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## References

- Dewald GW, Wyatt WA, Juneau AL, Carlson RO, Zinsmeister AR, Jalal SM, et al. Highly sensitive fluorescence in situ hybridization method to detect double BCR/ABL fusion and monitor response to therapy in chronic myeloid leukemia. *Blood* 1998;91:3357-65.
- van Dongen JJ M, Macintyre EA, Gabert JA, Delabesse E, Rossi V, Saglio G, et al. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease Report of the BIO-MED-1 Concerted Action: Investigation of minimal residual disease in acute leukemia. *Leukemia* 1999;13:1901-28.
- Hayette S, Tigaud I, Thomas X, French M, Perrin MC, Nicolini F, et al. Identification of a rare e6a2 BCR-ABL fusion gene during the disease progression of chronic myelomonocytic leukemia: a case report. *Leukemia* 2004;18:1735-6.
- Colla S, Sammarelli G, Voltolini S, Crugnola M, Sebastio P, Giuliani N. e6a2 BCR-ABL transcripts in chronic myeloid leukemia: is it associated with aggressive disease?. *Haematologica* 2004;89:611-3.
- Hochhaus A, Reiter A, Skladny H, Melo JV, Sick C, Berger U, et al. A novel BCR-ABL fusion gene (e6a2) in a patient with Philadelphia chromosome-negative chronic myelogenous leukaemia. *Blood* 1996;88:2236-40.
- Quentmeier H, Cools J, MacLeod RAF, Marynen P, Uphoff CC, Drexler HG. E6-a2 BCR/ABL1 fusion in T-cell acute lymphoblastic leukemia. *Leukemia* 2005;19:295-6.
- Melo JV. The diversity of BCR-ABL fusion proteins and their relationship to leukemia phenotype. *Blood* 1996;88:2375-84.
- Huntly BJ, Guilhot F, Reid AG, Vassiliou G, Hennig E, Franke C, et al. Imatinib improves but may not fully reverse the poor prognosis of patients with CML with derivative chromosome 9 deletions. *Blood* 2003;102:2205-12.
- Quintas-Cardama A, Kantarjian H, Talpaz M, O'Brien S, Garcia-Manero G, Verstovsek S, et al. Imatinib mesylate therapy may overcome the poor prognostic significance of deletions of derivative chromosome 9 in patients with chronic myelogenous leukemia. *Blood* 2005;105:2281-6.

## Multiple Myeloma

### Baseline Tc<sup>99m</sup>-MIBI scanning predicts survival in multiple myeloma and helps to differentiate this disease from monoclonal gammopathy of unknown significance

**We performed baseline Tc<sup>99m</sup>-MIBI scanning in 43 patients with multiple myeloma (MM) and in 31 with monoclonal gammopathy of unknown significance (MGUS) patients. We identified two groups of MM patients whose actuarial survival correlated with low or high MIBI scores. MGUS patients had normal or very low scores.**

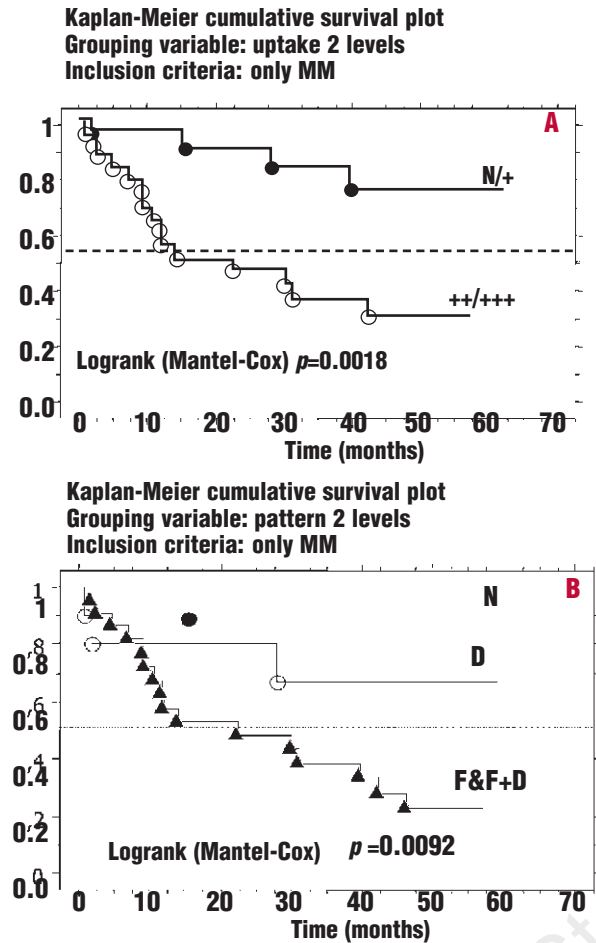
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The ability of Tc<sup>99m</sup>-MIBI scanning to detect bone marrow involvement in MM has been known since 1996<sup>1</sup> but is scarcely used. The aim of this prospective study was to determine whether Tc<sup>99m</sup>-MIBI uptake can be used as a prognostic factor in MM and whether it can differentiate between MM and MGUS.

We studied 43 MM and 31 MGUS patients (33 men and 41 women), aged between 43 and 83 years (mean 69±7.9). Tc<sup>99m</sup>-MIBI scanning and plain X-rays were performed before any therapy. The median follow-up was 57 and 44 months in the MM and MGUS groups, respectively. The Tc<sup>99m</sup>-MIBI scans were scored for intensity and pattern according to Pace;<sup>2</sup> there were four possible intensity levels (normal, +, ++ and +++) and four possible patterns (normal (N), focal (F), diffuse (D) and focal + diffuse (F+D)). Two specialists in nuclear medicine, blind to the patients' diagnosis, evaluated the patterns and scores. We also received the percentage of bone marrow cells and biochemical data related to prognosis: β<sub>2</sub>-microglobulin (0.7-1.8 μg/mL), C-reactive protein (0.01-0.5 mg/dL), albumin (3.4-4.8 g/dL), creatinine (0.5-1.3 mg/dL) and lactate dehydrogenase (80-480 U/L). The units and normal ranges in our laboratory are given in brackets. All symptomatic patients with active disease but two received VBCMP/ VBAD as first line therapy. Radiotherapy and/or autologous stem cell transplantation were applied when appropriate.

The patients were categorized into groups according to Tc<sup>99m</sup>-MIBI uptake and MIBI pattern and the differences between groups were analyzed with the Kruskal-Wallis test and stepwise multiple regression. We used the Kaplan-Meier method for survival analysis, and log rank (Mantel-Cox), Breslow-Gehan-Wilcoxon, Tarone-Ware, Peto-Peto-Wilcoxon and Harrington-Fleming tests to investigate differences between groups. In cases of a *p* value <0.05 in any test we assumed a difference in survival and the significance was checked with the post-hoc Bonferroni-Dunn test. MM and MGUS groups were analyzed separately and together. Table 1 shows the variables correlated with MIBI uptake and pattern with the Kruskal-Wallis test (A), and with multiple stepwise regression (B). Only β<sub>2</sub>-microglobulin, C-reactive-protein and lactate dehydrogenase were selected by multiple regression in MM patients. The percentage of bone marrow plasma cells was also selected if all the patients were considered together. Twenty-eight of the 31 MGUS patients had negative MIBI scores. Of the three cases with positive scores, two had a very low intensity (+) diffuse pattern and one a very low intensity (+) focal pattern



**Figure 1.** A. Multiple myeloma patients were categorized in two groups with good and poor prognosis. In a previous analysis (not shown) the survival curves of patients with ++ and +++ scores were almost identical and those with the normal and + scores were similar. B. Multiple myeloma patients were categorized into three groups according to scan patterns. In a previous analysis (not shown) the survival curves of patients with Focal and Focal+Diffuse patterns were similar.

(localized in a joint affected by active rheumatoid arthritis).

There was a significant difference in survival among MM patients depending on the intensity of MIBI uptake ( $p=0.0264$ ). The patients with ++ or +++ scores had a similar poor prognosis. If the MM patients were categorized into two groups (normal/+ and ++/+++), the significance was even greater ( $p=0.0018$ ) and the probability of survival at 57 months was  $74\% \pm 11.5$  in the former group and  $26.1\% \pm 10.2$  in the latter (Figure 1A). The difference in survival between these two groups was confirmed with the post-hoc Bonferroni-Dunn test ( $p=0.0072$ ).

There was also a difference in survival among MM patients depending on the pattern of the scan, but this difference was at the borders of statistical significance ( $p$  slightly less than 0.05 in four tests, but 0.0608 in the Breslow-Gehan-Wilcoxon test). We categorized MM patients into three groups according to Tc<sup>99m</sup>-MIBI pattern: normal; diffuse; focal together with focal and diffuse

**Table 1A.** Kruskal-Wallis test for MIBI uptake.

	MIBI uptake				MIBI pattern			
	MM h	p	ALL h	p	MM h	p	ALL h	p
Moncl. Ig (serum)	2.75	n.s.	18.850	0.0008	3.7	n.s.	15.38	0.0015
Moncl. Ig (urine)	3	n.s.	8.4	0.037	3.4	n.s.	9.47	0.0237
$\beta 2$ $\mu$ g	8.9	0.03	15.25	0.0016	9.514	0.0232	15.55	0.0014
C protein	9.23	0.026	14.87	0.0028	15.99	0.0011	20.85	0.0001
Albumin	2.2	n.s.	3.97	n.s.	2.062	n.s.	3.81	n.s.
Creatinin	9.25	0.026	4.82	n.s.	2.554	n.s.	5	n.s.
Lactate dehydrogenase	7.4	n.s.	6.96	n.s.	8.093	0.0441	6.3	n.s.
B.M. plasma cells	6.2	n.s.	26.1	0.0001	4.416	n.s.	24.08	0.0001

MM: only multiple myeloma patients; ALL: MGUS and MM patients; h: h corrected for ties; p: tied p-value.; n.s.: no significance.  $\beta$ -2-microglobulin and C-reactive protein show significantly different means in both MM and all patients. Bone marrow plasma cells and monoclonal component only are different if all patients were considered together. C protein: C reactive protein.

**Table 1B.** Step-wise multiple regression. Variables in model.

	MIBI uptake	
	Step 1	Step 2
MM & MGUS	Plasma cells $p < 0.0001$	—
Only MM	$\beta 2$ -microglobulin $p = 0.0059$	$p = 0.0343$
Lactate dehydrogenase		
	MIBI pattern	
	Step 1	Step 2
MM & MGUS	C-reactive protein $p < 0.0001$	$p < 0.0001$
Only MM	C-reactive protein $p = 0.0042$	—
Plasma cells		

(Figure 1B); the three groups are clearly separated and the statistical significance reaches the level of  $p=0.0203$  in the log rank Mantel-Cox test.

In previous studies Tc<sup>99m</sup>-MIBI has proven to be more sensitive than Tc<sup>99m</sup>-methylene diphosphonate and X-ray, and at least as sensitive as computed tomography, magnetic resonance imaging and positron emission tomography, with some discordant results regarding magnetic resonance.<sup>3-9</sup> However the prognostic significance of Tc<sup>99m</sup>-MIBI scanning has not been analyzed. Our results suggest that baseline Tc<sup>99m</sup>-MIBI scanning has prognostic significance, and that scores of marked intensity (++, +++) or advanced patterns (F and F+D) in the absence of inflammation or other pathologies exclude the diagnosis

of MGUS. Furthermore we have confirmed a previously reported correlation<sup>10</sup> between Tc<sup>99m</sup>-MIBI scanning results and laboratory data:  $\beta$ -2-microglobulin, C-reactive protein and lactate dehydrogenase. It would be incorrect to do a Cox-regression test in this small series to check whether these variables are independent of Tc<sup>99m</sup>-MIBI. Further studies should be done to establish the utility of Tc<sup>99m</sup>-MIBI in prognosis, staging and response to therapy in MM.

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Key words: multiple myeloma monoclonal gammopathy, prognosis,  
M protein, technetium Tc<sup>99m</sup> Sestamibi, radionuclide imaging.

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## References

1. Tirovola EB, Biassoni L, Britton KE, Kaleva N, Kouykin V, Malpas JS: The use of <sup>99m</sup>Tc-MIBI scanning in multiple myeloma. *Br J Cancer* 1996;74:1815-20.
2. Pace L, Catalano L, Pinto A, De Renzo A, Di Gennaro F, Califano C, et al. Different patterns of technetium-<sup>99m</sup> sestamibi uptake in multiple Myeloma. *Eur J Nucl Med* 1998; 25:714-20.
3. Wakasugi S, Noguti A, Katuda T, Hashizume T, Hasegawa Y. Potential of (<sup>99m</sup>Tc)-MIBI for detecting bone marrow metastases: *J Nucl Med* 2002;43:596-602.
4. Svaldi M, Tappa C, Gebert U, Bettini D, Fabris P, Franzelin F, et al. Technetium-<sup>99m</sup> sestamibi scintigraphy: an alternative approach for diagnosis and follow-up of active myeloma lesions after high-dose chemotherapy and autologous stem cell transplantation. *Ann Hematol* 2001;80:393-7.
5. Fonti R, Del Vecchio S, Zannetti A, De Renzo A, Di Gennaro F, Catalano L, et al. Bone marrow uptake of <sup>99m</sup>Tc-MIBI in patients with multiple myeloma. *Eur J Nucl Med* 2001;28:214-20.
6. Mileshekin L, Blum R, Seymour JF, Patrikeos A, Hicks RJ, Prince HM. A comparison of fluorine-18 fluoro-deoxyglucose PET and technetium-<sup>99m</sup> sestamibi in assessing patients with multiple myeloma. *Eur J Haematol* 2004;72:32-7.
7. Mirzaei S, Filipits M, Keck A, Bergmayer W, Knoll P, Koehn H, et al. Comparison of Technetium-<sup>99m</sup> MIBI imaging with MRI for detection of spine involvement in patients with multiple myeloma. *BMC Nucl Med* 2002;3:2.
8. Alper E, Gurel M, Evrensel T, Ozkocaman V, Akbunar T, Demiray M. <sup>99m</sup>Tc-MIBI scintigraphy in untreated stage III multiple myeloma: comparison with X-ray skeletal survey and bone scintigraphy. *Nucl Med Commun* 2003;24:537-42.
9. Alexandrakis MG, Kyriakou DS, Passam F, Koukouraki S, Karkavitsas N. Value of Tc-<sup>99m</sup> sestamibi scintigraphy in the detection of bone lesions in multiple myeloma: comparison with Tc-<sup>99m</sup> methylene diphosphonate. *Ann Hematol* 2001; 80:349-53.
10. Alexandrakis MG, Kyriakou DS, Passam FH, Malliaraki N, Christophoridou AV, Karkavitsas N. Correlation between the uptake of Tc-<sup>99m</sup> sestamibi and prognostic factors in patients with multiple myeloma. *Clin Lab Haematol* 2002;24:155-9.

## Chronic Lymphocytic Leukemia

### Low-dose intravenous alemtuzumab therapy in pretreated patients affected by chronic lymphocytic leukemia. A single center experience

**We report the efficacy and safety of intravenous low-dose alemtuzumab (10 mg three times weekly for 10 weeks) in 12 patients with relapsed or refractory chronic lymphocytic leukemia. Low-dose alemtuzumab induced significant responses in these patients (16% complete remission, 25% partial remission), with mild hematologic and extra-hematologic side effects and a low rate of infections, even in the presence of long-lasting severe immunosuppression.**

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Several studies have reported the efficacy of standard-dose alemtuzumab (30 mg three times weekly, administered by either the intravenous or the subcutaneous route) in previously treated patients with chronic lymphocytic leukemia (CLL), with overall response rates (ORR) ranging from 33% to 42%.<sup>1-3</sup> Although the response rates were high, standard dose alemtuzumab in refractory patients was associated with considerable hematologic and extra-hematologic toxicity.<sup>4,5</sup>

Recently, a pilot study with low-dose subcutaneous alemtuzumab (10 mg three times weekly for 18 weeks) in refractory CLL patients showed a high response rate (ORR: 50%) and a favorable toxicity profile.<sup>6</sup> We therefore administered low-dose alemtuzumab to 12 patients with pretreated CLL. We evaluated the efficacy (NCIWG criteria) of the treatment, the duration of response, the overall survival, the safety, the incidence of infectious complications and the immune recovery. All patients had been previously treated with at least two lines of chemotherapy (range 2-5). In four patients treatment with fludarabine was not attempted because of autoimmune hemolytic anemia or refusal. The median time from the last treatment to initiation of alemtuzumab therapy was 4 months (range 2-24 months) (Table 1).

Alemtuzumab was given intravenously at a dose of 3 mg on day 1; from day 3 the target dose was raised to 10 mg three times weekly for 30 administrations. Treatment was stopped if disease progressed or grade IV thrombocytopenia, infections or cytomegalovirus (CMV) reactivation occurred. Therapy was withheld if the neutrophil count fell below 500/ $\mu$ L, although granulocyte colony-stimulating factor was administered for grade IV neutropenia. CMV screening was conducted by weekly analysis of antigenemia and CMV DNA. Immunological subsets (CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>, CD16/56, CD19<sup>+</sup>) were studied before and after the end of treatment on days 60, 120, 180 and 240. Of the 12 patients, two (16%) obtained a CR and three (25%) achieved a PR, with an ORR of 41% (Table 2). The ORR was 83% in stage A/progressive or B/II disease compared to 0% in stage C/IV disease ( $p=0.01$ ). The ORR was 50% in patients with mutated V<sub>H</sub> genes and 29% in patients with unmutated V<sub>H</sub> genes ( $p=ns$ ). However, both CR were achieved in patients with mutated V<sub>H</sub> genes and minimal residual disease was not detectable in these two patients. Five patients who were refractory to previous chemotherapy did not achieve any response to alemtuzumab. The