Original article

Nuclear Medicine Communications

^{99m}Tc tetrofosmin scintigraphy in acute leukaemia: the relationship between marrow uptake of tetrofosmin and P-glycoprotein and chemotherapy response

Aysun Sükan[a], Zeynep Yapar[a], Berksoy Şahin[b], Oğuz Kara[b], A. Fuat Yapar[c], Salih Çetiner[d] and Mustafa Kibar[a]

Background The non-invasive detection of P-glycoprotein (Pgp) and multidrug resistance related proteins *in vivo*, will represent the greatest challenge in overcoming multidrug resistance. Although ^{99m}Tc tetrofosmin has been used previously as a myocardial perfusion agent, it is now also being used in the imaging of various tumours. In the current study, ^{99m}Tc tetrofosmin was used in the investigation of acute leukaemia.

Aim To show the uptake pattern of ^{99m}Tc tetrofosmin in the bone marrow of patients with acute leukaemia, and to ascertain the relationship between ^{99m}Tc tetrofosmin uptake and the level of Pgp expression and their relation to the response to chemotherapy. In addition, CD95, which is an indicator of apoptosis (programmed cell death), has also been assessed.

Materials and methods Pgp and CD95 were detected by using flow cytometry. Of the 27 acute leukaemia patients assessed, nine had previously received chemotherapy, and 18 had had an initial diagnosis. All patients had undergone ^{99m}Tc tetrofosmin scintigraphy, and their Pgp and CD95 levels had been determined. The same parameters were studied again for 14 patients. The responses to chemotherapy were assessed by patients' clinicians. A control group of 37 patients without bone marrow pathology was also studied in order to provide comparisons for the scintigraphy results. The control images were assessed only qualitatively.

Results In leukaemia patients the uptake of ^{99m}Tc tetrofosmin into bone marrow was found to be considerably higher than in control patients ($P=0.000$). An analysis of the relationship between Pgp, CD95, and the qualitative and quantitative tetrofosmin uptake ratios (URs) showed that there was an inverse correlation only between Pgp and the quantitative uptake ratio ($P=0.016$, $r=-0.461$). When the patients were grouped as 'good' and 'poor', as related to the chemotherapy response, there were no meaningful differences between these two groups regarding Pgp, CD95 and tetrofosmin URs ($P>0.05$). By evaluating the scintigraphic findings of the 'repeated' 14 patients, we showed that if the ^{99m}Tc tetrofosmin UR in the second imaging test was reduced by >0.08, the response to chemotherapy tended to be good. This method, based on follow-up scanning with tetrofosmin, showed a sensitivity of 83% and a specificity of 62% in the prediction of a 'good' response, if a decrease of 0.08 was taken into consideration.

Conclusion In this study, patients with acute leukaemia showed significant uptake of tetrofosmin into the bone marrow. The addition of basal and repeated ^{99m}Tc tetrofosmin scintigraphy to the management protocol for leukaemia could lead to the preferential determination of responses to chemotherapy, by evaluating whole bone marrow non-invasively. This method seems promising, but it needs further support from various similar investigations comprising more patients in order to confirm our results. *Nucl Med Commun* 25:777–785 © 2004 Lippincott Williams & Wilkins.

Nuclear Medicine Communications 2004, **25**:777–785

Keywords: acute leukaemia, ^{99m}Tc tetrofosmin, P-glycoprotein, chemotherapy response

Departments of [a]Nuclear Medicine, [b]Oncology, [d]Central Laboratory, Çukurova University Medical School, Adana, Turkey and [c]Department of Nuclear Medicine, Baskent University Medical School, Adana Hospital, Adana, Turkey.

Correspondence to Dr Aysun Sukan, Çukurova Üniversitesi Tıp Fakültesi, Nükleer Tıp Anabilim Dnli, Balcali, Adana, Turkey.
Tel/fax: +90 322 338 6486;
e-mail: aysunsukan@yahoo.com

Received 10 November 2003 **Accepted** 22 November 2003

Introduction

Leukaemia is one of the most effective targets of cancer chemotherapy. However, drug resistance is often observed during chemotherapy. In acute myelogenous leukaemia (AML), the multidrug resistance (MDR) phenotype has been shown to be positive in a ratio varying from 30% to 50% of patients [1,2]. Also, the expression of P-glycoprotein (Pgp) has frequently been observed in resistant types after chemotherapy [3]. In acute lymphoblastic leukaemia (ALL), the rate of MDR positivity is somewhat lower than that of AML. MDR has been shown to be positive in 22% of ALL patients at the

0143-3636 © 2004 Lippincott Williams & Wilkins DOI: 10.1097/01.mnm.0000134319.32279.0f

initial diagnosis [4]. However, this ratio has increased to 58% in chemoresistant patients or at relapse. The remission rate is shown to be higher in Pgp negative patients for both AML and ALL [4,5].

Several methods have been established for the determination of MDR1 with the hope of optimizing therapy protocols [6–10]. While some methods (e.g. reverse transcriptase polymerase chain reaction, slot blot, northern blot) have aimed to detect MDR1 messenger RNA, others (e.g. flow cytometry, immunohistochemistry) have aimed to show the increase of the protein expression of Pgp [11–14], but these methods may reveal discordant results, with each method having its own disadvantages [6,10,15–17].

Pgp is a 170 kDa transmembrane glycoprotein which is encoded by the *MDR1* gene and acts as an adenosine triphosphate dependent efflux pump to reduce the intracellular accumulation of many chemotherapeutic drugs [18,19]. Since Piwmca-Worms *et al.* [20] have shown that sestamibi is a substrate for Pgp, the use of organotechnetium cations in the detection of Pgp overexpression has become one of the major issues for nuclear medicine physicians.

^{99m}Tc tetrofosmin (^{99m}Tc 1,2-bis[bis(2-ethoxyethyl) phosphino]ethane) is an agent that was developed for myocardial perfusion imaging and has been shown to accumulate in viable tumour tissue [21–27]. The functional characteristics of this agent are similar to those of ^{99m}Tc sestamibi [28]. Like sestamibi, tetrofosmin has also been suggested to be a potent agent for the prediction of MDR in various tumour cell lines [29–32].

The aim of the current study was to show the uptake pattern of ^{99m}Tc tetrofosmin in the bone marrow of patients with acute leukaemia, and also to ascertain the relationship between ^{99m}Tc tetrofosmin uptake and the level of Pgp expression and their relation to chemotherapy response.

Patients, materials and methods

Patients and chemotherapy regimens

A total of 27 patients (11 female, 16 male; mean age 36.11 ± 13.30 years, range 15–73 years) were included in our study. Twenty patients had AML and seven had ALL. Of these, 18 patients were initially diagnosed, seven patients had relapsed and two had primary resistance after chemotherapy. All patients underwent ^{99m}Tc tetrofosmin scintigraphy before starting chemotherapy. One or 3 days after imaging, remission induction therapy including cytosine arabinoside (ARA-C), idarubicin, all-transretinoic acid (ATRA) for AML patients, and prednisolone, vincristine, idarubicin, L-asparaginase for ALL patients was started. The patients in relapse or primary resistant to chemotherapy were given the regimen including etoposide, ARA-C, idarubicin, mitoxantrone (MTZ), granulocyte-stimulating factor (GCSF) and fludarabine. Four to six weeks after chemotherapy, the responses of the patients were evaluated by the clinical physicians according to the findings of bone marrow biopsy and flow cytometry. Blasts under 5% in bone marrow and leukaemia markers under 25% indicated a good response to chemotherapy. Following analysis of the response, 14 patients underwent a second imaging with ^{99m}Tc tetrofosmin to evaluate the effect of the chemotherapy on the scintigraphic images. The expression of Pgp was measured simultaneously.

Control subjects

Thirty-seven control subjects (mean age 47.97 ± 16.71 years; range 7–73 years) were also studied with ^{99m}Tc tetrofosmin to compare the bone marrow distribution of tetrofosmin in leukaemia patients with that of normal subjects. Of these, 27 subjects were referred to our department for myocardial perfusion imaging and 10 were referred for bone scanning with bone lesions of which following biopsy specimens confirmed benign pathologies for all.

^{99m}Tc tetrofosmin imaging

The patients received intravenous injection of 740 MBq (20 mCi) ^{99m}Tc tetrofosmin. Fifteen minutes later, 5 min planar images (256 × 256 matrix) were obtained from anterior thorax, pelvis and knees, respectively. Then, whole-body imaging was performed. Control subjects were evaluated by planar images only. Scintigraphic examination was performed with a large-field-of-view gamma camera (Camstar, Starcam4000i, GE Medical Systems) equipped with a low energy, all-purpose collimator.

Data analysis

Tetrofosmin scans were interpreted by two nuclear medicine physicians blinded to the patients' clinical information. Interpretation was performed visually and quantitatively. Proximal humerus and femurs, sternum, anterior iliac crests, and proximal tibias (if any uptake was seen) were chosen for bone marrow analysis. To avoid false-positive results that could originate from the overlapping physiological uptake in the heart, kidneys and bowels, the vertebral column was not included in the analyses. Uptake of tetrofosmin in the bone marrow was evaluated against the uptake in the reference organ (adjacent soft tissue).

Visual analysis was performed using a qualitative five-point scoring system with the following criteria: bone marrow uptake < soft tissue uptake → 0; bone marrow

uptake = soft tissue uptake → 1; bone marrow uptake slightly exceeding soft tissue uptake → 2; bone marrow uptake moderately exceeding soft tissue uptake → 3; and bone marrow uptake significantly exceeding soft tissue uptake → 4.

In quantitative analysis, on the planar images, a manual region of interest (ROI) was set on the marrow region (lesion) and an ROI was set on the adjacent soft tissue (background). The uptake ratio (UR) was calculated by dividing the count density of the lesion by that of the background RO1.

Flow cytometry

Flow cytometric analysis was done 1–2 days before the tetrofosmin scans. By this method, Pgp and CD95 levels were assessed for all patients by using blood and bone marrow samples taken from them. The monoclonal antibody (mAb) UIC2 that was used is specific for the extracellular epitope of Pgp. Cells were labelled directly for flow cytometric analysis using the following procedure. One millilitre of blood was taken from each patient and put in EDTA coated tubes. The white cell count was adjusted to be 3000–10 000 cells/ml. For each patient two test tubes (polistren 7 × 12) were prepared, one as an isotopic control and the other for the determination of Pgp. Whole blood samples (100 µl) were put into the tubes. Pgp isotopic control, IgG2a-PE (Immunotech., Lod no. 34) was added to the first test tube, and Pgp-PE (Immunotech., Lod no. 09) from monoclonal antibody (20 µl) were added to the second tube. The test tubes were kept in the dark and at room temperature for 20 min.

Subsequently, these samples were passed through a TQ-Prep (Coulter) instrument. Each were added, automatically, by the instrument, as follows: the (A) solution (erythrocyte lysis solution) (600 µl), the (B) solution (leucocyte stabilizer) (260 µl), and lastly the (C) solution (cell wall fixer) (100 µl). Erythrocytes were extracted from the lysed blood samples. Leucocytes were prepared for flow cytometry without damaging their structures. The samples prepared using these procedures were analysed by flow cytometry (Epics-XL-Coulter) and the blast cell population was gated using scatter parameters. The percentage of Pgp positive cells was noted for each sample.

Statistical analysis

The difference in tetrofosmin scores between patients and control subjects was analysed by the multiple comparisons Scheffe test. The correlation between flow cytometry and tetrofosmin results were analysed by Spearman's rank correlation coefficient. According to response grouping, the baseline parameters for all the patients and the repeated parameters for 14 patients were compared by using the Mann–Whitney U test. A value of $P < 0.05$ was considered significant. The alterations of the parameters from the first study to the second were analysed by the Wilcoxon signed rank sum test. For determining the sensitivity and specificity of tetrofosmin alterations in predicting a good response to chemotherapy, the cut-off value of the decrease in UR (0.08) was determined according to receiver operating characteristics curve (ROC) analysis.

Results

^{99m}Tc tetrofosmin bone marrow uptake in patients versus that in control subjects

Due to the lower uptake in most bone marrow compared with adjacent soft tissue, the control groups were not evaluated quantitatively. When visually correlated, the accumulation of ^{99m}Tc tetrofosmin in the bone marrow of patients was significantly higher than that in control subjects ($P = 0.000$) (Table 1). While there was no increased tetrofosmin activity compared to background in normal subjects, increased tetrofosmin activity (grade 2 in 75, grade 3 in 49, and grade 4 in 15 sites) was seen in 139 of 194 bone marrow sites (71.6%) in the leukaemia group. When bone marrow sites were compared quantitatively, the highest tetrofosmin UR was seen in humerus and iliac crest, and least uptake was detected in sternum. The mean URs detected from humerus and iliac crest were significantly higher than those of femur and sternum ($P = 0.000$). The UR of femur was higher than that of sternum ($P = 0.000$).

Correlation between tetrofosmin uptake ratio, Pgp expression and CD95

There was a statistically significant inverse relationship between the levels of Pgp and quantitative URs for tetrofosmin ($P = 0.016$, $r = -0.461$). Although an inverse relationship between Pgp levels and visual tetrofosmin findings was observed, it was not statistically significant ($P = 0.439$, $r = -0.155$). No significant relationship

Table 1 Visual mean marrow uptake ratios of tetrofosmin in patients versus control subjects and quantitative mean uptake ratios with respect to the evaluated regions separately

Control or patient	Humerus	Sternum	Iliac crest	Femur	Tibia	Mean ± SD
Control (visual evaluation)	0.34 ± 0.45 (n=66)	0.24 ± 0.5 (n=33)	0.42 ± 0.51 (n=72)	0.38 ± 0.47 (n=72)	1 (n=4)	0.39 ± 0.29 (n=247)
Patient						
Visual evaluation	2.37 ± 0.83 (n=52)	1.88 ± 0.99 (n=26)	2.34 ± 1.01 (n=52)	1.92 ± 0.78 (n=54)	2.0 ± 1.0 (n=10)	2.13 ± 0.76 (n=194)
Quantitative evaluation	3.42 ± 0.66* (n=52)	1.66 ± 0.36 (n=26)	3.15 ± 0.89* (n=52	2.63 ± 0.54* (n=54)	2.90 ± 0.8* (n=10)	1.54 ± 0.31 (n=194)

*Sum of the values detected from the right and the left regions of interest.

Table 2 Results of the parameters evaluated in the patient population and responses to chemotherapy

Patient number	Diagnosis	Uptake ratio (mean)		P-glycoprotein	CD95	Response to chemotherapy
		Visual evaluation	Quantitative evaluation			
1	AML	3.00	2.32	36.20	49.40	Good
2	AML	3.66	2.02	28.20	–	Poor
3	ALL	1.00	1.00	37.70	–	Good
4	AML	1.85	1.44	38.50	22.50	Good
5	AML	2.71	1.32	28.90	81.80	Poor
6	AML	1.71	1.91	18.80	–	Good
7	ALL	1.55	1.31	36.20	68.60	Good
8	AML	1.57	1.49	14.80	26.00	Poor
9	AML	1.00	1.34	49.80	27.60	Poor
10	AML	3.57	2.06	25.00	–	Good
11	AML	3.42	1.71	60.40	1.50	Poor
12	AML	1.55	1.52	11.00	30.00	Poor
13	AML	2.85	1.71	32.70	12.80	Poor
14	AML	2.14	1.57	32.90	–	Good
15	AML	1.66	1.23	32.40	–	Good
16	AML	3.00	1.66	36.00	–	Good
17	AML	2.77	1.89	9.00	53.20	Poor
18	AML	0.85	1.12	41.20	–	Poor
19	AML	2.42	1.75	25.00	15.00	Poor
20	AML	2.00	1.30	45.50	85.50	Good
21	ALL	2.28	1.46	16.50	29.30	Good
22	AML	1.88	1.32	44.60	–	Good
23	ALL	1.71	1.13	36.00	53.50	Poor
24	ALL	1.66	1.26	33.10	39.50	Poor
25	ALL	2.00	1.45	44.60	–	Poor
26	AML	2.14	1.55	30.30	62.70	Poor
27	ALL	1.66	1.77	28.00	33.00	Poor

AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia.

between CD95 and the other parameters was detected ($P > 0.05$).

Repeated studies and response to chemotherapy

Nine of 27 patients studied had had previous chemotherapy before the study and 18 were initially diagnosed without previous chemotherapy. A good response ratio to the subsequent chemotherapy in patients without previous chemotherapy (8/17, 47%) was higher than that of those with chemotherapy (2/9, 22%). However, the difference was not statistically significant ($P > 0.05$).

Considering all 27 patients, 11 (41%) had good and 17 (63%) had poor responses to chemotherapy (Table 2). There were no statistically significant differences between baseline Pgp, CD9S, visual and quantitative URs of the patients with good and poor responses to chemotherapy ($P > 0.05$).

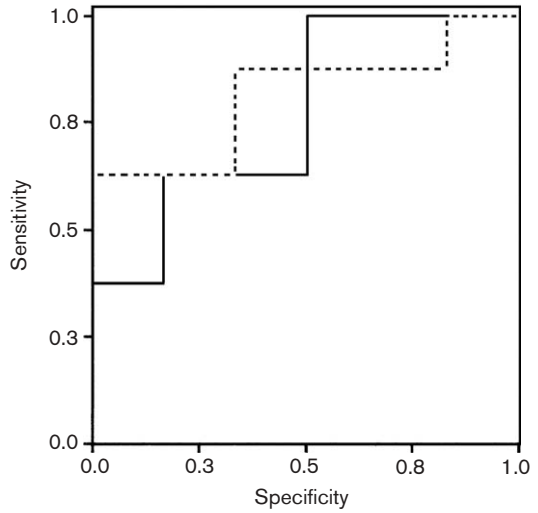
The tetrofosmin and flow cytometry studies for 14 patients were repeated after evaluation of the response to chemotherapy (Table 3). Of these 14 patients, six (42%) showed good response and eight (57%) showed poor response to chemotherapy. Patients with poor response to chemotherapy, Pgp, CD95, visual and quantitative URs did not show significant differences when the results for the initial and second studies were compared ($P > 0.05$), but visual and quantitative URs of the patients with good response to chemotherapy showed significant decreases in the repeated scans ($P < 0.05$).

When the alterations of the test parameters from the initial and repeated studies were analysed the response to chemotherapy was found to be good if the ^{99m}Tc tetrofosmin UR in the second imaging test was reduced by a value > 0.08. If a decrease of a value > 0.08 is taken into consideration, this method revealed a sensitivity of 83% and a specificity of 62% in the prediction of a good response (Fig. 1).

Discussion

Our study showed that the accumulation of tetrofosmin is significantly higher in the bone marrow of patients with acute leukaemia compared with that in normal subjects. Wakasugi *et al.* [33] have previously shown such an intense accumulation of sestamibi in patients with acute leukaemia. In that study, the authors used femoral bone marrow for the evaluations and found clearly visible sestamibi accumulation in all patients either at relapse or at initial diagnosis before chemotherapy, while none of the control subjects showed such uptake. Mild accumulation in the marrow was noted in only 12% (13/110) of their normal subjects, and 88% of them showed no detectable sestamibi accumulation. In accordance with these findings, increased tetrofosmin accumulation was not seen in the normal subjects in our study (Fig. 2). So, the appearance of an intensity similar to the adjacent soft tissue was attributed to normal haematopoietic activity. In most of the leukaemia patients included in the current study, uptake of tetrofosmin into bone marrow was clearly

Fig. 1



Receiver operating characteristics curve formed from the differences in tetrofosmin uptake ratios of the initial and second scans. When a decrease of a value >0.08 is taken into consideration, the method yields a sensitivity of 83% and a specificity of 62% in the prediction of a good response to chemotherapy. (–), Qualitative difference; (––), quantitative difference.

visualized even without an active growth plate (716%, 139/194).

The two normal subjects in whom the tibias were visualized with an intensity similar to the background were adolescents. From the physiological point of view bone marrow continues to grow until the adult pattern is reached, and therefore this was an expected finding in childhood. In contrast to normal adults, bone marrow is generally replaced by fat tissue in distal femur and tibia. Except for the two adolescents, there was no accumulation of tetrofosmin in the bone marrow of the tibias in the normal subjects. However, in our study, which consisted mostly of adults, the proportion of patients in whom the tibia was clearly visualized by tetrofosmin was rather high (37%, 10/27).

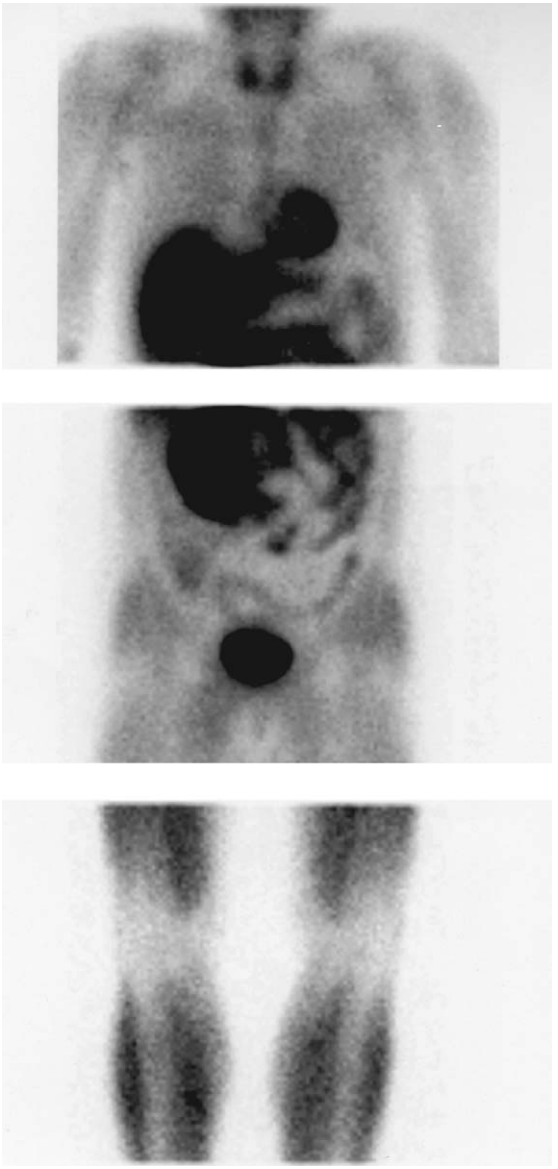
In the literature, there is no data concerning the distribution of tetrofosmin in the bone marrow of either leukaemia patients or normal subjects. Previous experience has focused on ^{99m}Tc sestamibi [33] and some authors have proposed that sestamibi has the potential to identify functional expression of Pgp in patients with acute leukaemia [34,35]. Although some differences were noted between sestamibi and tetrofosmin regarding membrane transport and intracellular localization, the uptake mechanisms of both lipophilic cationic complexes are associated with mitochondria, and accumulation of these tracers only occurs in viable and metabolically active cells [36]. In the light of this knowledge, the significantly increased uptake of tetrofosmin in the bone marrow of patients with acute leukaemia was attributed to the high mitochondrial density and membrane polarization in blast cells in our study.

Prior data have shown an inverse relationship between sestamibi uptake and Pgp expression in acute leukaemia [34,35]. A similar inverse relationship between Pgp expression and tetrofosmin uptake was found in our study. Although tetrofosmin was not investigated in leukaemia, some previous studies have revealed that tetrofosmin uptake decreases if Pgp expression increases in non-small cell lung cancer, breast cancer and malignant lymphoma [37–39]. In our study, the value of Pgp and tetrofosmin results were also investigated in predicting the response to chemotherapy. According to our results, Pgp does not offer a major independent role in this prediction. In contrast to our results, several reports suggest that there is an inverse correlation between Pgp expression and the response to chemotherapy in AML patients [40–42]. However, there are also reports in which Pgp is shown to have no prognostic value for AML [43,44]. Similar discordant results also exist for ALL [5,45]. Discordant results about the prognostic value of Pgp in leukaemia may be explained by several factors, including the variety of methods used for detecting Pgp, the simultaneous existence of multiple resistance mechanisms, the existence of Pgp in normal tissue or cells, the heterogenous expression of Pgp, and the expression Pgp without functional capacity [6,10,15].

While some of the methods directed to the detection of Pgp identify protein expression (e.g. flow cytometry and immunohistochemistry), others study the increase of MDR1 messenger RNA (e.g. RT–PCR, *in situ* hybridization). In addition to the differences in between the methods used, the differences in methodology and interpretation criteria also may be considered as sources of discordant results. Considering these reasons, the authors advise the use of at least two different methods simultaneously [34,46]. The use of flow cytometry alone may be considered as a limitation of the current study, for although the technique has the advantages of easier application and the ability to show heterogenous expression, it is not able to discriminate between malignant and normal cells. In addition, there is the possibility of contamination, which decreases the test specificity. The previous reports which were in favour or against our findings had used at least two different methods in the detection of Pgp.

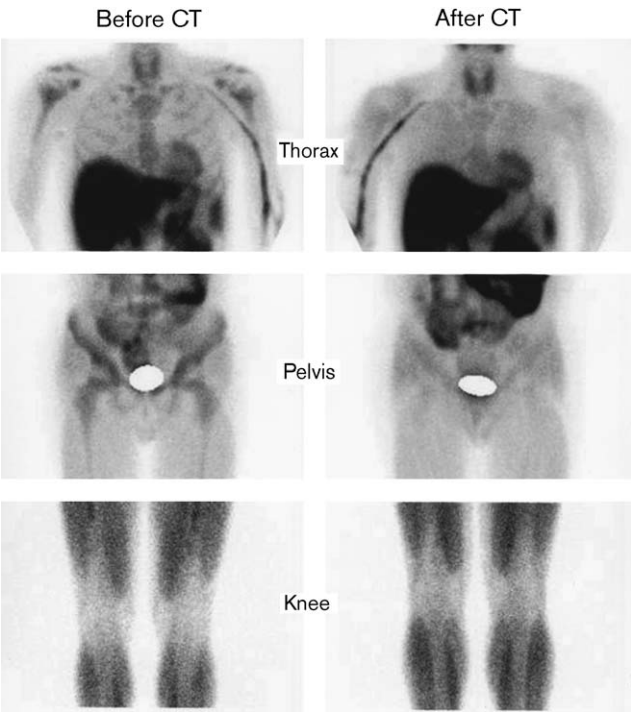
In our study, although an inverse correlation was found between Pgp expression and tetrofosmin uptake in bone marrow, baseline tetrofosmin URs did not reveal

Fig. 2



A 40-year-old control patient. Tetrofosmin uptake ratios in the sternum and iliac crests were assessed as equal to the background ($n=1$). No tetrofosmin uptake was visualized at the humerus and tibia ($n=0$). The mean qualitative uptake ratio was calculated as 0.42.

Fig. 3



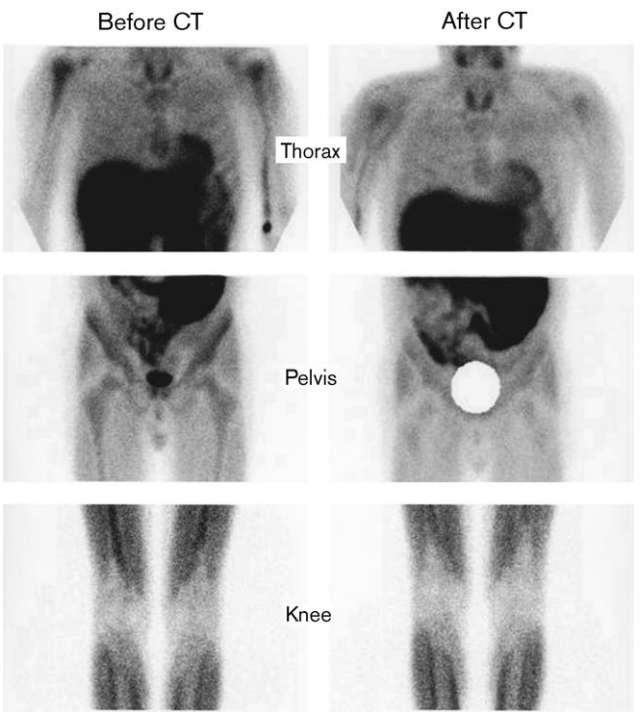
Uptake of ^{99m}Tc tetrofosmin, before and after chemotherapy (CT), into the bone marrow of a 43-year-old male patient with acute myeloid leukaemia. Prominent uptake in the proximal humerus, sternum, iliac crest and femur are seen before chemotherapy, and the mean qualitative and quantitative uptake ratios were calculated as 3.57 and 2.06, respectively. Expression of P-glycoprotein in blasts was measured as 22. The CD95 level could not be measured. The uptake ratios for ^{99m}Tc tetrofosmin were significantly decreased after chemotherapy and the mean qualitative and quantitative values were calculated as 2 and 1.41, respectively. The response to chemotherapy was evaluated as good.

meaningful differences between good and poor responders to chemotherapy. Prior data regarding the relationship between tetrofosmin uptake and chemotherapy response in lymphoma, breast and lung cancers contradicts our findings [37,47,48]. This may be explained by possible different behaviours of different cancers. According to our review of the literature, we did not find any report concerning response to chemotherapy in acute leukaemia carried out with either sestamibi or tetrofosmin. In the study by Wakasugi *et al.* [33], which investigated the value of sestamibi in predicting minimal residual disease by femoral marrow imaging, clearly visualized sestamibi accumulation was shown to be a potential marker for relapse (Fig. 2).

When the results of the second tetrofosmin quantitative URs were compared with the initial ones, a decrease of a value >0.08 in the second study could be a potential marker for a good response to chemotherapy. This interpretation criterion gave an acceptable accuracy of 71% (10/14) in predicting the response to chemotherapy (Figs. 3 and 4). Three patients with poor responses to chemotherapy showed false results by this method (patients 2, 4 and 7 in Table 3). The significant increase in the level of Pgp was thought to be a possible explanation for the reduced uptake of tetrofosmin for patient 4, but this explanation could not be applied to the other two patients. Therefore, different resistance

mechanisms that exist simultaneously in acute leukaemia were thought to be the possible reason. Zaman *et al.* [49] have described another efflux pump, multidrug resistance associated protein (MRP), which also extrudes lipophilic compounds from cells. Some investigators have discovered that tetrofosmin is also a substrate for MRP in gliomas, lung and nasopharengeal cancers [30,32,50]. Also, another protein (lung resistant protein, LPR) has been demonstrated to be an independent factor for predicting poor response to chemotherapy and reduced overall survival for AML [51]. It has been reported that expression of LRP is frequently observed in the patients in whom Pgp is expressed after resistance modulators are given [52].

Fig. 4



Uptake of ^{99m}Tc tetrofosmin, before and after chemotherapy (CT), into the bone marrow of a 40-year-old male patient with acute myeloid leukaemia. Prominent uptake in the proximal humerus, sternum, iliac crest and femur are seen before chemotherapy, and the mean qualitative and quantitative uptake ratios were calculated as 3 and 1.66, respectively. The CD95 level could not be measured. The uptake ratios for ^{99m}Tc tetrofosmin were significantly decreased after chemotherapy and the mean qualitative and quantitative uptake ratios were calculated as 2.28 and 1.28, respectively. The response to chemotherapy was evaluated as good.

Predicting response to chemotherapy and identifying resistance to it is a major challenge in the management of leukaemia patients. Despite the introduction of more effective and various kinds of drugs, the lack of response to chemotherapy threatens the success of cancer treatment. The identification of the MDR will certainly lead to better strategies and improve survival.

Conclusions

Our data show that ^{99m}Tc tetrofosmin imaging is a promising tool in the follow-up of leukaemia patients. A decrease of > 0.08 in the tetrofosmin uptake ratio in the bone marrow predicts good response to remission induction therapy, with an accuracy of 71%. Although further investigation with a larger number of patients is necessary to confirm our findings, the addition of baseline and follow-up ^{99m}Tc tetrofosmin scintigraphy to the leukaemia management protocol will lead to a non-invasive determination of the response to chemotherapy. Baseline Pgp levels and tetrofosmin uptake ratios in the bone marrow of leukaemia patients do not seem to be independent factors in the prediction of response to chemotherapy. However, an increased level of Pgp expression is correlated with a low accumulation of tetrofosmin in bone marrow of patients with acute leukaemia.

Table 3 Alterations in the results of the 14 patients who were studied twice, and the responses to chemotherapy

Patient number	Response	Pre-chemotherapy				Post-chemotherapy			
		P-glycoprotein	CD95	Nitel TO	Quantitative TURs	P-glycoprotein	CD95	Qualitative TURs	Nicel TO
1	Poor	31.10	39.50	1.66	1.26	38.80	–	1.33	1.36
2	Poor	28.20	49.40	3.00	2.32	18.50	24.80	3.22	1.73
3	Poor	41.20	–	0.85	1.12	46.00	70.00	1.00	1.18
4	Poor	14.80	26.00	1.57	1.49	61.90	82.20	1.42	1.37
5	Poor	32.70	12.80	2.85	1.71	23.00	65.00	2.00	1.75
6	Poor	11.00	30.00	1.55	1.52	37.00	62.00	1.71	1.57
7	Poor	49.80	27.60	1.00	1.34	40.00	52.40	1.00	1.17
8	Poor	36.00	53.50	1.71	1.13	37.90	–	1.28	1.05
Mean ± SD		30.85 ± 12.87	34.11 ± 14.24	1.77 ± 0.77	1.48 ± 0.39	37.88 ± 13.34	59.4 ± 19.57	1.62 ± 0.72	1.39 ± 0.26
9	Good	36.20	68.60	1.55	1.31	50.00	11.10	1.33	1.09
10	Good	25.00	–	3.57	2.06	41.80	40.20	2.00	1.41
11	Good	36.00	–	3.00	1.66	51.70	14.60	1.71	1.57
12	Good	45.50	85.50	2.00	1.30	12.40	–	1.71	1.22
13	Good	16.50	29.30	2.28	1.46	32.60	–	1.28	1.10
14	Good	38.50	22.50	1.85	1.44	24.00	10.00	2.00	1.14
Mean ± SD		32.95 ± 10.41	51.47 ± 30.45	2.37 ± 0.76	1.53 ± 0.28	35.41 ± 15.4	18.97 ± 14.28	1.67 ± 0.31	1.25 ± 0.19

References

1 Müller M, Lennartz K, Nowrousian M, *et al.* Improved flow cytometric detection of low P-glycoprotein expression in leukemic blast by histogram substraction analysis. *Cytometry* 1994; **15**:64–72.

2 Leith C, Chen I, Kopecky K, *et al.* Correlation of multidrug resistance (MDR) protein expression with functional dye/drug efflux in acute myeloid leukemia by multiparameter flow cytometry: identification of discordant MIR – /efflux + and MDR + /efflux – cases. *Blood* 1995; **86**:2329–2342.

3 Zhou D, Marie J, Subervilie A, Zittoun R. Relevance of MDR-1 gene expression in acute myeloid leukemia and comparison of different diagnostic methods. *Leukemia* 1992; **6**:879–885.

4 Canipos L, Guyotat D, Archimbaud E, *et al.* Clinical significance of multidrug resistance P-glycoprotein expression on acute nonlymphoblastic leukemia cells at diagnosis. *Blood* 1992; **79**:473–476.

5 Goasguen JE, Dossot JM, Farrel O, *et al.* Expression of multidrug associated P-glycoprotein (P170) in 59 cases *de novo* acute lymphoblastic leukemia: prognostic implications. *Blood* 1993; **81**:2394–2398.

6 Kessel D, Beck WT, Kukuruga D, Schulz V. Characterization of multidrug resistance by fluorescent dyes. *Cancer Res* 1991; **51**:4665–4670.

7 Noonan K, Beck C, Holzmayer TA. Quantitative analysis of MDR1 (multidrug resistance) gene expression in human tumors by polymerase chain reaction. *Proc Natl Acad Sci USA* 1990; **87**:7160–7164.

8 Longo R, Bensi L, Vecchi A, Messora C, Sacchi S. P-glycoprotein in acute myeloblastic leukemia analyzed by immunocytochemistry and flow cytometry. *Leukemia Lymphoma* 1995; **17**:121–125.

9 Ludescher C, Hilbe W, Eistere W, Huber C, Preuss E. Activity of Pgp in B-cell chronic lymphocytic leukemia determined by a flow cytometric assay. *J Natl Cancer Inst* 1993; **85**:1751–1757.

10 van der Heyden S, Gheuens E, DeBruijin E, Van Oosterom A, Maes R. P-glycoprotein: clinical significance and methods of analysis. *Crit Rev Clin Lab Sci* 1995; **32**:221–264.

11 Bechimol S, Ling V. P-glycoprotein and tumor progression. *J Natl Cancer Inst* 1994; **86**:814–816.

12 Riscin D, Ling V. Multidrug resistance in leukaemia. In: Freireicb EJ, Kantaijian H (editors). *Leukemia: Advances in Research and Treatment*. Boston, Dordrecht, London: Kluwer Academic Publishers; 1993, pp. 269–293.

13 Goldstein U, Galski H, Fojo A, William M, Lai SL, Gazdar A, *et al.* Expression of multidrug resistance gene in human cancers. *J Natl Cancer Inst* 1989; **81**:116–124.

14 Maria JP, Zittoun R, Sikiç BI. Multidrug resistance (mdrl) gene expression in adult acute leukemias: correlations with treatment outcome and *in vitro* drug sensitivity. *Blood* 1991; **78**:586–592.

15 Charpin C, Vielh P, Duffaud F, *et al.* Quantitative immunocytochemical assays of Pgp in breast carcinomas: correlation to messenger RNA expression and to immunohistochemical prognostic indicators. *J Natl Cancer Inst* 1994; **86**:1539–1545.

16 Cordon-Cardo C, O'Brien JP, Bocia J, Cassals D, Bertino JR, Melamed MR. Expression of the MDR gene product (Pgp) in human normal and tumor tissues. *J Histochem Cytochem* 1990; **38**:1277–1287.

17 Melefors O, Hentze MW. Translational regulation by mRNA/protein interactions in eucaryotic cells: ferritin and beyond. *Bioassays* 1993; **15**:85–90.

18 Juliano RI, Ling V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim Biophys Acta* 1976; **455**:152–162.

19 Lankelma J, Spoelstra EC, Dekker H, Broxterman HJ. Evidence of daunomycin efflux from multidrug resistant 2780AD human ovarian carcinoma cells against a concentration gradient. *Biochim Biophys* 1990; **1055**:217–222.

20 Piwmca-Worms D, Kronauge JF, Chiu ML. Uptake and retention of hexakis (2-methoxy isobutyl isonitrile) technetium(I) in cultered chick myocardial cells: mitocondrial and plasma membrane potential dependence. *Circulation* 1990; **82**:1826–1838.

21 Kostakoglu L, Uysal U, Özyar E, *et al.* A comparative study of technetium-99m sestamibi and technetium-99m tetrofosmin single-photon emission tomography in the detection of nasopharyngeal carcinoma. *Eur J Nucl Med* 1997; **24**:621–628.

22 Obwegeser R, Berghammer P, Rodrigues M, *et al.* A head-to-head comparison between technetium-99m-tetrofosmin and technetium-99m-MIBI scintigraphy to evaluate suspicious breast lesions. *Eur J Nucl Med* 1999; **26**:1553–1559.

23 Takekawa H, Shinano H, Tsukamoto E, *et al.* Technetium-99m-tetrofosmin imaging of lung cancer: relationship with histopathology. *Ann Nucl Med* 1999; **13**:71–75.

24 Hashimoto T, Takahashi K, Goto M, *et al.* Detection of malignant thymoma in primary tumour and metastatic lesions using ^{99m}Tc-tetrofosmin scintigraphy. *Radiot Med* 2001; **19**:169–172.

25 Adalet I, Kocak M, Oguz H, *et al.* Determination of medullary thyroid carcinoma metastases by ^{201}T1, ^{99m}Tc(V)DMSA, ^{99m}Tc-MIBI and ^{99m}Tc-tetrofosmin. *Nucl Med Commun* 1999; **20**:353–359.

26 Yapar Z, Kibar M, Ozbarlas S, *et al.* ^{99m}Tc tetrofosmin scintigraphy in musculoskeletal tumors: the relationship between P-glycoprotein expression and tetrofosmin uptake in malignant lesions. *Nucl Med Commun* 2002; **23**:991–1000.

27 Soricelli A, Cuocolo A, Varrone A, *et al.* Technetium-99m-tetrofosmin uptake in brain tumours by SPECT: comparison with thallium-201 imaging. *J Nucl Med* 1998; **39**:802–806.

28 Arbab AS, Koizumi K, Toyania K, *et al.* Uptake of technetium-99m-tetrofosmin, technetium-99m-MIBI and thallium-201 in tumour cell lines. *J Nucl Med* 1996; **37**:1551–1556.

29 Ballinger JR, Bannerman J, Boxen I, *et al.* Technetium-99m-tetrofosmin as a substrate for P-glycoprotein: *in vitro* studies in multidrug-resistant breast tumour cells. *J Nucl Med* 1996; **37**:1578–1582.

30 Perek N, Prevot N, Koumanov F, Frere D, Sabido O, Beauchesne P, Dubois F. Involvement of the glutathione S-conjugate compounds and the MRP protein in Tc-99m-tetrofosmin and Tc-99m-sestamibi uptake glioma cell lines. *Nucl Med Biol* 2000; **27**:299–307.

31 Muzzammil T, Moore MJ, Ballinger JR. *In vitro* comparison of sestamibi, tetrofosmin, and furifosmin as agents for functional imaging of multidrug resistance in tumours. *Cancer Biother Radiopharm* 2000; **15**:339–346.

32 Utsunomiya K, Ballinger JR, Piquette-Miller M, *et al.* Comparison of the accumulation and efflux kinetics of technetium-99m sestamibi and technetium-99m tetrofosmin in an MRP-expressing tumour cell line. *Eur J Nucl Med* 2000; **27**:1786–1792.

33 Wakasugi S, Ohta K, Hasegawa Y, Tatumi N, Nakamura H. Detection of minimal residual disease in acute leukemia by Tc-99m MIBI femoral imaging. *Clin Nucl Med* 2001; **26**:325–330.

34 Kostakoglu L, Guc D, Canpnar H, Kars A, Alper E, Kral P, *et al.* P-glycoprotein expression by technetium-99m-MIBI scintigraphy in hematologic malignancy. *J Nucl Med* 1998; **39**:1191–1197.

35 Ak, Aslan V, Vardareli E, Gülba Z. Assessment of the P-glycoprotein expression by ^{99m}Tc-MIBI bone marrow imaging in patients with untreated leukemia. *Nucl Med Commun* 2003; **24**:397–402.

36 Vallabhajosula S. Radiopharmaceuticals in oncology. In: Khalkhali L, Maublant JC, Goldsmith SJ (editors). *Nuclear Oncology – Diagnosis and Therapy*. Philadelphia: Lippincott Williams & Wilkins; 2001, pp. 31–62.

37 Shiau YC, Tsai SC, Wang JJ, Ho YJ, Ho ST, Kao CH. Predicting chemotherapy response and comparing with P-glycoprotein expression using technetium-99m tetrofosmin scan in untreated malignant lymphomas. *Cancer Lett* 2001; **170**:139.

38 Liu TJ, Tsai SC, Ho YJ, Sun SS, Kao CH. Comparison of the expression of P-glycoprotein, Ki-67, and P-53 to technetium-99m tetrofosmin mammoscintigraphic findings. *Cancer Invest* 2002; **20**:199–205.

39 Tabuenca MJ, Vargas JA, Varela A, *et al.* Inverse correlation between Tc-99m-tetrofosmin uptake and P-glycoprotein in non-small cell lung cancer. *J Nucl Med* 1999; **40**:1224–1225.

40 Wood P, Burgess R, MacGregor A, Liu Yin J. P-glycoprotein expression on acute myeloid blast cells at diagnosis predicts response to chemotherapy and survival. *Br J Haematol* 1994; **87**:509–514.

41 Zöchbauer S, Gsur A, Brunner R, Kyrle P, Lechner K, Pirker R. P-glycoprotein expression as unfavorable prognostic factor in acute myeloid leukemia. *Leukemia* 1994; **8**:975–977.

42 Basara N, Radosevic-Radojkovic N, Colovic M, Boskovic D, Rolovic Z. *In vitro* drug sensitivity of leukemic progenitors and P-glycoprotein expression in adult myeloid leukemia: correlation with induction treatment outcome. *Eur J Haematol* 1995; **55**:83–87.

43 Gruber A, Vitols S, Norgren S, *et al.* Quantitative determination of mdr 1 gene expression in leukaemic cells from patients with acute leukemia. *Br J Cancer* 1992; **66**:266–272.

44 Ino T, Miyazaki H, Isogai M, *et al.* Expression of P-glycoprotein in *de novo* acute myelogenous leukemia at initial diagnosis: results of molecular and functional assays, and correlation with treatment outcome. *Leukemia* 1994; **8**:1492–1497.

45 Pieters R, Huismas D, Lonen AH, *et al.* Relation of cellular drug resistance to resistance to long term clinical outcome in childhood acute lymphoblastic leukemia. *Lancet* 1991; **338**:399–403.

46 Beck WT, Grogan MT, William CL, Cordon-Cardo C, Parham DM. Methods to detect Pgp associated multidrug resistance in patient tumors: consensus recommendations. *Cancer Res* 1996; **56**:3010–1320.

47 Fukumoto M, Yoshida D, Hayase A, *et al.* Scintigraphic prediction of resistance to radiation and chemotherapy in patients with lung carcinoma: technetium 99m-tetrofosmin and thallium-201 dual single photon emission computed tomography study. *Cancer* 1999; **86**:1470–1479.
48 Kao CH, Ho YJ, Shen Y, Lee JK. Evaluation of chemotherapy response in patients with small cell lung cancer using technetium 99m-tetrofosmin. *Anticancer Res* 1999; **19**:2311–2315.
49 Zaman GJR, Flens MJ, van Leusden MR, *et al.* The human multidrug resistance associated protein MRP1 is a plasma membrane drug-efflux pump. *Proc Natl Acad Sci USA* 1994; **91**:8822–8826.
50 Chen WS, Luker KE, Dahlheimer JL, Pica CM, Luker GD, Piwmca-Worms D. Effects of MDR1 and MDR3 P-glucoproteins, MRP1, and BCRP/MXR/ABCP on the transport of ^{99m}Tc tetrofosmin. *Biochem Pharmacol* 2000; **60**:413–426.
51 Kostakoglu L. Multidrug resistance. Part B: other tumors. In: Khalkhal I, Maublant J, Goldsmith S (editors): *Nuclear Oncology – Diagnosis and Therapy.* Philadelphia: Lippincott Williams & Wilkins; 2001, pp. 73–82.
52 List AF, Spier CS, Grogan TM, *et al.* Overexpression of the major vault protein lung-resistance protein predicts treatment outcome in acute myeloid leukemia. *Blood* 1997; **20**:398–403.