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Abstract

Patients with multiple myeloma were treated chemotherapeutically with a combination of melphalan, ifosfamide, prednisolone, nitrosourea and vincristine (MIP-NV therapy). The M-protein kinetics during the course of MIP-NV therapy was studied. The kinetics of serum and urinary M-protein in the first cycle of the chemotherapy was classified into four patterns, and the mode of change in the M-protein level over the entire course of chemotherapy was classified into four prototypes. There were intimate relationships among M-protein kinetics patterns in the first cycle of the chemotherapy, the effect of the chemotherapy on M-protein reduction, maturity of myeloma cells, pretreatment labeling index and clinical stage of the disease. Moreover, analyzing the prototypes, it was found that both the time for maximum M-protein reduction and the rate of increase in the M-protein level after maximum M-protein reduction affected the survival time. To predict the effect of the chemotherapy on M-protein reduction and survival time, it was useful to analyze subgroups, which were classified according to the M-protein kinetics pattern in the first cycle, the time for maximum M-protein reduction and the rate of increase in the M-protein level after maximum M-protein reduction.

KEYWORDS: multiple myeloma, M-protein kinetics

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M-Protein Kinetics in Multiple Myeloma Treated with Melphalan, Ifosfamide, Prednisolone, Nitrosourea and Vincristine in Combination

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Patients with multiple myeloma were treated chemotherapeutically with a combination of melphalan, ifosfamide, prednisolone, nitrosourea and vincristine (MIP-NV therapy). The M-protein kinetics during the course of MIP-NV therapy was studied. The kinetics of serum and urinary M-protein in the first cycle of the chemotherapy was classified into four patterns, and the mode of change in the M-protein level over the entire course of chemotherapy was classified into four prototypes. There were intimate relationships among M-protein kinetics patterns in the first cycle of the chemotherapy, the effect of the chemotherapy on M-protein reduction, maturity of myeloma cells, pretreatment labeling index and clinical stage of the disease. Moreover, analyzing the prototypes, it was found that both the time for maximum M-protein reduction and the rate of increase in the M-protein level after maximum M-protein reduction affected the survival time. To predict the effect of the chemotherapy on M-protein reduction and survival time, it was useful to analyze subgroups, which were classified according to the M-protein kinetics pattern in the first cycle, the time for maximum M-protein reduction and the rate of increase in the M-protein level after maximum M-protein reduction.

Key words : multiple myeloma, M-protein kinetics

A distinguishing feature of multiple myeloma is that tumor cells produce M-protein. One can easily estimate the severity of the disease or the effect of therapy by measuring the serum or urinary M-protein level. The serum and urinary M-protein kinetics following chemotherapy with a combination of melphalan, ifosfamide, prednisolone, nitrosourea derivatives and vincristine (MIP-NV therapy) was studied with respect to the effectiveness of chemotherapy and the sur-

vival time. Factors which affected M-protein kinetics following chemotherapy were found to be clinical stage, cell maturity, pretreatment labeling index (LI%), time required for maximum M-protein reduction and the rate of increase in the M-protein level after maximum M-protein reduction.

Materials and Methods

Forty-eight patients who underwent MIP-NV therapy (1) were studied. The serum or urinary

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M-protein level was determined weekly as follows. The sera of patients were subjected to cellulose acetate membrane electrophoresis according to the method of Sezaki (2). The ratio of M-protein to total serum protein was determined with a densitometer (Densitron 200M, Joko Corp., Tokyo, Japan). The serum M-protein level (g/100 ml) was calculated from the total serum protein level (g/100 ml) and the ratio of M-protein to total serum protein. Five hundred ml of the urine was taken from the total daily urine and was concentrated to about 5 ml by ultrafiltration. Identification of urinary M-protein was performed by immunoelectrophoresis. The ratio of M-protein to total urinary protein was obtained as for the serum M-protein, and total M-protein excretion (g/day) was calculated.

Assessment of the response to chemotherapy was done after at least three cycles of chemo-

therapy. M-protein reduction was expressed as a percent of the pretreatment level. The remission time was defined as the period in which the M-protein level was less than 50% of the pretreatment level.

To classify serum M-protein kinetics in the first cycle of chemotherapy, 75% of the pretreatment M-protein level was defined as the upper limit for a reduction, and 125% of the pretreatment level was defined as the lower limit for an increase in patients who were treated with a full dose of MIP-NV. The 85% and 115% levels were used as the limits for patients who underwent mIP-aV therapy, where m and a represent melphalan and ACNU at doses reduced to one half of those in MIP-NV therapy. The M-protein kinetics were classified into four patterns as follows: Decreasing pattern, the M-protein level decreased and remained below 75% or 85% of

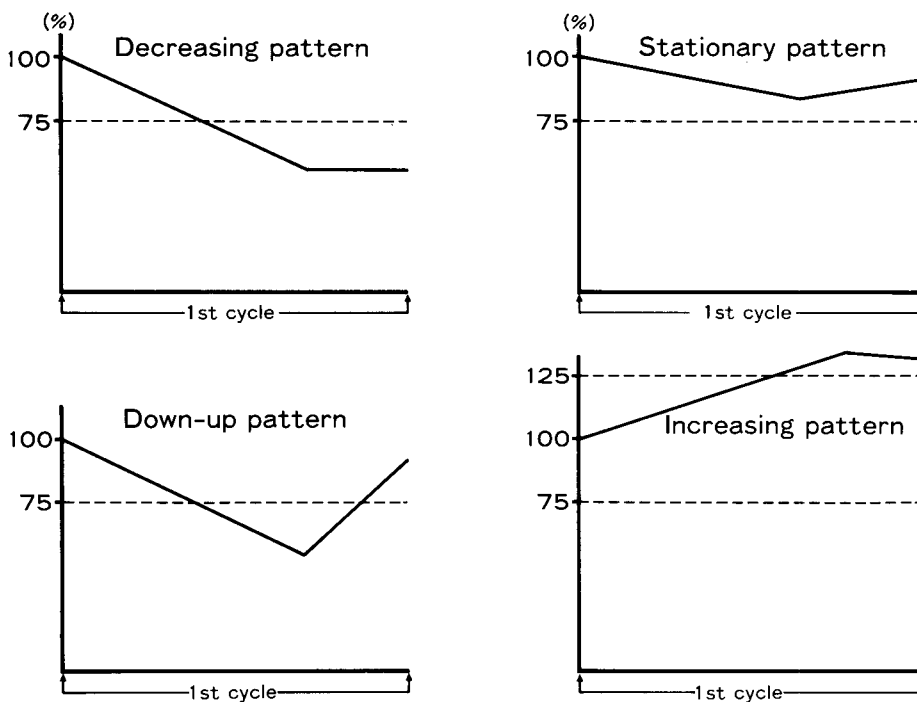


Fig. 1 Patterns of serum M-protein kinetics in patients with multiple myeloma in the first cycle of chemotherapy. Ordinate shows the change in M-protein level (%), which was calculated by the following equation.

$$\frac{\text{M-Protein level (g/100 ml)}}{\text{Pretreatment M-protein level (g/100 ml)}} \times 100$$

Abscissa shows the course of the first cycle of chemotherapy.

the pretreatment M-protein level; down-up pattern, M-protein level decreased once to below the 75% or 85% level, but again increased over that level; stationary pattern, the M-protein level varied from the 75% or 85% level to the 125% or 115% level; increasing pattern, the M-protein level increased to over the 125% or 115% level. These patterns are illustrated in Fig. 1.

The mode of M-protein kinetics was studied over the entire course of chemotherapy to death or at least for 33 months for survivors by determining the M-protein level every four months. The mode of M-protein kinetics was classified into four prototypes as follows. P_1 : The maximum M-protein reduction was more than 60%, and the slope of the linear plot of the M-protein increase to time after the lowest M-protein level was less than 0.5. P_2 : The maximum M-protein reduction was more than 60%, and the slope was more than or equal to 0.5. P_3 : The maximum M-protein reduction was less than 60%, and the slope was

more than or equal to 0.5. P_4 : The maximum M-protein reduction was less than 60%, and the slope was less than 0.5. These prototypes are illustrated in Fig. 2.

The maturity of the nucleus and cytoplasm of myeloma cells was examined by light microscopy according to Bernier and Graham (3). Both the nucleus and cytoplasm of 20 plasma cells from each bone marrow aspirate were scored as follows: immature, 0; intermediate, 1; and mature, 2. The labeling index of myeloma cells was determined by rapid scintillation autoradiography according to the method of Durie and Salmon (4). Plasma cells containing 5 grains or more over the nucleus were considered labeled. One thousand cells were counted to determine the labeling index (LI%). The average grain count of labeled cells was determined (5).

The median survival time from the start of chemotherapy was calculated using the method of Kaplan and Meier (6). Differences between sur-

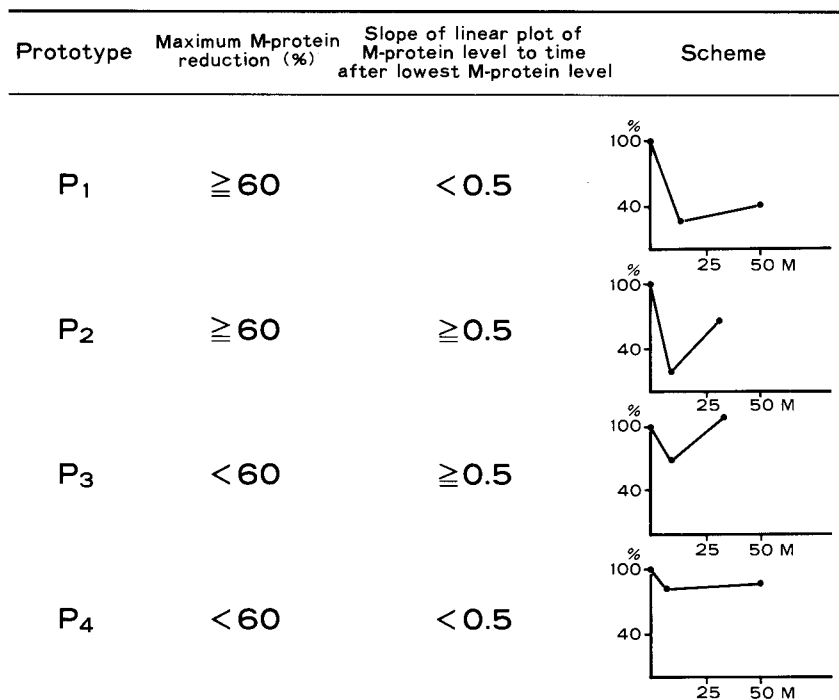


Fig. 2 Definition of prototypes of serum M-protein kinetics in patients with multiple myeloma over the entire course of chemotherapy. Ordinate of the scheme shows the reduction (%) of serum M-protein level compared to the pretreatment level. Abscissa shows the time in months (M).

vival curves were tested using a logrank test (7).

Results

The average remission time was 29 months for patients with an M-protein reduction of more than 60%. This remission time was significantly longer than the 11 months of patients with an M-protein reduction of 50 to 60% ($p < 0.005$).

The median survival time of previously untreated patients with an M-protein reduction of 25 to 60% at stage III_{A+B} was 14 months, which was significantly shorter than the 30 months of patients with an M-protein reduction of more than 60% ($p < 0.01$). On the other hand, the average survival time of three patients with an M-protein reduction of less than 25% was as long as 39 months. Except for patients with an M-protein reduction of less than 25%, the survival time of patients at stage III_{A+B} was shorter than that of patients at stage $I_A + II_A$ regardless of the M-protein reduction (Table 1).

The process of serum and urinary M-protein reduction following chemotherapy was evaluated simultaneously in five patients with urinary Bence Jones (BJ) protein excretion greater than 1.0 g/day. All cases but one

(T.Y.) showed the decreasing pattern of both serum and urinary M-protein reduction in the first cycle of chemotherapy.

In the case of T.Y., serum M-protein showed the stationary pattern, but urinary BJ protein showed the down-up pattern. The serum M-protein reduction was $86.7 \pm 11.6\%$ on the average, which was slightly lower than that of urinary BJ protein. Time for achieving a 50% reduction in serum M-protein was 5.3 ± 2.2 weeks on the average, which was longer than that for urinary BJ protein, though there was no statistically significant difference.

The maximum M-protein reduction in the first cycle was 64% in patients who showed the decreasing pattern and 43% in patients who showed the down-up pattern after treatment with the MIP-NV protocol. These reductions were greater than 44% ($p < 0.1$) and 20% ($p < 0.01$) in patients who were treated with the mIP-aV protocol and who showed the decreasing and down-up patterns, respectively.

In all patients treated with these protocols, 20 of 21 patients who showed the decreasing pattern, two of ten patients who showed the down-up pattern and three of 15 patients who showed the stationary pattern achieved an M-protein reduction of more

Table 1 Remission time and survival time of patients with different degree of M-protein reduction by combination chemotherapy^a

Reduction of M-protein (%) (r)	Number of patients ^b	Average remission time (months)	Average survival time (months)	
			Stage	
			$I_A + II_A$	$III_A + III_B$
$r < 25$	15(9)	—	36	39
$25 \leq r < 50$	6(4)	—	48	11
$50 \leq r < 60$	7(5)	11	56	20.5
$60 \leq r < 90$	6(6)	26	45	31.5
$90 \leq r$	12(9)	30	33.5	35.5

a: Patients were treated with melphalan, ifosfamide, prednisolone, nitrosourea and vincristine in combination(1). For details, see the text.

b: Numbers in parentheses indicate previously treated patients.

than 50%. The rate of achieving an M-protein reduction of more than 50% was thus especially high in the patients with the decreasing pattern. No patients with the increasing pattern achieved an M-protein reduction of more than 50% (Table 2).

There was no relationship between the survival time of patients at clinical stage I_A+II_A and the M-protein kinetics pattern in the first cycle of chemotherapy. In patients at stage III_A+III_B, the survival time of patients with the decreasing pattern was 32.5 months on the average, and that of patients with the down-up pattern was 19.7 months, but there was no statistically significant difference in the survival time.

In the first cycle of chemotherapy, the nucleus maturity score of patients with the stationary pattern was 1.63 ± 0.48 , which was higher than the 1.30 ± 0.50 of patients with the down-up pattern ($p < 0.01$) and also higher than the 1.26 ± 0.50 of patients with the decreasing pattern ($p < 0.001$). On the other hand, the cytoplasm maturity score of patients with the stationary pattern was 0.83 ± 0.49 , which was lower than the 1.12 ± 0.60 of patients with the decreasing pattern ($p < 0.01$). The score of patients with the down-up pattern was in between these two patterns. There was no statistically significant difference between the degree of maturity of myeloma cells and the prototype of M-protein kinetics during the

entire course of therapy.

The pretreatment LI% of patients with the decreasing pattern in the first cycle was 2.3 ± 1.3 , which was higher than the 0.8 ± 0.6 of patients with the stationary pattern ($p < 0.1$), but which was lower than the 6.2 ± 0.9 of patients with the down-up pattern ($p < 0.05$). The pretreatment grain count in patients with the stationary pattern was 5.5 ± 0.7 , which was lower than the 10.6 ± 4.5 of patients with the decreasing pattern ($p < 0.1$).

The pretreatment LI% in patients with a 25 to 50% reduction in M-protein was 5.3 ± 1.9 , which was higher than the 1.5 ± 1.7 of patients with an M-protein reduction of less than 25% ($p < 0.05$), but was not different from that of patients with an M-protein reduction of more than 50%. There was no difference in the pretreatment grain count with respect to the M-protein reduction. The LI% and grain count values gradually increased as time passed following chemotherapy in five and four of seven patients, respectively.

The maximum M-protein reduction (%) in patients with P₁ and P₂ was 82 ± 14 , which was higher than the 30 ± 10 of patients with P₃ and P₄ ($p < 0.001$). The rate of increase in the M-protein level in relation to time after the lowest M-protein level was 0.2 ± 0.2 in patients with P₁ and P₄, which was lower than the 1.5 ± 1.3 of pa-

Table 2 M-Protein kinetics pattern in the first cycle of chemotherapy and reduction of M-protein^a

Pattern	Number of Patients (N)	Maximum reduction of serum M-protein (%)	Number of patients with a 50% reduction in M-protein/N
Decreasing	14 (7)	64 (44)	14/14 (6/7)
Down-up	4 (6)	43 (20)	1/4 (1/6)
Stationary	13 (2)	19 (10)	2/13 (1/2)
Increasing	1 (1)	—	0/1 (0/1)

a: Forty-eight patients were treated with MIP-NV or mIP-aV protocol, and M-protein kinetics pattern and M-protein reduction in the first cycle of the therapy are shown. Numbers in the parentheses show patients treated with mIP-aV protocol. For details, see the text.

tients with P_2 and P_3 ($p < 0.05$). The median survival time of patients with P_1 was 52 months, which was longer than that of patients with P_2 and P_3 ($p < 0.005$). The survival time of two patients with P_4 was 47 months on the average, which was longer than that of patients with P_2 or P_3 (Table 3).

The time for maximum M-protein reduction ($> 60\%$) of all eight patients with P_1 was more than 6 months. The median survival time of five patients with P_2 , whose time for maximum M-protein reduction ($> 60\%$) was less than 6 months, was 18.5 months, which was shorter than the 49 months of 14 patients with $P_1 + P_2$ whose time for maximum M-protein reduction was more than 6 months ($p < 0.005$). In patients whose time for maximum M-protein reduction was more than 6 months, the median survival time of patients with P_1 (slope < 0.5) was 52 months, which was longer than the 29 months of patients with P_2 (slope ≥ 0.5) ($p < 0.005$).

Discussion

Nathans *et al.* (8) showed that the total daily production of paraprotein in a solid plasma cell tumor in mice was proportional to the weight of the tumor. Later, Fakhri and Hobbs (9), using an ascitic form of plasmacytoma in mice (MP-5663), showed that

the serum paraprotein level was directly related to the actual number of tumor cells. On the other hand, the urinary BJ protein level is not directly related to the tumor cell number as is the serum M-protein level because the urinary excretion of BJ protein represents the excess production that escapes renal catabolism. In patients who respond to MIP-NV therapy, the average reduction of urinary BJ protein is higher, and the time for achieving a 50% reduction in BJ protein is shorter, than the corresponding measures of serum M-protein. However, it is evident that the measurement of BJ protein excretion provides a reliable index of tumor mass change (10). Hobbs (10) reported that in 2% of all patients, tumor burden increased while M-protein decreased. In the present study, only one out of 48 patients, whose survival time was 37 months, showed a rapid decrease in the serum IgG(λ) M-protein level with a gradual increase in urinary BJ protein excretion during the progression of the disease for seven months before death. Except for that case, serial measurement of the M-protein level was thought to reliably demonstrate the progress or regress of the disease.

The M-protein reduction rate could be predicted from the M-protein kinetics pattern in the first cycle of chemotherapy. Especially in patients with the decreasing pat-

Table 3 Prototype of M-protein kinetics and survival time of previously untreated patients^a

Prototype	Number of patients	T ^{b,c}	Maximum reduction of M-protein (%)	Rate of increase in M-protein after the lowest M-protein level	Median survival time (months)
P_1	8	17 \pm 10	78 \pm 15	0.2 \pm 0.2	52
P_2	11	9 \pm 6	84 \pm 14	1.7 \pm 1.5	27
P_3	7	10 \pm 7	38 \pm 17	1.4 \pm 0.6	27
P_4	2	4	22 \pm 5	0.04 \pm 0.22	47

a: Serum M-protein kinetics of patients with multiple myeloma after chemotherapy was classified into four prototypes. For details of these prototypes and chemotherapy, see the text.

b: Time until the lowest M-protein level after the start of chemotherapy (months).

c: Values are expressed as mean \pm standard error of mean.

tern, the proportion of cases with an M-protein reduction of more than 50% was high (20/21 cases, Table 3). Thus, the M-protein kinetics pattern in the first cycle of chemotherapy was found to be a good indicator of the effect of chemotherapy on the M-protein level.

The myeloma cell nucleus was more immature in patients with the decreasing pattern in the first cycle than in patients with the stationary pattern ($p < 0.001$), and the nucleus was more immature in patients in stage III than in patients in stage $I_A + II_A$ ($p < 0.001$). These results offer an explanation of the intimate relationships between the decreasing pattern and stage III, and between the stationary pattern and stage $I_A + II_A$. These results also help to explain the long survival time of patients with the stationary pattern.

The pretreatment LI% correlated to the M-protein kinetics patterns in the first cycle, but it was not statistically different depending on clinical stage and survival time. From these results, the pretreatment LI% was thought to indicate the mode of response of the tumor in the early term after the start of chemotherapy. Hofmann *et al.* (5) reported a relationship of the grain count of the myeloma nuclei with the response to therapy. However, there was no clear relationship among the grain count, M-protein kinetics pattern in the first cycle, M-protein reduction and survival time.

Alexanian *et al.* (12) reported that patients who responded rapidly (*i.e.*, within two months) had a shorter median survival time than patients who responded later. Durie *et al.* (13) reported that the median survival time of patients reaching maximum regression ($> 50\%$ tumor mass reduction) within six months was shorter than that of patients requiring a longer time for maximal reduction. In the present study, among patients who had an M-protein reduction of

more than 50%, there was no difference in the median survival time between those who responded rapidly (within 10 weeks after the start of chemotherapy) and those who did not (1). However, in patients who achieved an M-protein reduction of more than 60% (prototypes P_1 and P_2), the median survival time of patients reaching maximum M-protein reduction within six months was significantly shorter than that of patients requiring longer than six months ($p < 0.005$). Moreover, the median survival time of patients with the less sharp increase in the M-protein level was longer than that of patients with the sharper increase. Thus, it was found that reaching the maximum M-protein reduction after more than six months and a slower increase in the M-protein level after the maximum M-protein reduction affected the survival time more strongly than achieving an M-protein reduction of more than 60%.

Induction chemotherapy should be continued for at least six months if the disease does not relapse, and intensive maintenance chemotherapy is considered to be necessary to decrease the rate of increase in the M-protein level.

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