Prognostic and diagnostic effects of high serum midkine on patients with hepatocellular carcinoma

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Abstract

Background

Midkine (MK) is a soluble cytokine, and its serum levels strongly correspond with protein expression levels in tumors. This study aimed to clarify the clinicopathological and prognostic significance of serum MK (s-MK) in patients with hepatocellular carcinoma (HCC).

Methods

Serum samples were obtained before surgery from 123 patients with HCC who underwent surgery between 2012 and 2020. Based on the receiver operating characteristics curve, the best cutoff value for s-MK in differentiating HCC from healthy cases was 426 pg/mL. Patients’ clinicopathological variables and overall survival were compared between the s-MK-positive group and the s-MK-negative group.

Results

The sensitivity, specificity, and accuracy of s-MK were 82.1%, 97.4%, and 88.0%, respectively. An s-MK-positive status was significantly associated with the number of tumors (≥2). The positivity rate of s-MK was significantly higher than that of α-fetoprotein and protein induced by vitamin K absence-II. In total, only 28% of the patients were positive for s-MK. The s-MK positive group showed significantly worse overall survival than the s-MK negative group. The multivariate analysis revealed that an s-MK-positive status was independently associated with poor prognosis.

Conclusion

s-MK was useful in detecting early HCC. A s-MK-positive status was associated with the number of tumors and was an independent prognostic risk factor.

Introduction

Midkine (MK) is a pleiotropic growth-binding protein initially found to be highly upregulated during embryogenesis, thereby playing a key role in neuronal differentiation [1, 2]. Previous studies have shown that MK exhibits antiapoptotic and angiogenic activities leading to enhanced cell proliferation in tumor entities. Since MK is a soluble cytokine, its serum levels strongly correspond with protein expression levels in tumors [3]. Serum MK (s-MK) was proposed as a potential biomarker for different tumor entities including hepatocellular carcinoma (HCC).

Serum α-fetoprotein (AFP) is the only diagnostic marker recommended in the HCC guidelines. However, its diagnostic performance is unsatisfactory, with low sensitivity and specificity. To improve the diagnosis of HCC, advances in biomarker detection techniques have led to the identification of many new biomarkers, such as autoantibodies and s-MK [4–6]. s-MK, an emerging serum biomarker, activates several cell surface receptors to participate in modulating various biological activities and is significantly increased
in HCC [7]. s-MK has been proposed as a promising serum biomarker for HCC diagnosis. Although several studies have estimated the diagnostic value of s-MK for HCC, the results are inconsistent [8–12]. Precise clinicopathological analyses including AFP and protein induced by vitamin K absence-II (PIVKA-II) have not been published.

An s-MK-positive status has been reported to be associated with poor prognosis in some solid tumors such as colorectal cancer [13] and nonsmall cell lung cancer [14], whereas an s-MK-positive status has been reported to be not a poor prognostic factor in esophageal [15] and gastric cancers [16]. The correlation between an s-MK-positive status and the prognosis of patients with HCC has not been published.

Therefore, this study aimed to clarify the clinicopathological and prognostic significance of an s-MK-positive status in patients with HCC.

**Methods**

**Patients**

This study was registered as UMIN000014530. Serum samples were obtained before surgery from 123 patients with HCC who underwent surgery at Omori Medical Center, Toho University School of Medicine, between January 2012 and December 2020. A total of 123 patients with histologically proven primary HCC were enrolled. The patient cohort consisted of 87 male (70.7%) and 36 female (29.3%) patients, with a median age of 69 (range, 40–85) years. To ensure the complete absence of the influence of the previous cancer, those with active coexisting cancer, i.e., synchronous coexisting cancer or metachronous cancer within five disease-free years, were excluded. The final HCC stage was assessed pathologically following the tumor–node–metastasis classification criteria of the eighth edition of the International Union against Cancer [17]. Tumors associated with distant metastasis including peritoneal dissemination were considered not resectable. Hepatectomy followed the treatment algorithm described in Japanese guidelines [18, 19].

**Data collection and analyses for serum biomarkers**

Serum samples were obtained before surgery and stored at −80°C until analysis. Serum samples of healthy controls, with no previous malignant disease and without hepatitis B or C infection, were obtained from Biobank Japan. The average age of the control group (n = 77) was 52 years, with a male-to-female ratio of 50:39.

Clinicopathological characteristics, AFP, and PIVKA-II were included in the analysis. Preoperative variables, pathological characteristics, postoperative status, and survival were entered into a spreadsheet and imported into a dedicated database. The prognostic value and clinical utility of s-MK for HCC diagnosis were estimated. Overall survival was calculated from the time of surgery until death or study conclusion.
Enzyme-linked immunosorbent assay kits for human MK (CDYELISA, Immuno-probe Ltd., Saitama, Japan) were used for detecting s-MK according to the manufacturer’s protocol. The cutoff value for s-MK was fixed at 426 pg/mL based on the receiver operating characteristic curve (Fig. 1A).

Patients' clinicopathological variables, demographics, tumor characteristics, and overall survival were compared between the s-MK-positive group and the s-MK-negative group. The cutoff values were 10.0 ng/mL and 40.0 mAU/mL for AFP and PIVKA-II, respectively, following the assay kit manufacturer's instructions.

Statistical analysis

Statistical analyses were performed by JMP version 12 (SAS Institute, Cary, NC, USA). Between-group comparisons of the clinicopathological variables were performed using Fisher's exact probability test. Overall survival was calculated by the Kaplan–Meier product limit estimate. Between-group differences in survival were compared by the log–rank test. Significant predictors were identified by univariate and multivariate analyses using Cox proportional hazard models, and hazard ratios with 95% confidence intervals (CIs) were calculated. A $P$ value of $< 0.05$ was considered statistically significant.

Results

Sensitivity and specificity of serum MK levels

By the ROC curve, the best cutoff point was determined to distinguish the HCC group using s-MK. The area under the curve for s-MK was 0.973 (95% confidence interval [CI] 0.903–0.992) (Fig. 1A). According to the curve, the best cutoff value for s-MK in differentiating HCC from healthy cases was 426 pg/mL. At this value, the sensitivity, specificity, and accuracy were 82%, 97%, and 88%, respectively. The mean s-MK levels in the HCC and healthy control groups were 781 ± 678 and 224 ± 101 pg/mL, respectively (Fig. 1B, $P < 0.01$).

Comparison of clinicopathological characteristics between the s-MK-positive group and the s-MK-negative group in patients with HCC

Of the 123 patients, 101 (82%) were positive for s-MK (> 426 pg/mL) (Table 1). An s-MK-positive status was significantly associated with hepatitis B virus negativity and number of tumors ($\geq 2$) but not with the liver reserve or liver background.

Positivity rates of s-MK and AFP according to TNM stages

The positivity rates of the s-MK were significantly higher than those of AFP and PIVKA-II ($P < 0.05$, Fig. 2A). In total, only 28% (34 of 123) of the patients were positive for s-MK. In patients with stage I/II, only 33% (21 of 63) were positive for s-MK (Fig. 2B). Even in patients with stage III/IV, only 22% (13 of 60) were positive for s-MK (Fig. 2C).
Figure 3A shows the positivity rates of s-MK, AFP, and PIVKA-II at each TNM stage. In stage I, the positivity rate for s-MK was significantly higher than those of AFP and PIVKA-II (83% vs. 31% vs. 31%, \( P < 0.05 \)); similarly, 86%, 50%, and 43% (\( P < 0.05 \)) for stage II; 76%, 39%, and 63% (\( P < 0.05 \), not significant) for stage III; and 56%, 78%, and 56% (not significant) for stage IV, respectively.

The positivity rate of the combined use of s-MK and AFP + PIVKA-II was significantly higher than that of AFP + PIVKA-II (93% vs. 65%, \( P < 0.05 \), Fig. 3B). In stage I, the positivity rate of the combined use of s-MK and AFP + PIVKA-II was significantly higher than that of AFP + PIVKA-II (94% vs. 51%, \( P < 0.05 \)). Moreover, in stage II, the positivity rate of the combined use of s-MK and AFP + PIVKA-II was significantly higher than that of AFP + PIVKA-II (100% vs. 79%, \( P < 0.05 \)). In stage III, the positivity rate of the combined use of s-MK and AFP + PIVKA-II was significantly higher than that of AFP + PIVKA-II (88% vs. 67%, \( P < 0.05 \)).

**Prognostic effect of s-MK, AFP, and PIVKA-II status on overall survival**

The 5-year overall survival according to the s-MK, AFP, and PIVKA-II status is shown in Fig. 4. Although no significant difference was found in the overall survival according to the AFP status (Fig. 4B, \( P = 0.315 \)), the s-MK-positive group showed significantly worse overall survival than the s-MK-negative group (Fig. 4A, \( P = 0.007 \)). Similarly, the PIVKA-II-positive group showed significantly poorer overall survival than the PIVKA-II-negative group (Fig. 4C, \( P < 0.001 \)).

Figure 5 shows the comparison of overall survival at stages I/II and III/IV according to the s-MK, AFP, and PIVKA-II status. Regarding the prognostic effect of the s-MK status, the s-MK-positive group in stage I/II showed slightly worse overall survival than the s-MK-negative group (Fig. 5A, \( P = 0.116 \)). The s-MK-positive group in stage III/IV showed significantly worse overall survival than the s-MK-negative group (Fig. 5B, \( P = 0.048 \)). No significant difference was found in the overall survival according to the AFP status (Fig. 5C and D, \( P = 0.818 \), \( P = 0.127 \)). On the contrary, a significant difference was found in overall survival according to the PIVKA-II status (Fig. 5E and 5F, \( P = 0.015 \), \( P = 0.007 \)).

**Univariate and multivariate analyses of overall survival**

In the univariate analysis, the Child–Pugh classification (B), liver damage (B), PIVKA-II-positive status, and s-MK-positive status were significantly associated with poor prognosis (Table 2). In the multivariate analysis, a PIVKA-II-positive status (\( P = 0.002 \); HR = 3.759; 95% CI 1.600–9.603) and s-MK-positive status (\( P = 0.006 \); HR = 5.157; 95% CI 1.483–32.553) were independently associated with poor prognosis.

**Discussion**

The positivity rate of s-MK was 82% for HCC. The positivity rate of the combined use of s-MK and AFP + PIVKA-II was significantly higher than that of AFP + PIVKA-II. An s-MK-positive status was associated with the number of tumors. The s-MK-positive group showed poor overall survival.
An s-MK-positive rate was not associated with stage, and this tendency was similar to the pattern of serum autoantibodies, as previously reported [5, 6]. It may be that s-MK is induced not only by cancer but also by various factors such as inflammation and hemodynamics [20]. At present, even in HCC, which has multistage carcinogenesis, at which stage that s-MK is induced is unclear. Karim et al. reported that the s-MK level was significantly elevated in the HCC group compared with the healthy control group and the liver cirrhosis group [21]. Therefore, s-MK is useful to detect early-stage cancer, and it may be useful in the follow-up of patients with cirrhosis.

s-MK was associated with the number of tumors but not with liver background or tumor size. Of the 23 patients with multiple tumors, the positivity rates for s-MK, AFP, and PIVKA-II were 100%, 69%, and 43%, respectively. This may be because MK plays an important role in cell proliferation, survival, migration, angiogenesis, and carcinogenesis [22, 23]. Whether s-MK is a cause or a consequence of multiple tumors is unclear. However, given that an s-MK-positive status is a poor prognostic factor, an s-MK-positive status may reflect the biological grade of the tumor.

The prognostic effect of s-MK on various cancers was not consistent. In this study, we, first, evaluated the prognostic effect of s-MK on HCC. An s-MK-positive status was an independent risk factor for poor overall survival. The poor prognostic effect of an s-MK-positive status in HCC suggests the high biological malignancy of s-MK-positive HCC cells, given the lack of correlation between an s-MK-positive status and cirrhosis. MK-positive cancer cells have been reported to be associated with antiapoptotic function, and resistance to chemotherapy after HCC recurrence may contribute to poor prognosis [24].

Among its limitations, first, no data were available for evaluating the association between s-MK positivity and the immunoreactivity of cancer cells. Since several previous studies have reported that s-MK concentrations were significantly associated with immunoreactivity, MK expression in cancer cells may likewise be associated with s-MK [25, 26]. Second, this study only focused on the preoperative s-MK and had no data of postoperative monitoring. Therefore, we could not capture changes in s-MK levels before and after surgery. Actually, the s-MK level was reported to decrease significantly after surgery in esophageal cancer [26].

In conclusion, s-MK was a convenient and useful serum biomarker to detect HCC even in patients with stage I/II regardless of LC. A s-MK-positive status was associated with the number of tumors and was an independent prognostic risk factor. Considering the malignant potential of s-MK-positive HCC, more intensive follow-up is necessary after surgery.

**Abbreviations**

α-fetoprotein  
AFP  
Hepatocellular carcinoma  
HCC  
midkine
Declarations

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Author contributions


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Conflict of interest

The authors have no conflict of interest associated with this study.

Ethics statement

All study participants provided consent for future analyses of their blood samples for research. The protocol for this study was approved by the ethics committee of the Toho University (Approval nos. M22211, M21038_20197_19213, and A18103_A17052_A16035_A16001_26095_25024_24038_22047). Patients provided written informed consent before enrolment. The study was registered in the UMIN Clinical Trials Registry (UMIN000014530) and was conducted following the guidelines of the Declaration of Helsinki and the Japanese Ethical Guidelines for Clinical Research.

References


Tables

Table 1 and 2 are available in the Supplementary Files section.

Figures
Figure 1

A: Receiver operating characteristic curve showing the diagnostic performance of serum midkine for discriminating the hepatocellular carcinoma group from the healthy group.

B: Serum midkine expression is upregulated in the hepatocellular carcinoma group compared with the healthy group. Data are shown in a box-and-whisker plots (median, 25th, and 75th percentile, range, and extreme values outside the range).
Figure 2

Relationship between positive serum tumor marker findings in patients with hepatocellular carcinoma for all patients (A), stage I/II (B), and stage III/IV (C).
Figure 3

Positivity rates of serum tumor markers in hepatocellular carcinoma. (A) Comparison of the positivity rates of serum tumor markers. (B) Comparison of the positivity rates between AFP/PIVKA-II and AFP/PIVKA-II/Midkine. AFP, α-fetoprotein; PIVKA, protein induced by vitamin K absence I.
Figure 4

Comparison of overall survival between the positive and negative midkine groups (A), AFP groups (B), and PIVKA-II groups (C). AFP, α-fetoprotein; PIVKA, protein induced by vitamin K absence.
Figure 5

Comparison of overall survival between the positive and negative midkine groups for stage I/II (A); that is, midkine for stage III/IV (B), AFP for stage I/II (C), AFP for stage III/IV (D), PIVKA-II for stage I/II (E), and PIVKA-II for stage III/IV (F). AFP, α-fetoprotein; PIVKA, protein induced by vitamin K absence

Supplementary Files

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- Table1.jpg
- Table2.jpg