>6</td>
</tr>
<tr>
<td>Ischiorectal abscess</td>
<td>1</td>
</tr>
<tr>
<td>Infected axillary lymph node</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial pericarditis</td>
<td>1</td>
</tr>
<tr>
<td>Sinusitis</td>
<td>1</td>
</tr>
<tr>
<td>No extrapulmonary infection</td>
<td>4*</td>
</tr>
</tbody>
</table>

*One patient with renal failure, one with noninfected hematoma and two with lymphoma had unexplained colonic activity in the absence of colonic disease.
where the images performed 48 hr postinjection were the most useful.

The tracer $^{111}$In-HigG has an advantage over $^{67}$Ga citrate in that it does not accumulate in lymphoma, which was present in three of our patients. This has also been noted in previous studies on HIV-negative patients with lymphoma (8,15). Like $^{67}$Ga-citrate, the presence of $^{111}$In-HigG in the chest of a patient with bronchopulmonary Kaposi's sarcoma is indicative of superimposed infection, and is particularly useful when chest x-ray is abnormal and therefore limited in its use diagnostically. Tracer $^{111}$In-HigG activity at sites of noninfected inflammation have been reported and occurred in one of our patients with splenic-bed hematomata (8). Two patients with lymphoma had colonic $^{111}$In-HigG activity but no evidence of lymphoma or of infection in the colon on biopsy. The cause is unknown but has been reported in patients with neutropenia after administration of $^{111}$In-HigG and $^{99m}$Tc-HigG in patients with lymphoma (14) and may be due to protein leakage in the lumen of these patients (15).

The ability to image intrapulmonary infection at 48 hr postinjection, confirmed by significantly increased lung-to-heart ratio of $^{111}$In-HigG activity, may offer advantages over $^{67}$Ga-citrate where images at 72 hr postinjection may be needed to confirm presence of chest infection (1,14). Furthermore, the estimated radiation burden to patients from $^{111}$In-HigG is less than that from $^{67}$Ga-citrate (16,17). However, before $^{111}$In-HigG can replace $^{67}$Ga-citrate as the agent of choice in identifying infection in HIV-positive patients, a more direct comparison of the two agents is required.

This study demonstrates that $^{111}$In-HigG, which has proven diagnostic utility in neutropenic patients, can accurately identify infection from a wide range of pathogens within and outside the lungs in HIV-positive patients and patients with AIDS who present with respiratory symptoms or pyrexia of undetermined origin. If subsequent studies confirm these findings, $^{111}$In-HigG may replace $^{67}$Ga-citrate as the scintigraphic method of choice in these patients.

REFERENCES