Usefulness of serum 5-S-cysteinyl-dopa as a biomarker for predicting prognosis and detecting relapse in patients with advanced-stage malignant melanoma

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ABSTRACT

With the recent development of novel molecular-targeted drugs for advanced-stage malignant melanoma, including RAF and MEK inhibitors and immune checkpoint blockers, the early detection of relapse is important for managing patients with malignant melanoma. In this study, we retrospectively analyzed two conventional serum biomarkers, 5-S-cysteinyl-dopa and lactate dehydrogenase, in patients with malignant melanoma (n = 140) who were treated at a single Japanese institute from June 2007 to June 2015. At initial hospital visit, serum 5-S-cysteinyl-dopa levels were significantly increased in patients with stages III (n = 38) and IV (n = 20) MM compared with patients with stages 0–II (n = 62) MM. In addition, in patients with stages III and IV MM, serum 5-S-cysteinyl-dopa levels of >15.0 nmol/L at initial hospital visit correlated with a poor prognosis. In 11 of 14 patients whose disease progressed during follow-up (mostly from stages III to IV), serum 5-S-cysteinyl-dopa levels exceeded the normal limit of 10.0 nmol/L during the clinical detection of distant metastases. These results indicate the usefulness of measuring serum 5-S-cysteinyl-dopa levels at initial hospital visit and during follow-up for early and effective therapeutic interventions using newly developed molecular-targeted drugs.

INTRODUCTION

Malignant melanoma (MM) is one of the most aggressive cancers and has a large variance in mortality rate between early and metastatic disease stages.[1] In recent years, the treatment of advanced-stage MM has evolved to include the use of RAF and MEK inhibitors and immune checkpoint blockers.[2, 3] Therefore, the early detection of metastatic MM after the surgical resection of primary MM is an important requirement.

Although various tumor biomarkers for MM have been developed, [4-6] 5-S-cysteinyl-dopa (5-S-CD) and lactate dehydrogenase (LDH) are the only serum biomarkers that are routinely measured in Japan.

Here, we retrospectively analyzed serum 5-S-CD and LDH levels in patients with MM who were treated at a single Japanese institute and compared the usefulness of measuring these two biomarkers. Furthermore, we aimed to evaluate the diagnostic value of 5-S-CD for predicting prognosis and detecting relapse.

METHODS

Patients

We measured serum 5-S-CD and LDH levels in 140 patients with MM on 710 occasions from June 2007 to June 2015 at the Department of Dermatology, Okayama University

Hospital. None had amelanotic melanoma. Serum 5-S-CD levels were measured in 120 patients at their initial hospital visit; of these patients, serum LDH levels were measured in 119. Clinical staging was performed according to the 2009 American Joint Committee on Cancer Melanoma Staging and Classification System. [1] The characteristics of the 120 patients were as follows: mean age, 64.1 years; 49 males and 71 females; and median follow-up duration, 24.2 months (range, 1–114 months). Twenty-nine (24.2%) patients died during follow-up, of which 20 died because of melanoma.

Measurement of serum 5-S-CD levels

Serum 5-S-CD levels were measured using high-performance liquid chromatography in SRL Inc. (Tokyo, Japan) according to a minor modification of a previously published method. [7] The reference range described in the supplier's report was 1.5–8.0 nmol/L. However, as previously described, 10.0 nmol/L was considered to be the cut-off value for the serum 5-S-CD level. [8, 9]

Measurement of serum LDH levels

Serum LDH levels were determined using conventional methods in the Department of Laboratory Medicine, Okayama University Hospital. The reference range was 124–222

IU/L.

Statistical analyses

All statistical analyses were performed using the IBM SPSS Statistics version 20.0 (Chicago, IL, USA). Serum 5-S-CD and LDH levels were compared among groups of patients with different stages of MM using the Mann–Whitney *U* test. The statistical significance level was determined at $P < 0.01$. Melanoma-specific survival (MSS) was calculated from the date of the first visit to our hospital to either the date of death because of melanoma or the date of the last follow-up. The Kaplan–Meier method was used to evaluate MSS, and the log-rank test was used to compare survival curves. The statistical significance level was determined at $P < 0.05$.

Ethics

This study was approved by the Ethics Committee of the Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences and Okayama University Hospital (No. 1509-018).

RESULTS

Serum 5-S-CD and LDH levels at initial hospital visit according to the disease stage

In 120 patients, serum 5-S-CD levels were obtained at initial hospital visit. Compared with patients who had early stage MM (stages 0–II; *n* = 62), serum 5-S-CD levels were significantly elevated in patients with stages III (*n* = 38, P = 0.00063) and IV (*n* = 20, P = 0.00073) MM. In a broader comparison between early and advanced-stage MM, serum 5-S-CD levels were found to be significantly elevated in patients with advanced-stage MM (III and IV) (P < 0.0001; Fig. 1a). These results indicated that serum 5-S-CD was a useful marker in advanced-stage MM but not in early stage MM.

Serum LDH levels were obtained at initial hospital visit for 119 of 120 patients (one patient with stage 0 was excluded). Serum LDH levels were not significantly increased in patients with advanced-stage MM (III and IV) compared with those with early stage MM (stages 0–II). However, serum LDH levels were significantly increased only in patients with stage IV MM (P = 0.0033) compared with those with early stage MM (Fig. 1b). These results indicate that serum 5-S-CD levels were superior to serum LDH levels as indicators for discriminating between early and late stage MM.

In addition, we calculated the sensitivity of the two biomarkers for each disease stage. As shown in Table 1, the sensitivity of serum 5-S-CD levels were higher than that of serum LDH levels for advanced-stage MM, whereas the sensitivity of both biomarkers

was similar for early stage MM.

Prognostic discriminative performance of serum 5-S-CD levels

We also evaluated the significance of serum 5-S-CD levels in MM prognosis. Among the patients in whom serum 5-S-CD levels were measured at initial hospital visit, 58 had an advanced-stage MM (stage III or IV). Of these, three patients died because of other diseases; excluding these three patients, MSS was calculated for the remaining 55 patients with an advanced-stage MM. The Kaplan–Meier method was used to determine five different 5-S-CD threshold concentrations with a range of 8.0–30.0 nmol/L (Fig. 2). Among these five analyses, a threshold value of 15.0 nmol/L was the only point at which 5-S-CD could significantly distinguish between patients with good and poor prognoses (Fig. 2c). This finding indicates a significant risk for poor prognosis in patients with advanced-stage MM whose serum 5-S-CD levels were >15.0 nmol/L at initial hospital visit.

Serum 5-S-CD levels during follow-up reflect MM relapse

Serum 5-S-CD levels were measured in 92 of 140 patients during follow-up. Of these 92 patients, 31 had a relapse. In 14 patients, serum 5-S-CD levels were measured during

the period between 2 months before and 2 months after recurrence or detection of metastases. Serum LDH levels were also measured in 12 of these patients. No patients had only LDH levels evaluated. As shown in Table 2, 11 of 14 patients had high serum 5-S-CD levels (>10.0 nmol/L). In patients 1 and 2, serum 5-S-CD levels did not increase around the relapse time. Only three of 12 patients had higher than normal serum LDH levels, whereas only one patient (No. 1) had elevated serum LDH levels with serum 5-S-CD levels not >10.0 nmol/L; seven patients (Nos. 4, 6, 7, 8, 9, 13, and 14) had serum 5-S-CD levels of >10.0 nmol/L with normal serum LDH levels. When these two biomarkers were combined, MM relapse could be detected in 12 of 14 (86%) patients.

DISCUSSION

Several serum biomarkers have been developed for MM. Among them, the melanoma-inhibitory activity protein and S-100 proteins, including S-100B protein, are popular protein biomarkers. Melanoma tumor cells secrete these proteins, and their serum levels reflect the disease stage, prognosis, disease-free survival, and overall survival. [5, 6] A characteristic of MM is melanin biosynthesis. Melanin-associated metabolites found in the urine or serum, such as 5-S-CD and 6-hydroxy-5-methoxyindole-2-carboxylic acid, have also been considered useful biomarkers for MM. [8] Of these metabolites, 5-S-CD is

the best marker that reflects the progression of MM. [10] Our study results reveal that serum 5-S-CD levels are sensitive biomarkers for advanced-stage MM, predict poor prognosis if >15.0 nmol/L at initial hospital visit, and are good predictors of relapse during follow-up.

Previous studies have demonstrated the significance of the serum 5-S-CD level as a tumor marker, particularly in patients with advanced-stage MM. [9, 11–13] In a multicenter study conducted in Japan for 10 years, Wakamatsu et al. [9] analyzed data from 218 patients on 2648 occasions and demonstrated that the serum 5-S-CD level is a sensitive and specific marker for stage IV MM. Bánfalvi et al. [12] conducted a similar single-center study in Hungary and demonstrated a significant difference in serum 5-S-CD levels between stages I and III MM and between stages II and III MM. Furthermore, Bánfalvi et al. [13] confirmed that serum 5-S-CD and S-100B protein are most sensitive in patients with stage IV MM. Our study also demonstrated a significant difference in serum 5-S-CD levels between early and advanced-stage MM, which is consistent with the results of previous studies. Defining an appropriate cut-off value is of great importance for the clinical application of tumor biomarkers. Considering seasonal variations in serum 5-S-CD levels, Wakamatsu et al. [14] decided on a cut-off value of 10.0 nmol/L for serum 5-S-CD levels. In a related study, serum 5-S-CD levels for 33 healthy control subjects were 4.3 ± 1.8

(mean ± SD) nmol/L. [8] When this cut-off value was used, serum 5-S-CD levels in all 33 healthy control subjects were <10.0 nmol/L. [9,10] In our study, more than half of the patients with advanced-stage MM had abnormal serum 5-S-CD levels with a sensitivity much higher than that of serum LDH levels when a cut-off value of 10.0 nmol/L was used.

Certain threshold values of tumor biomarkers may help in identifying patients at a risk. For example, serum prostate-specific antigen (PSA) levels are well-known and effective biomarkers for prostate cancer, having a cut-off value (defined from the healthy population) of approximately 4.0 ng/mL. However, according to the National Comprehensive Cancer Network guidelines for prostate cancer, a PSA level of <10.0 ng/mL is defined as a low-risk group, 10.0–20.0 ng/mL as an intermediate-risk group, and those with a level >20.0 ng/mL as a high-risk group. [15] Similarly, our study results demonstrate that serum 5-S-CD levels may be used as risk indicators for MM. Serum 5-S-CD levels of >15.0 nmol/L at initial hospital visit may indicate poor prognosis in patients with stages III and IV MM, although this needs to be confirmed in future large-scale studies.

With the recent development of novel therapies for advanced-stage MM, detecting relapse has become particularly important. In one multicenter study in Japan, 42 of 49 (86%) patients with visceral metastases had serum 5-S-CD levels of >10.0 nmol/L; in 16

of those 49 (33%) patients, the elevation of serum 5-S-CD levels preceded a clinical detection of visceral metastases. [9] In our study, serum 5-S-CD levels exceeded the normal limit of 10.0 nmol/L in 11 of 14 (79%) patients around the time of clinical detection of relapse, whereas abnormal serum LDH levels were observed in only 25% of patients. These results indicate that serum 5-S-CD levels are likely to be sensitive biomarkers for detecting disease progression.

Serum 5-S-CD levels are spuriously increased by solar radiation, ingestion of *Agaricus* extract, and chronic renal failure. [14, 16, 17] None of our patients had serious renal failure or had ingested *Agaricus* extract. It is difficult to precisely estimate the effects of solar radiation or seasonal variation on serum 5-S-CD levels in patients with MM, particularly in those with advanced-stage MM. However, as mentioned in an earlier study, a cut-off value of 10.0 nmol/L for serum 5-S-CD levels was defined to adjust for seasonal variations. [14]

Diem et al. [18] recently reported that serum LDH levels could be useful biomarkers for early response to treatment or disease progression in patients with MM undergoing anti–programmed cell death 1 therapy. They also recently demonstrated that an increasing baseline serum LDH level is independently associated with poor survival in patients treated with ipilimumab. [19] Two other studies have confirmed the correlation of high

baseline serum LDH levels with poor prognosis in patients undergoing ipilimumab treatment. [20, 21] Regarding our results, which indicate a higher sensitivity of serum 5-S-CD levels than that of serum LDH levels in advanced-stage MM, serum 5-S-CD levels may be superior to serum LDH levels in predicting treatment response and disease progression in patients receiving immune checkpoint inhibitors. Further studies are necessary to confirm this. Nevertheless, our study suggests the importance of measuring serum 5-S-CD levels at initial hospital visit and during follow-up to facilitate early and effective administration of newly developed molecular-targeted therapies.

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Figure legends

Fig 1. (a) Serum 5-S-cysteinyl-dopa (5-S-CD) levels at initial hospital visit according to the malignant melanoma (MM) stage are shown (n = 120). Compared with early stage MM (0–II), serum 5-S-CD levels were significantly elevated in stages III (P = 0.00063) and IV (P = 0.00073) MM. In the comparison between early and advanced-stage MM (III and IV), serum 5-S-CD levels significantly increased in advanced-stage MM (P < 0.0001). (b) Serum lactate dehydrogenase (LDH) levels at initial hospital visit (n = 119) according to the disease stage are shown. Serum LDH levels are not significantly increased in stage III MM and advanced-stage MM compared with early stage MM (P = 0.16 and 0.014, respectively). Only in the comparison between early stage MM and stage IV MM, serum LDH level was significantly increased (P = 0.0033).

Fig. 2. Significance of serum 5-S-cysteinyl-dopa (5-S-CD) levels at initial hospital visit. For patients with advanced-stage malignant melanoma (MM; stages III and IV; n = 55), the Kaplan–Meier curves are drawn at threshold values of (a) 8.0 nmol/L, (b) 10.0 nmol/L, (c) 15.0 nmol/L, (d) 20.0 nmol/L, and (e) 30.0 nmol/L. Log-rank test showed a significant difference (P = 0.015) only at a threshold value of 15.0 nmol/L.

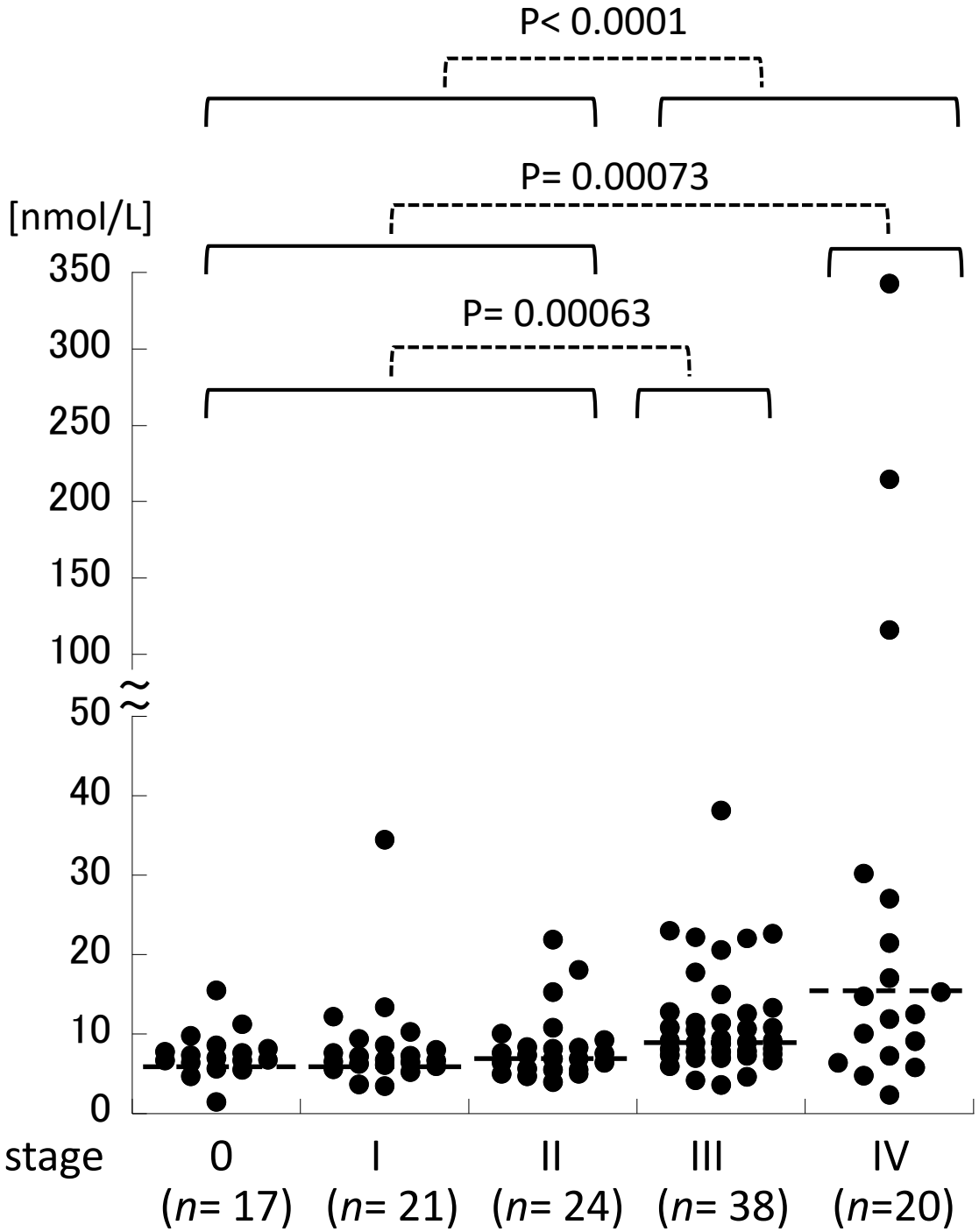
P< 0.0001
P= 0.00073
P= 0.00063
[nmol/L]
350
300
250
200
150
100
50
40
30
20
10
0
stage
0
(n= 17)
I
(n= 21)
II
(n= 24)
III
(n= 38)
IV
(n=20)

Figure 1 (a)

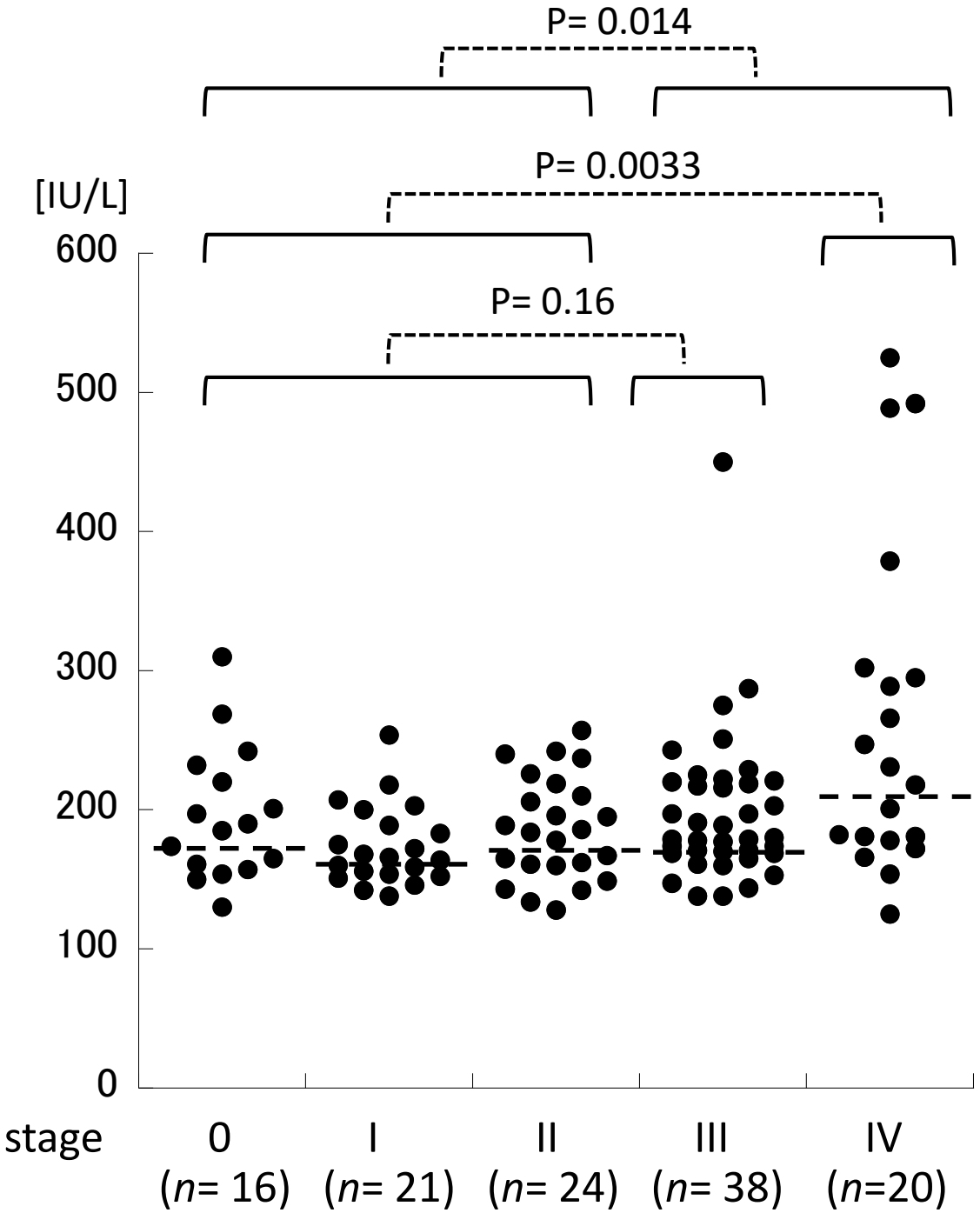
P= 0.014
P= 0.0033
P= 0.16
[IU/L]
600
500
400
300
200
100
0
stage
0
(n= 16)
I
(n= 21)
II
(n= 24)
III
(n= 38)
IV
(n=20)

Figure 1 (b)

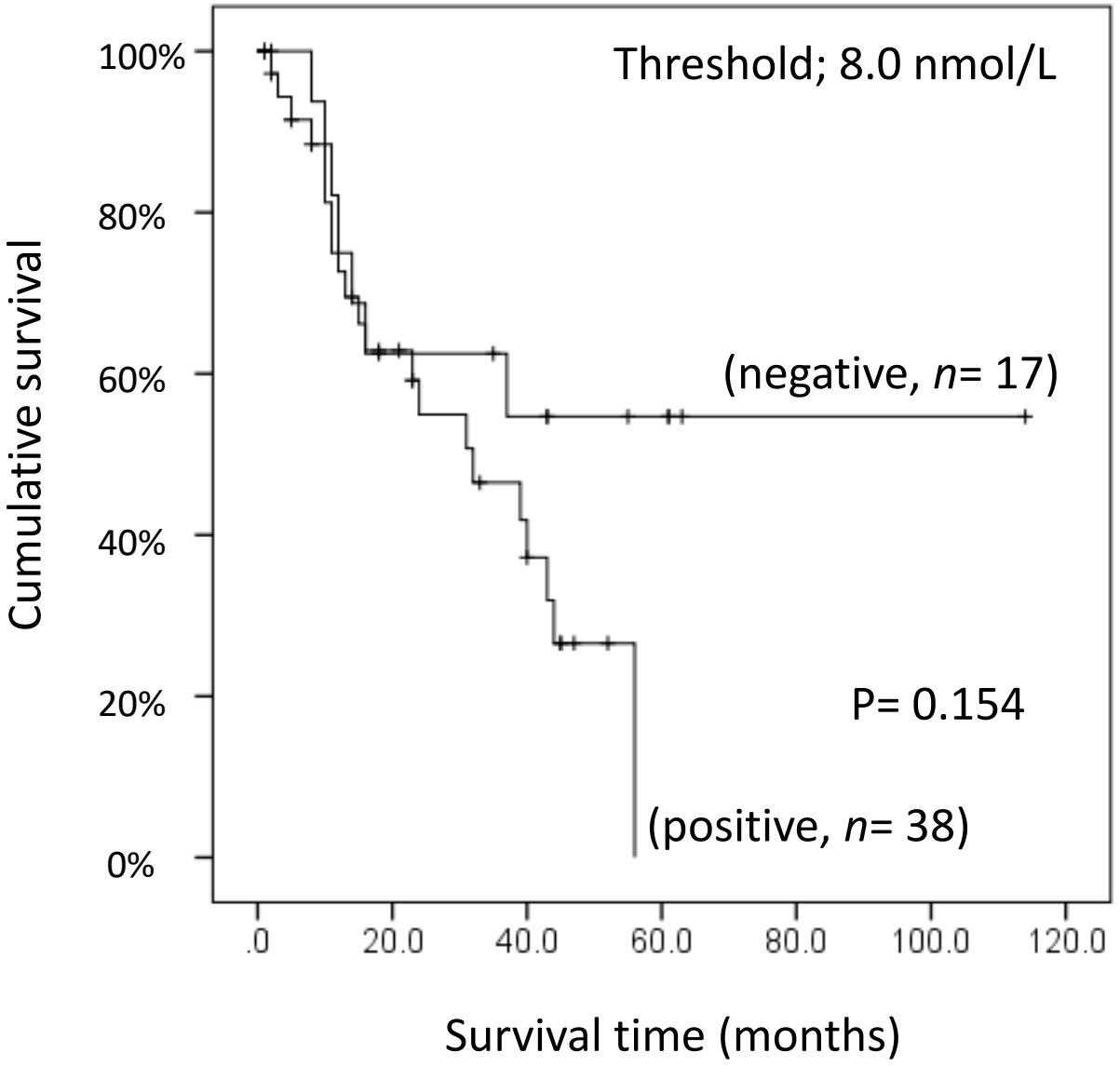
Threshold; 8.0 nmol/L
(negative, n= 17)
P= 0.154
(positive, n= 38)
Cumulative survival
100%
80%
60%
40%
20%
0%
.0
20.0
40.0
60.0
80.0
100.0
120.0
Survival time (months)

Figure 2 (a)

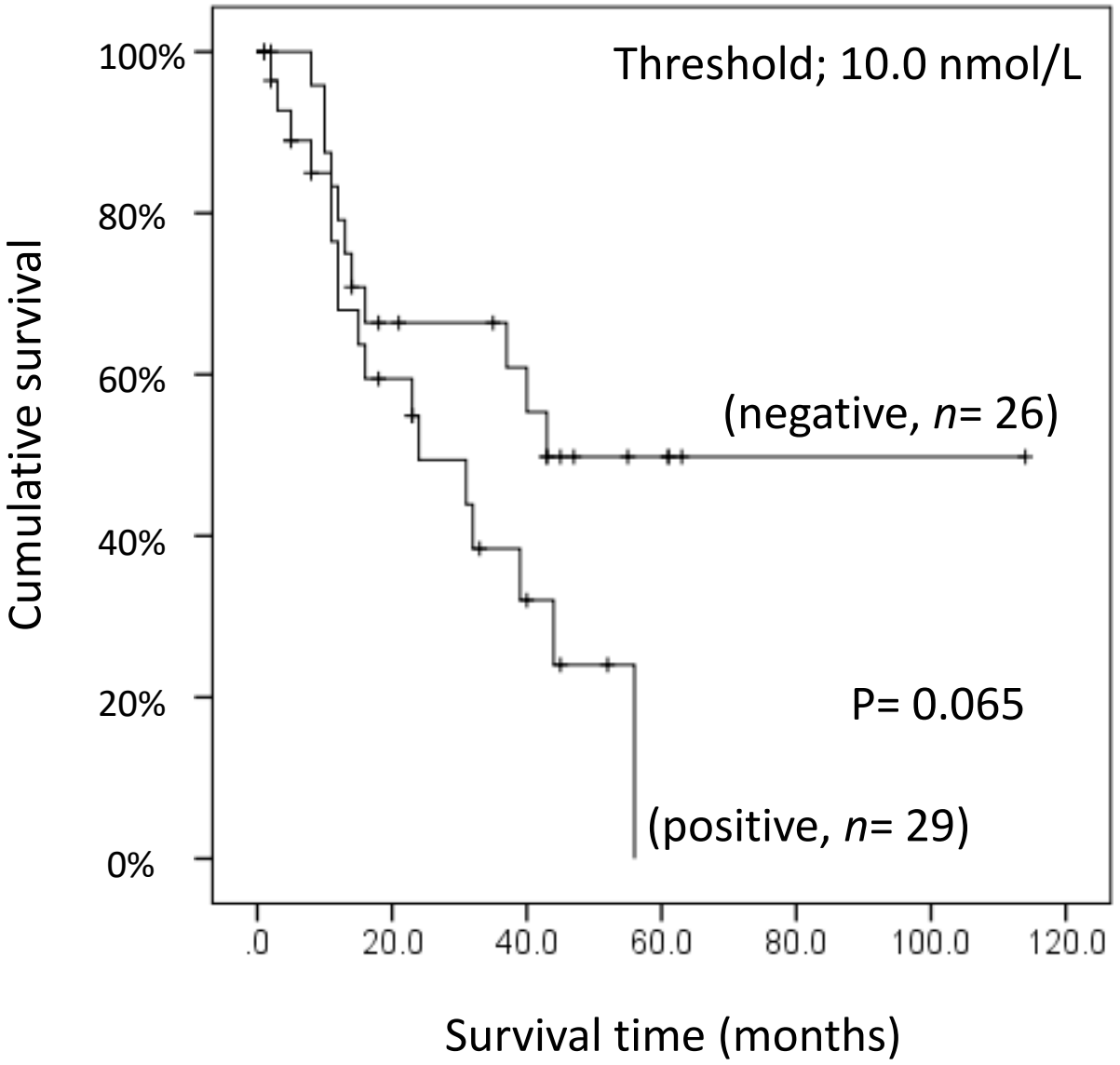
Threshold; 10.0 nmol/L
(negative, n= 26)
P= 0.065
(positive, n= 29)
Cumulative survival
100%
80%
60%
40%
20%
0%
.0
20.0
40.0
60.0
80.0
100.0
120.0
Survival time (months)

Figure 2 (b)

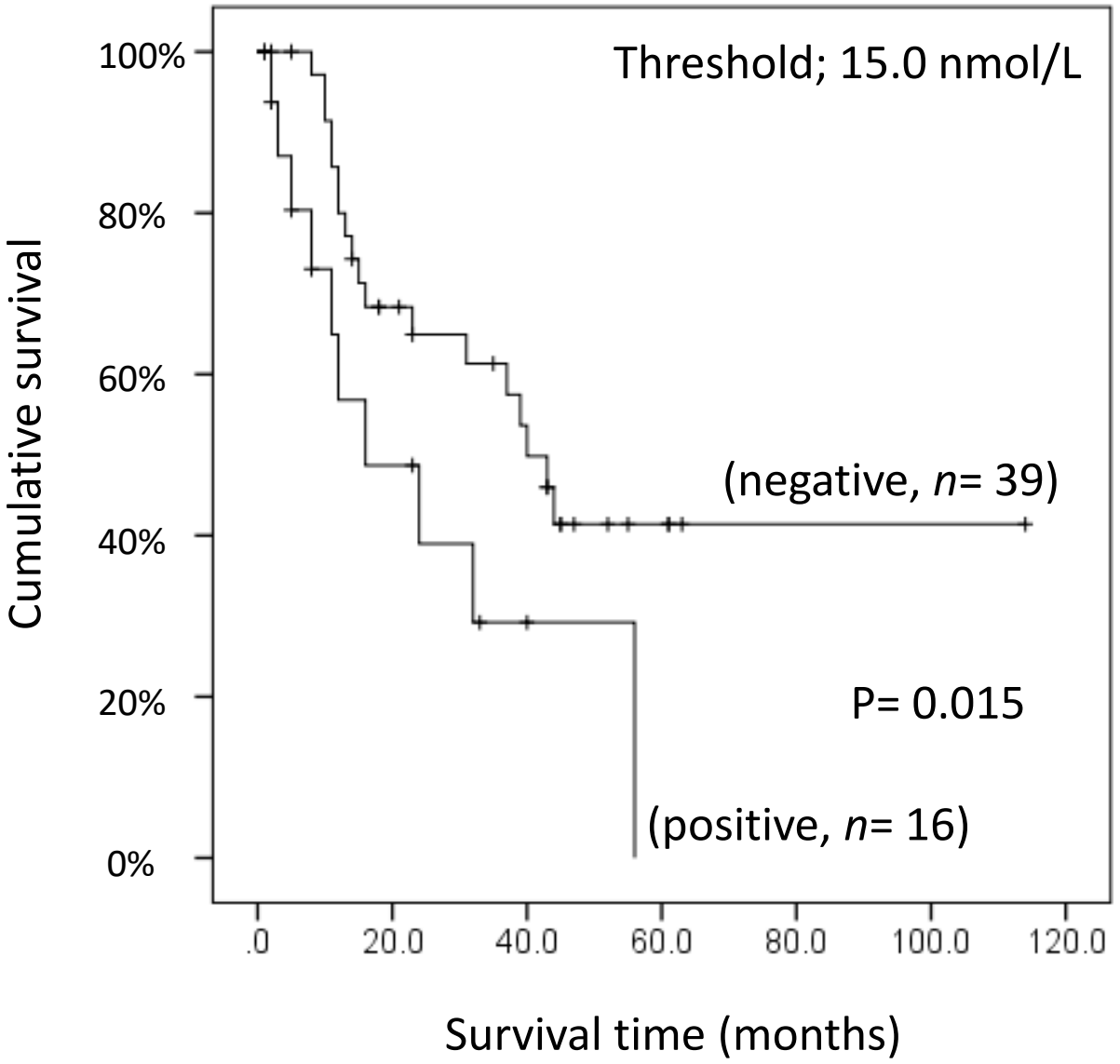
Threshold; 15.0 nmol/L
(negative, n= 39)
P= 0.015
(positive, n= 16)
Cumulative survival
100%
80%
60%
40%
20%
0%
.0
20.0
40.0
60.0
80.0
100.0
120.0
Survival time (months)

Figure 2 (c)

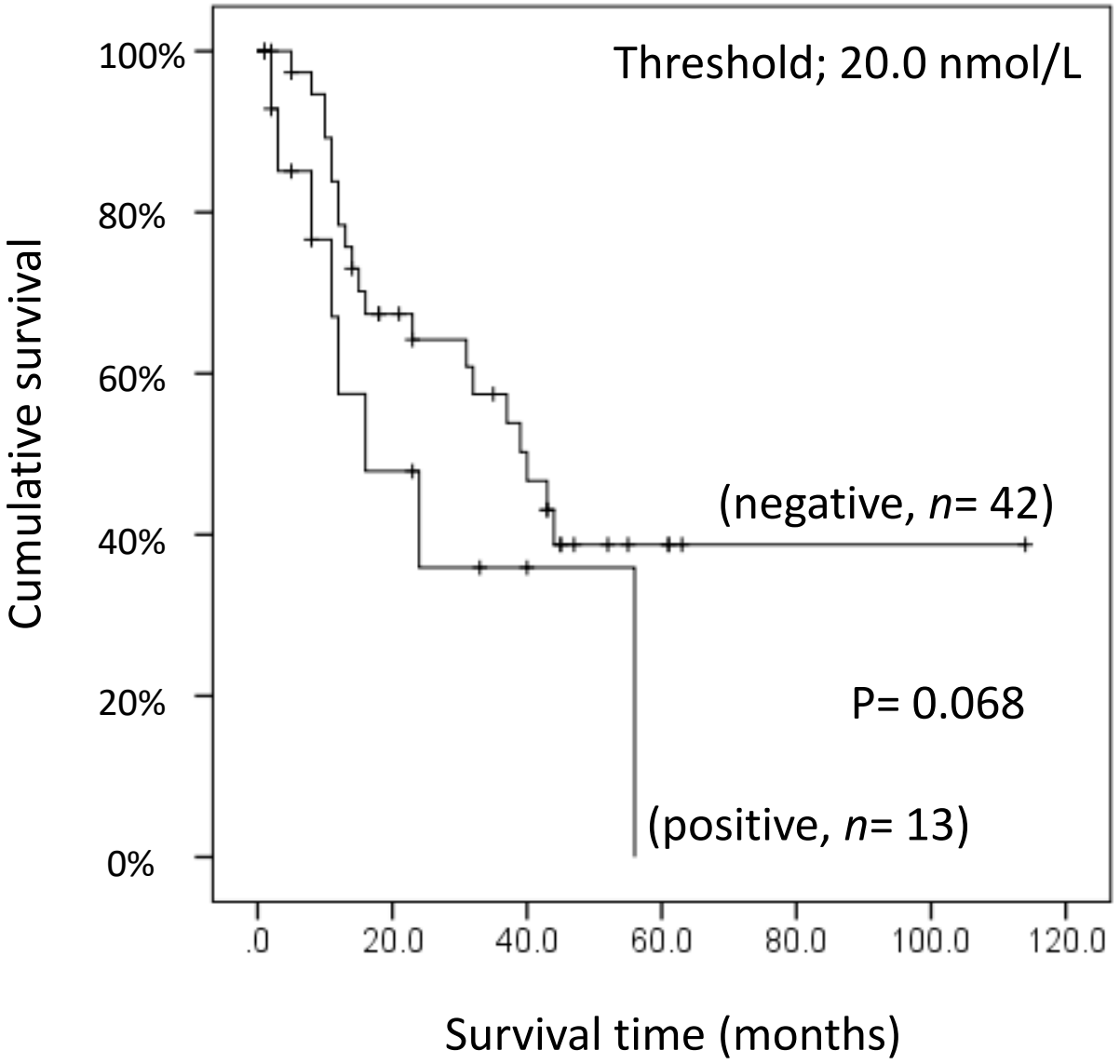
Threshold; 20.0 nmol/L
(negative, n= 42)
P= 0.068
(positive, n= 13)
Cumulative survival
100%
80%
60%
40%
20%
0%
.0
20.0
40.0
60.0
80.0
100.0
120.0
Survival time (months)

Figure 2 (d)

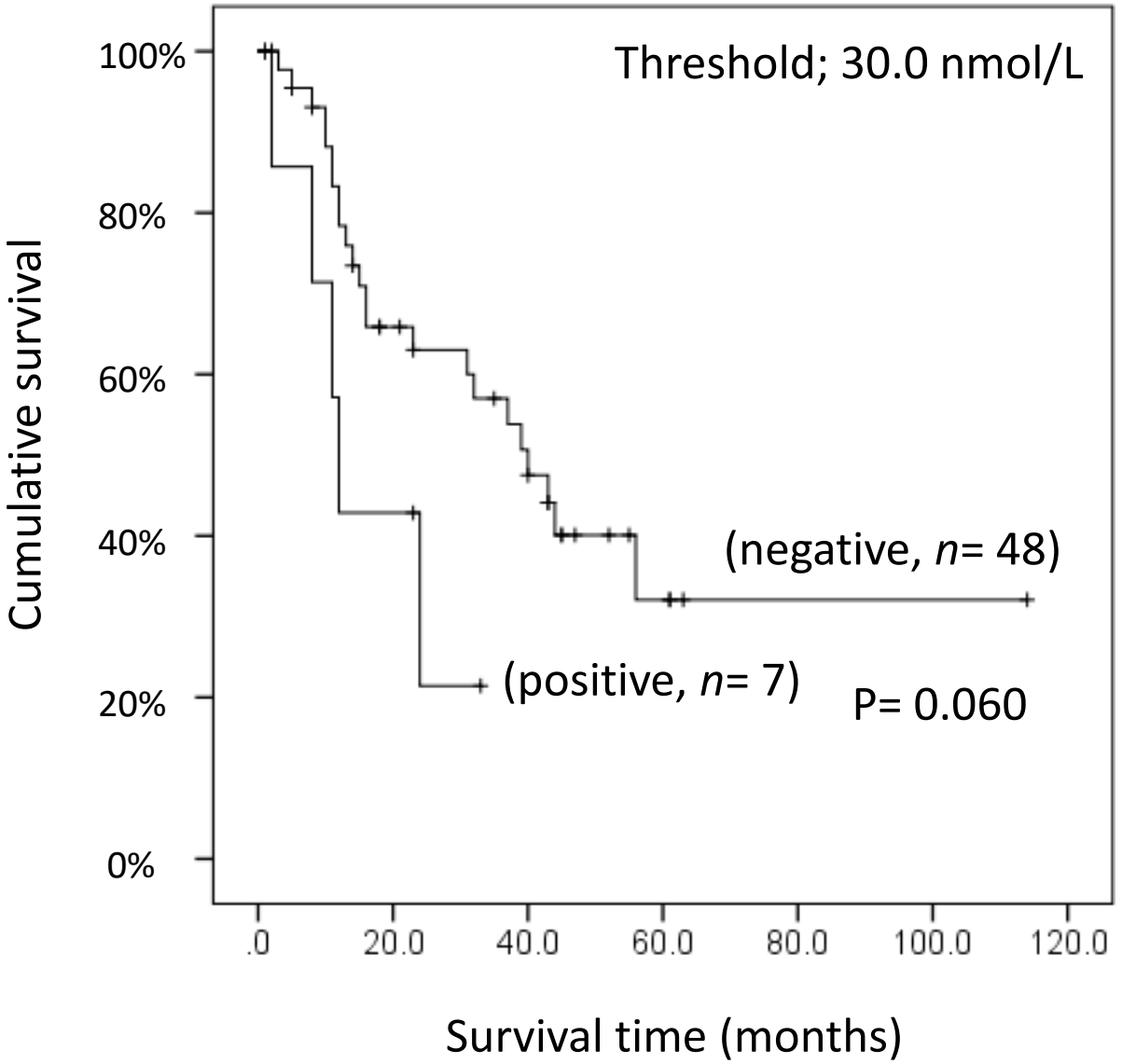
Threshold; 30.0 nmol/L
(negative, n= 48)
(positive, n= 7)
P= 0.060
Cumulative survival
100%
80%
60%
40%
20%
0%
.0
20.0
40.0
60.0
80.0
100.0
120.0
Survival time (months)

Figure 2 (e)

Sensitivity of 5-S-CD and LDH for each stage

	stage 0	stage I	stage II	early disease (stage 0+ I+ II)	stage III	stage IV	advanced disease (stage III+ IV)
5-S-CD	11.8%	19.0%	20.1%	18.0%	42.1%	70.0%	51.7%
LDH	25.0%	4.8%	20.1%	16.4%	21.1%	50.0%	31.0%

Table 1

Marker levels and profile of 14 MM patients with relapse

Case No.	Age	Sex	Stages before and after relapse	5-S-CD [nmol/L] (Cut-off; 10.0 nmol/L)	LDH [IU/L] (Cut-off; 222 IU/L)
1	63	F	IIIA→IV	4.4	296
2	64	M	IIIC→IV	8.1	203
3	69	M	IIIA→IV	121.9	600
4	58	F	IIIC→IV	15.2	131
5	68	M	IIIA→IV	44.6	160
6	78	M	IIIC→IV	24.2	N/A
7	66	F	IIIB→IV	10.1	187
8	70	M	IIIC→IV	36.9	193
9	87	F	IIIB→IV	23.3	194
10	71	F	IIIB→IV	40.7	N/A
11	64	F	IIB→IV	41.2	237
12	79	F	IIIB→IV	7.9	195
13	61	F	IB→IIIB	33.8	168
14	59	F	IIIC→IV	15.3	181

*N/A means “not assayed”.
*Underlined values are exceeding cut-off.

Table 2