
Ji-An Liang¹, Yu-Chien Shiau², Shih-Neng Yang¹, Fang-Jen Lin¹,³, Albert Kao⁴ and Cheng-Chun Lee⁴

Departments of ¹Radiation Therapy and Oncology and ⁴Medical Research, China Medical College Hospital, Taichung, ²Department of Nuclear Medicine, Far Eastern Memorial Hospital and Institute of Biomedical Engineering, College of Electrical Engineering, National Taiwan University, Taipei and ³Department of Radiation Therapy and Oncology, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan

Received September 7, 2001; revised December 10, 2001; accepted January 7, 2002

Background: The purpose of this study was to predict chemotherapy response in untreated malignant lymphomas using technetium-99m methoxyisobutylisonitrile (Tc-MIBI) scan.

Methods: Twenty-five patients with malignant lymphoma were studied before receiving chemotherapy. Early Tc-MIBI scan was performed 10 min after intravenous injection of Tc-MIBI. Immunohistochemical analyses were performed on multiple non-consecutive sections of the biopsy specimens to determine Pgp expression. Chemotherapy response was evaluated in the first 1–2 years after completion of treatment by clinical and radiological methods.

Results: The mean tumor-to-background ratio of the 15 patients with good response (3.3 ± 0.6) was significantly higher than that of the 10 patients with poor response (1.2 ± 0.1). Among the 15 patients with good response to chemotherapy, all had positive Tc-MIBI scan results but negative Pgp expression. Among the 10 patients who had poor response to chemotherapy, all 10 had negative Tc-MIBI scan, but six patients had positive Pgp expression and four had negative Pgp expression. Significant differences were found in the incidences of good and poor responses determined by Tc-MIBI scan and Pgp expression. However, there were no significant differences in the incidences of good and poor responses for other prognostic factors.

Conclusion: Compared with other prognostic factors, early Tc-MIBI scan more accurately predicts chemotherapy response in patients with malignant lymphoma.

Key words: lymphoma – technetium-99m methoxyisobutylisonitrile – chemotherapy response – P-glycoprotein expression

INTRODUCTION

The major therapeutic modality for all histologies and stages of non-Hodgkin’s lymphoma (NHL) is chemotherapy, except for radiation for indolent clinical stage I and plus radiation for aggressive clinical stage I. The primary therapeutic modality for Hodgkin’s disease (HD) is chemotherapy and radiotherapy (1–3). Resistance to chemotherapeutic agents is a major cause of treatment failure in malignant lymphoma. Therefore, the ideal chemotherapeutic goal in malignant lymphoma is to achieve the highest response with the lowest possible morbidity due to chemotherapy side effects.

Recently, it was reported that multidrug resistance gene 1 (MDR1) encoding human P-glycoprotein (Pgp) may play an important role in multidrug resistance of malignant lymphoma (4–8). Determinations of Pgp expression at the time of diagnosis provide valuable information for the design of treatment protocols (4–8). Therefore, before initiating chemotherapy, it is important to determine accurately the presence of Pgp in malignant lymphoma, to achieve a satisfactory chemotherapy response. Technetium-99m methoxyisobutylisonitrile (Tc-MIBI) has been considered a potential tumor imaging agent for detecting malignant lymphomas and evaluating chemotherapy response (9–13). In addition, from a review of the literature, Pgp recognizes certain chemotherapeutic agents as a substrate and prevents accumulation of some lipophilic cationic radiopharmaceuticals such as Tc-MIBI (14–19). However, there have been no studies to support the relationship between
Tc-MIBI scan results and Pgp expression in predicting chemotherapy response in malignant lymphomas.

Therefore, the aim of this study was to compare Tc-MIBI scan results, immunohistochemical analyses of Pgp expression, other prognostic factors and chemotherapy response in patients with untreated malignant lymphoma.

**PATIENTS AND METHODS**

**PATIENTS**

This was a retrospective study. A total of 25 non-consecutive patients (15 men, 10 women; age range 25–65 years; mean age 46.2 ± 12.3 years) with untreated malignant lymphoma (12 with HD and 13 with NHL) were studied with Tc-MIBI scan before chemotherapeutic intervention (Table 1). At diagnosis,
all malignant lymphomas were categorized according to the REAL classification (20). No MALT-type malignant lymphoma was included in this study. After Tc-MIBI scan, all patients received multiagent chemotherapy according to lymphoma type and clinical stage. Chemotherapy regimens for patients with HD included nitrogen mustard (mechlorethamine) (dose 6 mg/m², days 1, 8), vincristine (dose 1.4 mg/m², days 1, 8), procarbazine (dose 100 mg/m², days 1–14) and prednisolone (dose 40 mg/m², days 1–14) (MOPP), alternating with doxorubicin (dose 25 mg/m² i.v., days 1, 15), bleomycin (dose 10 units, days 1, 15), vinblastine (dose 6 mg/m², days 1, 15) and dacarbazine (dose 375 mg/m², days 1,15) (ABVD) (1–3). Patients with NHL were treated with cyclophosphamide (dose 750 mg/m² i.v., day 1), doxorubicin (dose 50 mg/m² i.v., day 1), vincristine (dose 1.4 mg/m² i.v., maximum 2 mg, day 1) and prednisolone (dose 100 mg/m² p.o., days 1–5) (CHOP) protocols (1–3). Five HD patients in early stages and three indolent NHL patients with lower aggressiveness in early stages received additional radiotherapy after chemotherapy. The informed consent to participate in this study program was obtained from all the patients prior to initiation of this study.

**INTERPRETATION OF CHEMOTHERAPY RESPONSE**

Chemotherapy response was evaluated in the first 1–2 years after completion of treatment by clinical and radiological methods (9,21). Evaluation criteria were as follows: (1) complete response = no evidence of disease; (2) partial response = \( \geq 50\% \) decrease in the sum of the products of the maximum perpendicular diameters of all measurable lesions, no evidence of progression in any lesion and no new lesions; (3) no response = \(< 25\% \) increase in the sum of the products of the maximum perpendicular diameters of all measurable lesions, no evidence of progression in any lesion and no new lesions; and (4) progressive disease = \( \geq 25\% \) increase in the sum of the products of the maximum perpendicular diameters of all measurable lesions and/or the appearance of new lesions. We defined complete and partial responses as good response, while no response and progressive disease were defined as poor response.

**TECHNETIUM-99m METHoxyISOBUTYLISONITRILE SCAN**

There was a delay of 30 min from the time of oral intake of 500 mg of perchlorate to the start of the imaging procedure to prevent abnormal uptake of free Tc-99m pertechnetate. A commercial MIBI preparation [maximum 5.56 Gb (150 mCi) in ~1–3 ml] was obtained from Dupont (Cardiolite). The labeling and quality control procedures were carried out according to the manufacturer’s instructions. Labeling efficiencies were all \( >95\% \). Each patient was positioned supine on the imaging table with the chest strapped to prevent motion. Ten minutes after intravenous injection of 740 MBq (20 mCi) of Tc-MIBI, early static images of the supradiaphragmatic regions were obtained in the anterior and posterior projection. The equipment consisted of a large field-of-view gamma camera fitted with a low-energy, high-resolution collimator. A single 20% energy window was set at 140 keV and 500K counts were obtained for each static image.

**VISUAL AND QUANTITATIVE INTERPRETATIONS**

Only supradiaphragmatic lesions were evaluated. Intraabdominal and inguinal lesions were not studied owing to physiological tracer accumulation in intra-abdominal organs. For quantitative assessment, tumor-to-background (T/B) ratios were obtained from Tc-MIBI scans. For neck and axillary lesions, regions of interest (ROIs) were drawn over the largest lesion area (tumor) with abnormal activity and the contralateral normal side (background). Background activity for mediastinal lesions was defined as any normal soft-tissue activity in the thorax. For visual interpretation, two experienced physicians reviewed the images. If Tc-MIBI uptake was axillary \( \geq \) soft-tissue background activity, Tc-MIBI scan was considered positive (Figs 1 and 2).

**IMMUNOHISTOCHEMICAL STAINING OF P-GLYCOPROTEIN**

Formalin-fixed paraffin sections (5 μm) were deparaffinized in an oven at 50°C for 40 min and hydrated with various concentrations of ethanol–water dilutions. Endogenous peroxidase was blocked by 3% hydrogen peroxide for 15 min. Antigen
retrieval was performed by treatment with enzyme digestion in 0.1% trypsin in PBS at room temperature for 5 min and inhibition with 10% skim milk in PBS for 5 min. The sections were incubated for 2 h with primary antibody JSB-1 (50 μg/ml) (Boehringer Mannheim Biochemica, Germany) at 1:50 concentration in a moist chamber at 37°C for 2 h. After three 5 min washes in PBS buffer, detection of the primary antibody was performed with a link antibody according to the manufacturer’s instructions (DAKO LSAB_2 System, Peroxidase; Dako, Carpinteria, CA) (12,15). All specimen evaluations were performed on a Nikon microscope (AFX-DX) using an ocular magnification of ×20 and an eyepiece grid. Positive cells were quantified by evaluating four randomly selected high-power fields (minimum 800 tumor cells). Based on the established criteria in the literature (12,16), Pgp expression was defined as follows: negative = (i) when there was a complete absence of staining or (ii) scattered or focal positive cells <10% of the specimen with weak staining, and positive = diffuse positive cells ≥10% of the specimen with weak or strong staining (Fig. 3).

**Statistical Analyses**

T/B ratio was expressed as mean ± standard deviation (SD). The Mann–Whitney U-test was used to test for differences in T/B ratios between patients with good response and poor response. Chi-squared tests were used to assess the differences in good and poor response incidences between patients with positive Tc-MIBI scan results and patients with negative Tc-MIBI scan results, patients with positive Pgp expression and patients with negative Pgp expression, patients with HD and patients with NHL, patients with stage I–II and patients with stage III–IV diseases, patients aged >40 years and patients aged ≤40 years and patients with and without B symptoms (Table 2). However, there were no significant differences in the incidences of good and poor responses between patients with positive Tc-MIBI scan results and patients with negative Tc-MIBI scan results and between patients with positive Pgp expression patients and patients with negative Pgp expression (Table 2).

**RESULTS**

The detailed data for the patients are shown in Table 1. The mean T/B ratio of the 15 patients with good response (3.3 ± 0.6) was significantly (p < 0.01) higher than that of the 10 patients with poor response (1.2 ± 0.1) (Table 1). Among the 15 patients with good response to chemotherapy, all (100%) had positive Tc-MIBI scan results and negative Pgp expression. Among the 10 patients with poor response to chemotherapy, all (100%) had negative Tc-MIBI scan results but six (60%) patients had positive Pgp expression and four (40%) had negative Pgp expression. Significant differences were noted in the incidences of good and poor responses between patients with positive Tc-MIBI scan results and patients with negative Tc-MIBI scan results and between patients with positive Pgp expression patients and patients with negative Pgp expression (Table 2). However, there were no significant differences in the incidences of good and poor responses between HD and NHL patients, stage I–II and stage III–IV patients, patients aged >40 years and patients aged ≤40 years and patients with and without B symptoms (Table 2).
DISCUSSION

In a review of the literature, only one published report (10) was found concerning the use of Tc-MIBI scan to predict chemotherapy response in malignant lymphoma. In that report, 17 children with positive results and significantly higher T/B ratio had a complete response to chemotherapy. Another seven children who had negative Tc-MIBI scan results and a significantly lower T/B ratio had partial or no response to chemotherapy, irrespective of the lymphoma type. Our results can support these findings. However, in that study, Pgp expression and other prognostic factors were not analyzed.

The tumor uptake mechanism of Tc-MIBI has been suggested to involve binding to cytosol in the tumor cell (22). The cationic charge and lipophilicity of Tc-MIBI, mitochondrial and plasma membrane potentials of tumor cells and cellular mitochondrial content can all play a significant role in tumor uptake of this agent (22) or the uptake may be caused by indirect phenomena such as increased tumor blood flow and capillary permeability. In addition, the retention of Tc-MIBI in cells depends on the activity of the 170 kDa Pgp coded on the MDR1 gene, which functions as an ATP-dependent efflux pump for many cytotoxic substances, mostly lipophilic cations. Other studies (14–19) demonstrated the relationship between Tc-MIBI tumor uptake and Pgp expression and implied some potential for Tc-MIBI scintigraphy as a non-invasive imaging test for assessing Pgp expression. Low and high Tc-MIBI tumor uptakes are thought to be consistent with relatively high and low Pgp expressions, respectively (9,11,12) and the mechanism of chemotherapy resistance in malignant lymphomas is thought to involve MDR-Pgp expression (4–8). Therefore, in this study, delayed chest imaging was not considered necessary.

Compared with other prognostic factors, such as lymphoma type, stage, and B symptoms, early Tc-MIBI scan more accurately predicts chemotherapy response in patients with malignant lymphoma. However, it is a major limitation of this study that it seems to be difficult to evaluate prognostic factors based on a smaller number and mixture of patients. Therefore, further studies with homogeneous and a larger number of patients and simultaneous measurement of MRP expression are necessary to confirm our findings.

References

9. Kao CH, Tsai SC, Wang JJ, Ho YJ, Ho ST, Changlai SP. Technetium-99m-sestamethoxyisobutylisonitrile scan as a predictor of chemotherapy response in malignant lymphomas compared with P-glycoprotein expres-

Table 2. Distributions of Tc-MIBI scan results, age, tumor type, tumor stage, B symptoms and Pgp expression according to chemotherapy response

<table>
<thead>
<tr>
<th>Chemotherapy response</th>
<th>Tc-MIBI scan results</th>
<th>Age (years)</th>
<th>Type</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>≤40</td>
<td>&gt;40</td>
</tr>
<tr>
<td>Good</td>
<td>15</td>
<td>0</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Poor</td>
<td>0</td>
<td>10</td>
<td>&lt;0.0001</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemotherapy response</th>
<th>Stage</th>
<th>B symptoms</th>
<th>Pgp expression</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I–II</td>
<td>Yes</td>
<td>Positive</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>III–IV</td>
<td>No</td>
<td>Negative</td>
<td>6</td>
</tr>
<tr>
<td>Good</td>
<td>5</td>
<td>0</td>
<td>0.8611</td>
<td>6</td>
</tr>
<tr>
<td>Poor</td>
<td>3</td>
<td>7</td>
<td>0.3268</td>
<td>6</td>
</tr>
</tbody>
</table>


