Serum Midkine as a Prognostic Biomarker for Patients With Hepatocellular Carcinoma

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Key Words: Midkine; Hepatocellular carcinoma; Prognostic marker; Enzyme-linked immunosorbent assay

Abstract

Gene expression profiles of paired hepatocellular carcinoma (HCC) and adjacent noncancerous liver tissue samples revealed preferential expression of midkine in HCC. This finding suggested the clinical usefulness of midkine measurement in serum for monitoring HCC treatment response, recurrence, and progression. A prospective study in 285 patients, 144 in complete remission and 141 at risk for developing de novo HCC, was conducted. The changes in serum midkine level were in parallel with disease activity in about 81% of patients with HCC. The study also revealed that rapidly rising serum midkine levels occurred in patients in the terminal stage of HCC. The rising rate of serum midkine levels was inversely correlated with remaining survival days. However, serum midkine measurement did not detect emergence of new HCC in most patients in complete remission and in high-risk people without a history of HCC. Serum midkine levels can be useful to monitor HCC progression, and a sharp rise signals the approach of end of life in patients with HCC.

Hepatocellular carcinoma (HCC) is the most common type of cancer in the world and has been the leading cause of cancer death in many countries.1 Although α-fetoprotein (AFP) is a widely used biomarker for the diagnosis and follow-up of HCC, the AFP level is not increased in 40% of patients with HCC.2 The sensitivity and specificity of AFP for the diagnosis of HCC are 60% and 90%, respectively.2-6 Identification of additional novel biomarkers to improve the diagnosis of and prognosis in HCC is needed. Midkine, a member of a highly conserved and developmentally regulated gene family, has a critical role in cell growth, survival, migration, angiogenesis, and carcinogenesis.7 The expression of midkine is up-regulated in neuroblastoma, gastrointestinal cancers, bladder cancer, breast cancer, and HCC.8-11 A higher midkine level in peripheral blood was associated with a poor outcome in patients with neuroblastoma, oral squamous cell carcinoma, gastric cancer, and endometrial carcinoma.12-15

Although the levels of midkine were reported to be elevated in peripheral blood samples from patients with HCC and the level was low or undetectable in chronic hepatic disease, its value in prognostication of survival, early detection of HCC, and monitoring of disease activity remains unclear.16 Through our study of gene expression profiles of paired HCC and adjacent noncancerous liver tissue samples in 18 patients, we found that midkine was differentially expressed in HCC and could serve as a useful biomarker for the diagnosis of and prognosis in HCC (unpublished study, 2005). Therefore, we conducted a prospective study to assess the clinical usefulness of serum midkine levels in patients with HCC or patients at high risk...
for the development of HCC. The results of our study are reported herein.

**Materials and Methods**

**Patients and Samples**

Patients who had a tissue diagnosis of HCC and active or inactive disease were eligible. Patients with concurrent second primary cancers or advanced liver cirrhosis (Child-Pugh C) were excluded. During 2006 and 2007 we recruited 165 eligible patients who had active HCC for a longitudinal study to correlate changes of serum midkine levels with HCC disease activity. Blood samples were obtained when patients returned to the outpatient clinic for regular follow-up or were admitted for treatment. The treatment included transcatheter hepatic artery chemoembolization, radiofrequency ablation, or percutaneous ethanol injection therapy. If patients had exposure to heparin within 48 hours of blood sample collection, blood samples were not collected for the study. Other clinical characteristics are summarized in Table 1.

In this study, we also enrolled 285 patients at risk for the development of HCC. Of the 285 patients, 144 were in complete remission after curative treatment and 141 patients did not have a prior diagnosis of HCC but had positive risk factors for the development of HCC, including carrying the hepatitis B surface antigen, positive anti–hepatitis C virus antibody, and/or liver cirrhosis. They were regularly followed up for the possible development of HCC. Serum samples from the patients were obtained during follow-up. All pertinent clinical characteristics of this cohort are summarized in Table 1. The clinical characteristics of patients who donated 18 pairs of HCC tissues and adjacent noncancerous liver tissue for a gene expression profiling study and of patients who donated 1-time serum samples for research are also included in Table 1.

The procedures of our study were conducted in accord with ethical standards established by the institution in which the experiments were performed in accord with the Helsinki Declaration of 1975. Written informed consents were obtained from all participating patients.

**Serum Midkine Measurement by ELISA**

Serum midkine levels were measured by enzyme-linked immunosorbent assay (ELISA) using antibodies and recombinant human midkine purchased from R&D Systems, Minneapolis, MN. For the assay, each well of a 96-well ELISA plate was coated overnight with 50 μL of 5 μg/mL goat antihuman midkine antibody in phosphate buffered saline (PBS). After 3 washings with PBS containing 0.05% polysorbate 20 and blocking with 50 μL of dilution buffer (PBS containing 2% bovine serum albumin and 0.05% polysorbate 20) at room temperature, 50 μL of patient serum samples diluted 2× was added into each well in duplicate. Recombinant human midkine was used as standard. Our initial study showed that concentrations of midkine standard ranging from 0.05 to 50 ng/mL were suitable to construct a standard curve for the assay. After 90 minutes of incubation, the plates were washed 3 times. Thereafter, each well was incubated

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**Table 1**

**Clinical Characteristics of Cohorts Included in the Study**

<table>
<thead>
<tr>
<th>Cohort/Study</th>
<th>Paired HCC and Adjacent Noncancerous Liver Tissue (n = 18)/Differential Gene Expression</th>
<th>Archival Serum Samples From Patients With HCC (n = 85)/Correlation Between Serum Midkine and AFP Levels</th>
<th>Patients With HCC Undergoing Treatment and Follow-up (n = 165)/Longitudinal Correlation Between Serum Midkine and HCC Activity</th>
<th>High-Risk Patients for HCC (n = 285)/Longitudinal Study for Recurrence or De Novo Occurrence of HCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>15 (83)</td>
<td>65 (76)</td>
<td>122 (73.9)</td>
<td>182 (63.9)</td>
</tr>
<tr>
<td>F</td>
<td>3 (17)</td>
<td>20 (24)</td>
<td>43 (26.1)</td>
<td>103 (36.4)</td>
</tr>
<tr>
<td>Median (range) age (y)</td>
<td>53 (29-73)</td>
<td>—</td>
<td>64 (30-86)</td>
<td>56 (23-88)</td>
</tr>
<tr>
<td>Median (range) follow-up (d)</td>
<td>—</td>
<td>—</td>
<td>363 (35-878)</td>
<td>467 (91-781)</td>
</tr>
<tr>
<td>HBsAg+</td>
<td>12 (67)</td>
<td>57 (67)</td>
<td>92 (55.8)</td>
<td>184 (64.6)</td>
</tr>
<tr>
<td>Anti-HCV antibody+</td>
<td>4 (22)</td>
<td>15 (18)</td>
<td>53 (32.1)</td>
<td>63 (22.1)</td>
</tr>
<tr>
<td>HBsAg+ and anti-HCV+</td>
<td>1 (6)</td>
<td>1 (1)</td>
<td>7 (4.2)</td>
<td>8 (2.8)</td>
</tr>
<tr>
<td>Serologic status unknown</td>
<td>0 (0)</td>
<td>2 (2)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>—</td>
<td>—</td>
<td>104 (63.0)</td>
<td>135 (47.4)</td>
</tr>
<tr>
<td>Complete remission after treatment of HCC+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>144 (50.5)</td>
</tr>
</tbody>
</table>

AFP, α-fetoprotein; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

* Data are given as number (percentage) unless otherwise indicated.

† Treatment included surgery (n = 100), transcatheter hepatic artery chemoembolization (n = 40), radiofrequency ablation (n = 2), and percutaneous ethanol injection therapy (n = 2).

‡ The other 141 patients did not have HCC and were regularly followed up for the development of de novo HCC.
with 50 μL of biotinylated goat antihuman midkine antibody (0.4 μg/mL) for 60 minutes. After 3 washes, each well was incubated with 50 μL of streptavidin–horseradish peroxidase conjugate (0.5 μg/mL; Pierce Biotechnology, Rockford, IL) for 30 minutes at room temperature. Finally, each well was developed with 100 μL of O-phenylenediamine dihydrochloride chromogenic substrate (Sigma, St Louis, MO), and optical density at 450 nm was measured using a SPECTRAmax PLUS plate reader (Molecular Devices, Sunnyvale, CA).

To validate the assay, a pooled normal serum sample from 10 healthy adults was assayed at 3 dilutions (2×, 4×, and 8×). In addition, this pooled serum sample spiked with recombinant midkine to a concentration of 50 ng/mL was similarly assayed at 3 different dilutions. A parallel relationship between the diluted samples and the standard curve was noted. The expected concentration of midkine was obtained for the spiked pooled normal serum sample. The interassay variation was also evaluated using 2 serum samples that contained different levels of midkine. These 2 samples were assayed on 5 dates during a period of 3 months. The average concentrations of midkine were 0.9 and 18.2 ng/mL for the 2 samples, and their coefficients of variation were 20% and 8%, respectively. The lower detection limit of the assay judged from the lowest measurable point of the standard curve was 0.05 ng/mL.

### Midkine for Monitoring HCC Occurrence, Recurrence, and Progression

Diagnosis of HCC was based on the criteria proposed by the Barcelona-2000 EASL conference. Occurrence was defined as the development of de novo HCC diagnosed by biopsy in patients at risk for the development of HCC during regular follow-up. Recurrence was defined as development of new HCC in patients in complete remission after curative therapy for HCC. Change in HCC disease activity was evaluated by multiphasic spiral computed tomography, dynamic contrast-enhanced magnetic resonance imaging, sonography, serum AFP measurement, and/or clinical parameters. The therapeutic effect of transcatheter hepatic artery chemoembolization was evaluated by an experienced radiologist (Z.H.Y.L.) according to the pattern of lipoidal retention, tumor necrosis, and contrast enhancement of residual viable tumor. Complete regression was defined as complete absence of all known preexisting HCC without new lesions. Partial response was defined as more than 50% reduction of total tumor load by comparing 2 imaging studies before and after treatment with an interval of no less than 4 weeks after treatment. Progression of HCC was determined according to radiographic findings, changes in the serum AFP level, and deterioration of the clinical condition as defined in **Table 21**. Patients with disease status that did not meet the definition of progression, partial response, or complete regression were regarded as having stable disease.

### Effect of Intravenous Administration of Heparin on Midkine Levels in Peripheral Blood Samples

To study the effect of heparin on midkine levels in peripheral blood samples, we first conducted a study on how intravenous bolus administration of 2,500 U of heparin (LEO Pharma, Princes Risborough, England) affected the blood midkine level. Blood samples were collected before and at different time points after heparin administration (15, 30, 60, 90, and 120 minutes). Next, we studied the effect of increasing doses of heparin (1,000, 1,750, 2,500, and 3,500 U) on midkine levels in peripheral blood samples. This study was performed in a healthy male adult volunteer (body surface area, 1.94 m²). This volunteer was given a designated dose of heparin once a week. Blood samples were collected immediately before and 30 minutes after intravenous heparin injection.

### Gene Expression Profiling and Reverse Transcriptase–Polymerase Chain Reaction of Midkine for Paired HCC and Adjacent Noncancerous Liver Tissues

Gene expression of midkine in 18 pairs of HCC and adjacent noncancerous liver tissue samples was obtained from the microarray database at the Koo Foundation Sun Yat-Sen Cancer Center, Taipei, Taiwan. The gene expression profiles of these tissue samples were determined by isolation of total RNA from approximately 50 mg of fresh frozen tissues using Trizol reagent (Invitrogen, Carlsbad, CA) and purified with the RNeasy Mini Kit (Qiagen, Valencia, CA). Hybridization targets were prepared from 8 μg of total RNA according to the Affymetrix protocol (Affymetrix, Santa Clara, CA) and hybridized to Affymetrix U133A GeneChips. Immediately following hybridization, the hybridized microarray underwent automated washing and staining in an Affymetrix GeneChip Fluidics Station 400 using the protocol EukGE-WS2v4. Thereafter, microarrays were scanned with an HP GeneArray scanner (Hewlett-Packard, Palo Alto, CA). The expression intensity of each gene was scaled to a trimmed mean of 500, logarithmically transformed to base 2, and normalized using quantile normalization.

### Table 21

**Criteria Used to Define Disease Progression in Patients With HCC**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiographic findings</td>
<td></td>
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<tr>
<td>New intrahepatic HCC</td>
<td>Size ≥2 cm</td>
</tr>
<tr>
<td>Preexisting, apparently viable HCC</td>
<td>≥30% increase in diameter</td>
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<tr>
<td>Vascular invasion</td>
<td>New portal or hepatic vein invasion</td>
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<tr>
<td>Extrahepatic lesion</td>
<td>New biopsy-proven HCC metastasis</td>
</tr>
<tr>
<td>AFP level</td>
<td>Progressive increase of serum AFP level</td>
</tr>
<tr>
<td>Clinical condition</td>
<td>Hepatic decompensation and/or death</td>
</tr>
</tbody>
</table>

AFP, α-fetoprotein; HCC, hepatocellular carcinoma.
For confirmation of the results obtained from microarrays, TaqMan quantitative reverse transcriptase–polymerase chain reaction (Applied Biosystems, Carlsbad, CA) was performed on the same RNA samples, with forward primer (GCGGCGTGGGTTTCC), reverse primer (GGCTCCAAACTCCTTCTTCCA), and probe (FAM-CCTGCACCGGATGCG-MGBNFQ). The expression data were normalized by using a housekeeping gene, HPRT1 (catalog No. Hs99999909_m1, Applied Biosystems).

Results

Differential Midkine Gene Expression in HCC

Analysis of the gene expression profiles from 18 pairs of HCC and their adjacent isogenic noncancerous liver tissue samples showed significant differential expression of midkine between HCC and adjacent noncancerous hepatic tissues.

Figure 1A. Midkine gene expression was consistently increased in all paired HCC tissue samples. In contrast, the expression of the AFP gene was increased in only 10 of 18 paired HCC tissues (Figure 1B). The remaining 8 pairs that did not show significant change had low expression of the AFP gene in HCC tissue representing AFP– tumors (Figure 1B). The increased differential expression of midkine in HCC was further confirmed by quantitative reverse transcriptase–polymerase chain reaction (Figure 1C). These findings suggest that establishment of a patient-specific serum midkine baseline for longitudinal follow-up could be a more effective approach for the detection of early HCC occurrence or recurrence.

Serum Midkine and AFP Levels in Patients With HCC

To explore the potential clinical usefulness of serum midkine levels, we measured the levels of serum midkine and AFP in 85 archival serum samples of patients with HCC. The reference range of midkine established from serum samples

Figure 1. Differential expression of midkine (A) and α-fetoprotein (AFP; B) genes between hepatocellular carcinoma tissue (PHCC) and adjacent noncancerous liver tissue (PN) in 18 paired samples. The results were obtained from microarray study of fresh frozen surgical specimens. C. Differential expression of the midkine gene was confirmed by quantitative reverse transcriptase–polymerase chain reaction. mRNA, messenger RNA.
of 53 healthy adults was 0.25 ± 0.24 ng/mL (mean ± 2 SD). When a midkine level of 0.5 ng/mL was used as the upper limit of the reference range, we found that 21 patients with HCC (25%) had elevated midkine and AFP levels; 14 (16%) had elevated midkine levels only; 28 (33%) had elevated AFP levels only; and 22 (26%) did not have elevation of midkine and AFP levels. The results indicate that serum midkine levels may be used in conjunction with AFP levels to increase the sensitivity of HCC detection.

To assess the specificity of serum midkine levels for HCC detection, we measured serum midkine levels in 72 patients with newly diagnosed HCC and 120 patients who had various benign and malignant gastrointestinal diseases. The 72 patients with newly diagnosed HCC were part of the cohort used for the longitudinal follow-up study to correlate serum midkine levels with HCC disease activity (Table 1). Among the 120 patients without HCC, elevated serum midkine levels (>0.5 ng/mL) were found in 45% of patients with gastric cancer (5/11), 50% of patients with pancreatic cancer (3/6), and 20% of patients with cirrhosis (2/10; Figure 3). In contrast, elevated serum midkine levels were found in 60% of patients with newly diagnosed HCC (43/72; Figure 3). These results demonstrated that the midkine level, although not specific, is more likely to be elevated in patients with HCC.

Longitudinal Serum Midkine Measurement for Monitoring HCC Disease Activity

As shown in Figure 1A, there was some overlap between HCC samples and adjacent noncancerous liver tissue samples for midkine expression. Nevertheless, midkine expression was consistently increased in HCC compared with adjacent noncancerous liver tissue samples within the same patient. This finding suggested that longitudinal measurements of serum midkine levels in the same person could be a more effective way of monitoring HCC disease activity. To evaluate this possible clinical application, we studied changes in serum midkine levels over time in 165 patients with HCC and correlated changes in midkine levels with the clinical courses. Among 165 patients with HCC, 122 had disease progression, 41 had stable disease, and 2 had partial response after treatment according to the predefined criteria.

The association between serum midkine levels over time and disease activity could be positive or negative. A positive correlation was defined as parallel correlation between the change in serum midkine level and clinical course.

Figure 2: Scatter plot of α-fetoprotein (AFP) and midkine levels in archived serum samples from 85 patients with hepatocellular carcinoma. Dashed and solid lines indicate the upper limit of reference ranges for serum AFP and midkine levels, respectively. AFP levels are given in conventional units; to convert to Système International units (μg/L), multiply by 1.0.

Figure 3: Serum midkine levels measured in patients with newly diagnosed hepatocellular carcinoma (HCC) and various neoplastic and nonneoplastic gastrointestinal diseases. There were 72 patients with newly diagnosed HCC, 24 with colorectal cancer, 5 with colonic adenoma, 11 with gastric cancer, 8 with esophageal cancer, 6 with pancreatic cancer, 6 with liver adenoma, 7 with fatty liver, 26 with peptic ulcer, 17 with gallstones, and 10 with cirrhotic liver.
(progression, regression, or stable disease). If the change in serum midkine level did not show parallel correlation with the clinical course, the change was regarded as negative. Representative examples of positive and negative correlation are shown in Figure 4 and Figure 5. Among the 165 patients, 134 had a positive correlation (81.2%) and 31 had a negative correlation (18.8%).

Rapidly Rising Serum Midkine Levels and Patient Death
During the course of this study, we noticed that rapidly rising serum midkine levels always took place shortly before the death of patients due to HCC progression (Figure 4). In our longitudinal follow-up study, 108 patients died of disease progression. We then determined the rising rates of serum midkine levels before death. The rising rate of midkine levels
was calculated from the initial measurement of the rapid rise and the last measurement before death, as shown in Figure 6A. We conducted a correlation study between the rising rates of serum midkine levels and the days between the initial rise and death Figure 7A. Regression analysis showed a significant inverse power correlation between the 2 parameters ($r = 0.61; P < .0001$). The majority of patients with the midkine level increasing at a rate more than 0.1 ng/mL per day died within 100 days after the initial continuous rise in the midkine level (Figure 7B).

**Serum Midkine Levels for Monitoring Recurrence and New Occurrence of HCC**

To evaluate whether serum midkine levels can be used to monitor the recurrence of HCC in patients in complete remission after curative therapy ($n = 144$) or the occurrence of de novo HCC in patients at risk for the development of HCC ($n = 141$; Table 1), we conducted a longitudinal follow-up study in the 285 patients. During the follow-up period, recurrence developed in 13 patients and de novo HCC developed in 2. Among the 13 patients in whom recurrence developed during follow-up, 3 (23%) had increased serum midkine levels 2, 5, and 12 days, respectively, before a positive diagnostic imaging study. For the 2 patients in whom de novo HCC developed, we were unable to detect a significant rise of the serum midkine level before positive detection by an imaging study. The diameters of the 13 recurrent HCCs ranged from 1.3 to 5.6 cm, with a median diameter of 2.2 cm at the time of detection. The diameters of the 2 de novo HCCs were 1.5 and 3 cm. These results show that the longitudinal measurements of serum midkine levels were unable to detect the development of new HCC in patients in complete remission or the development of de novo HCC in high-risk people.

**Effect of Intravenous Administration of Heparin on Serum Midkine Levels**

As reported previously, heparin can increase peripheral blood midkine levels through the release of bound midkine from the vascular endothelial surface in patients undergoing hemodialysis. We conducted a study to determine the dose response of intravenous bolus administration of heparin on
serum midkine levels. The results showed that the plasma midkine level was highly elevated after heparin administration and reached a peak level 30 minutes after heparin injection [Figure 8A]. Furthermore, the increased plasma midkine level after heparin injection was proportional to the increasing dose of heparin [Figure 8B]. Heparin at a dose up to 3,500 U still could not induce the maximal release of midkine into peripheral blood.

Discussion

In this study, we evaluated the role of serum midkine as a biomarker for monitoring disease recurrence and progression in patients with HCC. We also determined whether longitudinal measurements of serum midkine levels could be used for the early detection of de novo HCC in high-risk patients. Our study was prompted by the finding that midkine messenger RNA showed significant differential expression between HCC tissue samples and adjacent noncancerous liver tissue samples. When levels of midkine gene expression were compared between HCC tissue and adjacent noncancerous liver tissue as 2 separate groups, overlap between the 2 groups was apparent (Figure 1A). However, comparison of midkine gene expression between tissue samples of HCC and adjacent noncancerous liver tissue samples showed consistently increased expression of the midkine gene in HCC tissue. This finding suggested that establishment of a baseline level of midkine in each person and use of this baseline value for longitudinal follow-up could be a clinically useful approach to monitor HCC disease activity.

We conducted a study in 285 patients at risk for the development of recurrent or de novo HCC (Table 1). Unfortunately, our study failed to show that longitudinal serial serum midkine measurement is effective to monitor recurrence or de novo occurrence of HCC. As reported previously, midkine is a heparin-binding protein and can bind to heparan sulfate on the surface of vascular endothelial cells. Moreover, intravenous administration of heparin can increase the serum midkine level. Therefore, a lack of sensitivity in using serum midkine levels to detect early recurrence or de novo occurrence of HCC could be because most of the midkine secreted by HCC binds to the vascular endothelial surface. To confirm this possibility, we studied how plasma midkine levels were affected by intravenous administration of different doses of heparin. The results of our study showed that plasma midkine levels were highly elevated after heparin administration and reached peak levels at 30 minutes after injection (Figure 8A). Furthermore, the adsorbed midkine can be effectively released into blood by increasing doses of heparin (Figure 8B).

The findings confirm that there are 2 pools of midkine in the vascular space. One pool is freely circulating in blood, and the other is bound on the vascular endothelial surface and releasable by heparin. As shown in Figure 8A, midkine levels in peripheral blood can be increased by 1,200-fold after heparin administration. Thus, the vascular endothelial surface has a high capacity to adsorb most of
the secretion of midkine. Such adsorption can dampen the rise of midkine levels in peripheral blood after secretion. Consequently, midkine is insensitive for the detection of de novo occurrence or early recurrence of HCC as observed in our study. Our results suggest that the sensitivity may be improved by releasing all of the adsorbed midkine from the vascular endothelial surface into the circulation with heparin administration. Unfortunately, the present study showed that a dose of heparin up to 3,500 U remains insufficient to release midkine to a maximum. Thus, the use of heparin to release midkine for measurement of the total amount of midkine within the intravascular space is impractical and cannot be safely used.

Despite the insensitivity of serum midkine levels for the detection of the emergence of new HCC, we found that longitudinal follow-up of serum midkine levels could be useful for monitoring treatment response and disease progression in HCC. A parallel relationship between a change in the serum midkine level and HCC disease activity was observed in about 80% of patients with HCC. Although midkine and AFP can be used to monitor HCC activity in patients with AFP+ HCC (Figure 4A), the dynamic range of serum midkine levels is not as high as that of AFP levels (Figures 4A and 5A). Thus, midkine levels seem less effective than AFP levels for monitoring changes in disease activity in patients with AFP+ HCC. Nevertheless, longitudinal serial measurements of midkine at regular intervals could be useful to monitor disease progression in patients with AFP– HCC (Figure 4B).

It is interesting that our study revealed that serum midkine levels rose sharply in most patients as they approached the end of life (Figure 4). The rate of increase was inversely correlated with the remaining duration of survival (Figure 7). The exact cause for the rapid rise in serum midkine levels shortly before death is unclear. It is probable that midkine is secreted in increasing amount as HCC continues to grow at the terminal stage. When the secreted total midkine level becomes sufficiently high and saturates all of the binding sites on vascular endothelial cells, the serum midkine level is no longer dampened by its binding to heparan sulfate on the vascular endothelial surface. Consequently, the midkine level in the blood begins to rise sharply. Such an event signals uncontrolled tumor growth, large tumor burden, and the end of life. Alternatively, the rapid increase of midkine levels might result from the loss of midkine binding sites on vascular endothelium during the terminal stage of the disease. Unfortunately, studies to determine the exact cause of the rapid rise of midkine levels are technically prohibitive at present. Regardless of the exact cause of the rapid terminal rise in the serum midkine level, longitudinal measurement of serum midkine levels can be a useful prognostic biomarker for prediction of the impending death of patients with HCC.

The results of this study demonstrated that longitudinal regular follow-up of serum midkine levels together with imaging studies can be used to monitor disease progression in AFP+ HCC. The sharp rise of serum midkine levels in patients with HCC signals the approaching end of life. Longitudinal measurement of serum midkine levels is not sufficiently sensitive for the detection of newly emerged HCC in high-risk people or patients in complete remission.

References


