Abstract. C4b-binding protein α-chain (C4BPA) was previously identified as a novel serum biomarker for pancreatic ductal adenocarcinoma (PDAC). To apply this biomarker for clinical diagnosis, a lectin ELISA was established to measure serum fucosylated (Fuc)-C4BPA levels in 45 patients with PDAC, 20 patients with chronic pancreatitis (CP) and 50 healthy volunteers (HVs) in one training and three validation sets. The lectin ELISA developed in the current study exhibited satisfactory within-run (2.6-6.7%) and between-day (1.8-3.6%) coefficient of variations. Serum Fuc-C4BPA levels in patients with PDAC (0.54±0.27 AU/ml) was significantly higher than that in HVs (0.21±0.06 AU/ml; P<0.0001) and patients with CP (0.25±0.03 AU/ml; P<0.0001). Additionally, serum Fuc-C4BPA levels in preoperative patients were significantly decreased compared with postoperative patient sera (P<0.0003). The receiver operating characteristic (ROC) curve analyses revealed that the area under the curve (AUC) of Fuc-C4BPA (0.985) was higher than that of carbohydrate antigen (CA)19-9 (0.843), carcinoembryonic antigen (0.548) and total C4BPA (0.875) (P<0.0001). To analyze the clinical significance of Fuc-C4BPA, the ability of Fuc-C4BPA to predict lymph node metastasis was compared with that of CA19-9. The AUC of serum Fuc-C4BPA levels (0.703) was significantly higher than that of serum CA19-9 levels (0.500) in patients with PDAC (P<0.0001). The current study established a novel lectin ELISA for measuring serum Fuc-C4BPA levels. Thus, Fuc-C4BPA has potential clinical applications owing to its high diagnostic value in PDAC.

Introduction

Pancreatic ductal adenocarcinoma (PDAC), a malignant tumor, is associated with poor prognosis, which is mainly due to the difficulties in early diagnosis and the low efficacy of adjuvant therapy. There is a need to develop methods for early diagnosis and multimodal treatment involving surgical resection and chemo-radiotherapy to improve the clinical outcomes of patients with PDAC. The development of mass spectrometry (MS)-based proteomics technology has facilitated the detection of disease biomarkers (1). This technology, which involves the analysis of peptides and proteins in biological samples, has enabled the identification of many biomarker candidates in the human plasma or serum and can provide cancer-specific diagnostic information (2,3).

A comprehensive analysis of glycoproteins in the serum samples of patients with PDAC using Tandem Mass Tag™ with MS identified C4b-binding protein α-chain (C4BPA) as a novel serum PDAC biomarker (4). C4BPA is reported to be markedly upregulated in patients with clear-cell ovarian cancer (5) and non-small-cell lung cancer (6). This indicated the diagnostic value of serum C4BPA in different cancers. However, the objective comparison of serum C4BPA levels is difficult because of the use of different measurement methods. High reproducibility and specificity are the key parameters for establishing useful clinical biomarkers, large-scale validation of these markers, and high-throughput screening for such markers.

Fucosylation and sialylation are major glycosylation events in post-translational protein modifications, which are involved in the pathogenesis of inflammation and cancer (7). In fucosylation, the transfer of fucose from GDP-fucose to a molecule is catalyzed by fucosyltransferases. Sialylation involves the addition of a sialic acid unit to the end of an oligosaccharide chain.
In a glycoprotein. The glycoproteins, alpha-fetoprotein-L3 (AFP-L3; fucosylated AFP) and carbohydrate antigen 19-9 (CA19-9; sialyl Lewis A antigen) are used as tumor markers in hepatocellular carcinoma (8) and PDAC (9), respectively. Thus, glycoproteins are potential serum diagnostic biomarkers for cancer. Previous studies have reported that the fully-sialylated C4BPA levels are upregulated in the serum of patients with ovarian cancer (10). However, there are no studies on the importance of serum fucosylated (Fuc-) C4BPA in patients with cancer. Therefore, we hypothesized that the serum Fuc-C4BPA level has diagnostic and clinical significance in patients with PDAC.

In this study, we established a novel hybrid enzyme-linked immunosorbent assay (ELISA) for measuring Fuc-C4BPA using Lens culinaris agglutinin (LCA)-lectin, which specifically binds to fucose. We demonstrated that serum Fuc-C4BPA is a potential diagnostic biomarker for PDAC with clinical significance.

Materials and methods

Serum samples of participants. The inclusion criterion for the study was patients aged 20-85 who have been histologically diagnosed with PDAC or chronic pancreatitis (CP). Patients with other malignancies in the active phase were excluded from the study. For the training set, we measured the serum Fuc-C4BPA level in 19 patients with PDAC, 10 patients with CP, and 40 age and gender-matched healthy volunteers (HVs). For the validation set 1, the Fuc-C4BPA levels were comparatively analyzed in nine pairs of pre- and post-operative (3-4 weeks post-surgery when the serum levels of C-reactive protein returned to the normal range) sera obtained from patients with PDAC who underwent curative surgery. Additionally, the diagnostic values of conventional tumor markers, such as CA19-9, carcinoembryonic antigen (CEA), total C4BPA, and Fuc-C4BPA were investigated in the validation set 2. In the validation set 3, the serum Fuc-C4BPA levels were comparatively analyzed among 50 HVs, 20 patients with CP, and 45 patients with PDAC to examine the correlation between the serum C4BPA levels and the clinicopathological features of patients with PDAC (Table I). The blood samples from patients with PDAC or CP were collected at the Department of General Surgery, Chiba University Hospital between May 2011 and March 2019. The samples from HVs were collected at the Department of General Surgery, Kashiwado Hospital. The procedures for sample collection and processing were performed as previously reported (11). The ethics committee of each institute approved the protocol (approval number: 2155 for Chiba University, and 007 for Kashiwado Hospital). Written informed consent was obtained from all the patients and HVs. All data from the participants were fully anonymized, and the study was performed according to the guidelines of the Declaration of Helsinki 1975.

Western blot analysis. To examine the specificity of the anti-C4BPA antibody, recombinant human C4BPA protein (Abnova) and serum sample of a patient with PDAC were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis using a 10-20% gradient gel (DRC, Tokyo, Japan) in the absence of β-mercaptoethanol. The resolved proteins were transferred to a polyvinyl difluoride membrane. To minimize nonspecific binding, the membrane was incubated with blocking One (Nacalai Tesque). The membrane was washed thrice with PBST (phosphate-buffered saline (PBS) containing 0.1% (v/v) Tween-20) and incubated with anti-C4BPA polyclonal antibodies (LifeSpan Biosciences, Inc.) for 1 h at room temperature. Next, the membrane was washed thrice with PBST and incubated with rabbit horseradish peroxidase (HRP)-conjugated anti-mouse immunoglobulin secondary antibody (Dako Japan) for 1 h at room temperature. The immunoreactive bands were visualized using Pierce Western Blotting substrate (Thermo Fisher Scientific). Precision plus protein dual-color standards (Bio-Rad Laboratories, Inc.) were used as internal references.

Immobilization of antibody to a polystyrene microtiter plate. The anti-C4BPA polyclonal antibodies in PBS were dispensed into a 96-well polystyrene microtiter plate (Thermo Fisher Scientific) at a concentration of 0.5 mg/well and incubated for 1 day at 4°C. The plate was washed thrice with PBS containing 0.05% Tween-20. Next, the wells of the microtiter plate were coated with 20% NOF102 containing 10% sucrose for one day at 4°C, followed by drying for seven days at 4°C. The microtiter plate was maintained at 4°C until use.

LCA-lectin ELISA conditions. First, 100-µl aliquots of 100-fold diluted serum samples were added in duplicates to each well of a microtiter plate washed with PBS. The samples were incubated at room temperature for 1 h and washed thrice. HRP-conjugated LCA-lectin (J-Chemical, Inc.) in PBS containing 0.05% Tween-20 (100 µl) was added to each well and the samples were incubated at room temperature for 30 min. The plate was washed thrice and 100 µl of TMB solution (Fujifilm Wako Pure Chemical Corporation) was added to each well. After incubation at room temperature for 10 min, 100 µl of stop solution was added. The absorbance of the mixtures was measured at 450 nm.

Statistical analysis. Numerical data are presented as the means ± standard deviations. All statistical analyses, including linear regression analysis and Pearson’s correlation coefficient calculation were performed, and receiver operator characteristic (ROC) and the area under the ROC curves (AUC) were calculated using SPSS v.19.0 (SPSS). For non-parametric data, the means of the two groups were compared using Mann-Whitney U test, Welch’s t-test. The means of the two groups among three groups were compared using Dunn’s nonparametric comparison for post hoc testing after a Kruskal-Wallis test. The means of four groups were compared the differences using ANOVA test and the Tukey-Kramer post hoc test. The differences were considered significant at P<0.05.

Results

Establishment and characterization of a hybrid LCA-lectin ELISA for measuring Fuc-C4BPA. We first developed a sandwich ELISA system, which has a higher sensitivity than a previously reported ELISA system (4), for measuring total C4BPA levels. The anti-C4BPA antibody was used to detect recombinant human C4BPA protein and serum C4BPA in a
This antibody was used as a capture antibody to establish a novel hybrid LCA-lectin ELISA for measuring the Fuc-C4BPA levels. A standard curve was generated based on the absorbance value of the diluted C4BPA in the HEK293T cell lysate (OriGene Technologies, Inc.) to evaluate the correlation between the absorbance values and the C4BPA concentrations. The working concentration range of Fuc-C4BPA was 0.0-2.0 AU/ml. The assay results revealed a linear correlation between the absorbance values and the C4BPA levels at a concentration range of 0.0-2.0 AU/ml (y=1.0009x, r²=0.9988, P<0.0001; Pearson's correlation). The detection limit was estimated by assaying the zero concentration eight times and was defined as the C4BPA ‘zero’ concentration + 3 SD. The limit of Fuc-C4BPA detection was 0.13 AU/ml. To examine the within-run and between-run reproducibility, the precision of the assay was examined at Fuc-C4BPA concentrations of 0.23 and 1.39 AU/ml. The within-run coefficients of variation (CVs) were determined using eight replicates of each sample. The assay was repeated on five different days to determine the between-assay CVs (two replicates of each sample per day). The within-run and between-run CVs were 2.6-6.7 and 1.8-3.6%, respectively.

Finally, interference was assessed in the samples containing 0.23 AU/ml of Fuc-C4BPA. Potential interference materials were added to the sera at various concentrations. There was no substantial interference from hemoglobin (up to 5,000 mg/l), free bilirubin (up to 207 mg/l), diaturobilirubin (up to 204 mg/l), chyle (up to 1,400 formazine turbidity units, equal to 1,176 mg/l triglycerides), ascorbic acid (up to 500 mg/l), and rheumatoid factor (up to 500 U/l).

Fucosylated C4BPA levels are upregulated in the serum of patients with PDAC. The established LCA-lectin ELISA was used to examine the serum Fuc-C4BPA levels in 69 serum samples (40 age-matched HVs, 10 patients with CP, and 19 patients with PDAC) of the training set (Table I). As shown in Fig. 2A, the serum level of Fuc-C4BPA in patients with PDAC (0.61±0.33 AU/ml) was significantly higher than that in HVs (0.21±0.06 AU/ml; P<0.0001) and patients with CP (0.25±0.03 AU/ml; P<0.0001). Additionally, the serum level of Fuc-C4BPA in patients with CP was higher than that in HVs (P<0.01). Next, the Fuc-C4BPA levels were measured in nine pairs of pre- and post-operative sera in the validation set 1. Interestingly, the serum Fuc-C4BPA level significantly decreased in all nine patients after the curative operation (Fig. 2B) (P=0.0019; Wilcoxon signed rank test).

Fucosylated C4BPA levels have a higher PDAC detection accuracy than total C4BPA levels. To examine the discriminatory power of serum Fuc-C4BPA and total C4BPA levels, we compared the levels of Fuc-C4BPA and total C4BPA among 17 patients with PDAC and age-matched benign controls comprising 10 HVs and 10 patients with CP in the validation set 2. The serum levels of total C4BPA in patients with PDAC (87.7±41.7 µg/ml) were significantly higher than those in HVs (38.9±8.4 µg/ml) (P<0.001) and patients with CP (43.4±11.5 µg/ml) (P=0.01). The HVs and patients with CP did not exhibit significant differences in the serum levels of total C4BPA (P=0.35) (Fig. 3A). Meanwhile, the serum levels of Fuc-C4BPA in patients with PDAC (0.54±0.22 AU/ml) were significantly higher than those in HVs (0.20±0.04 AU/ml; P<0.0001) and patients with CP (0.25±0.03 AU/ml; P<0.0001) in the validation set 2 (Fig. 3B). The serum levels of total C4BPA and Fuc-C4BPA were not significantly correlated in this sample set (P=0.61, r=−0.13; Pearson’s correlation) (Fig. 3C).

Next, the receiver operating characteristic (ROC) curves were generated to evaluate the ability of Fuc-C4BPA, total

---

**Table I. Clinical characteristics of participants.**

| Patient set | Age, mean ± SD | Sex |
|-------------|----------------|-----|
| Training set |                |     |
| HVs (n=40)  | 65±5           | 25  | 15 |
| CP (n=10)   | 51±14          | 9   | 1  |
| PDAC (n=19) | 68±10          | 11  | 8  |
| Validation set 1 |          |     |
| Pre-/post-operative sera of PDAC (n=9) | 69±13 | 5 | 4 |
| Validation set 2 |          |     |
| HVs (n=10)  | 66±5           | 5   | 5  |
| CP (n=10)   | 58±12          | 9   | 1  |
| PDAC (n=17) | 63±11          | 9   | 8  |
| Validation set 3 |          |     |
| HVs (n=50; training set + validation set 2) | 67±5 | 30 | 20 |
| CP (n=20; training set + validation set 2) | 55±13 | 18 | 2  |
| PDAC (n=45; training set + validation set 1+2) | 66±11 | 25 | 20 |

HV, healthy volunteer; CP, chronic pancreatitis; PDAC, pancreatic ductal adenocarcinoma; M, male; F, female.
SOGAWA et al: FUCOSYLATED C4BPA PREDICTS LYMPH NODE METASTASIS IN PANCREATIC CANCER

C4BPA, CA19-9, and CEA levels to distinguish patients with PDAC from non-cancer (HVs and CP) participants. The cutoff values of these four markers were set at levels (mean + 2 SD) that yielded a specificity of 97.7% when compared with those of the participants without cancer (HVs and CP patients); 61.3 µg/ml, 0.297 AU/ml, 34.2 U/ml, and 7.3 ng/ml for total C4BPA, CA19-9, and CEA levels to distinguish patients with PDAC from non-cancer (HVs and CP) participants. The cutoff values of these four markers were set at levels (mean + 2 SD) that yielded a specificity of 97.7% when compared with those of the participants without cancer (HVs and CP patients); 61.3 µg/ml, 0.297 AU/ml, 34.2 U/ml, and 7.3 ng/ml for total
C4BPA, Fuc-C4BPA, CA19-9, and CEA, respectively. The area under the ROC curve (AUC) of Fuc-C4BPA, total C4BPA, CA19-9, and CEA was 0.982, 0.853, 0.862, and 0.607, respectively, in the validation set 2 (Fig. 3D). These results suggest that serum Fuc-C4BPA expression is a potential novel diagnostic biomarker for PDAC.

Fuc-C4BPA can identify the CA19-9 false-negative cases among patients with PDAC. To validate the diagnostic value of serum Fuc-C4BPA levels in PDAC, the PDAC sample size in the validation set 3 was increased to 45 from the training set and the validation sets 1 and 2. The Fuc-C4BPA levels in patients with PDAC (0.62±0.33 AU/ml) were significantly higher than those in HVs (0.21±0.03 AU/ml; P<0.0001) and patients with CP (0.25±0.03 AU/ml; P<0.0001) (Fig. 4A). The AUC of Fuc-C4BPA and CA19-9 was 0.985 and 0.853, respectively. This indicated that serum Fuc-C4BPA is a better diagnostic PDAC biomarker than serum CA19-9 (Fig. 4B; P<0.001). The accuracy of a single serum biomarker is limited in cancer detection. To increase the detection rate, additional biomarkers are often tested in a clinical setting. CA19-9 reacts with a monoclonal antibody directed against sialyl Lewis A antigen. Hence, Lewis A antigen-negative patients do not exhibit increased levels of CA19-9 (12). To overcome this limitation, the level of duke pancreatic monoclonal antigen type 2 (DUPAN-2) is measured because it is recognized by a monoclonal antibody directed against sialyl Lewis C antigen, which is the precursor of sialyl Lewis A antigen (13). We examined the ability of Fuc-C4BPA to identify the CA19-9 false-negative cases among patients with PDAC. Among the 16 CA19-9-negative patients, the percentage of the Fuc-C4BPA-positive cases (12/16; 75%) was significantly higher than that of DUPAN-2-positive cases (3/16; 18.8%) (P=0.0015; Chi-square test) (Fig. 4C). These results indicated that Fuc-C4BPA can identify the CA19-9 false-negative cases among patients with PDAC.

Fucosylated C4BPA predicts pathological lymph node (LN) metastasis in patients with PDAC. Next, we investigated the correlation between the levels of Fuc-C4BPA and the clinicopathological features of patients with PDAC. Among the major clinical parameters, such as factors contributing to the TNM classification system of malignant tumors, the
tumor stage was not correlated with the levels of Fuc-C4BPA and CA19-9 (Table II). Pathological LN metastasis (pN+) was positively correlated with high serum Fuc-C4BPA levels in the validation set 3 (P=0.035; Welch’s t-test) (Fig. 5A). Furthermore, the ability of Fuc-C4BPA and CA19-9 levels to predict LN metastasis was comparatively evaluated. The AUC of serum Fuc-C4BPA levels (0.703) was significantly higher than that of serum CA19-9 levels (0.500) (P<0.001) (Fig. 5B). These findings suggested that Fuc-C4BPA is a better predictor of LN metastasis than CA19-9 in patients with PDAC.

**Discussion**

Although several novel biomarkers have been identified using proteomic analysis, most of them are not available for clinical diagnosis. Generally, biomarkers are identified through comparative analysis of the disease and control groups based...
that the serum Fuc‑C4BPA levels were significantly higher in patients with lymph node metastasis than in patients without lymph node metastasis in the validation set 3. (B) The area under the curve of serum Fuc-C4BPA levels (0.703) was significantly higher than that of serum carbohydrate antigen 19-9 levels (0.500), which indicated the ability of Fc-C4BPA to predict lymph node metastasis. Fuc-C4BPA, fucosylated C4b-binding protein α-chain; pN+, patients with lymph node metastasis; pN−, patients without lymph node metastasis; LN, lymph node; AUC, area under the curve; CA19-9, carbon antigen 19-9.

Cancer-related immunocompetent cells or complement factors in the tumor microenvironment are associated with cancer progression (17). The contribution of aberrant fucosylation to the interactions within the tumor microenvironment remains poorly understood. In this study, we demonstrated that the serum Fuc-C4BPA levels were significantly upregulated in patients with PDAC. Additionally, the serum Fuc-C4BPA levels in the post-operative patients were significantly lower than those in the preoperative patients. Fucosylation is reported to be upregulated at an early stage of colon carcinogenesis (18). However, other studies have reported that de‑fucosylation through genetic mutation promotes the escape from natural killer cell‑mediated tumor surveillance and the development of malignant characteristics in certain types of advanced cancer (19). To understand the role of fucosylation during cancer progression from early to late-stage, the mechanisms underlying fucosylation or de‑fucosylation induced by the interaction between tumor cells and molecules in the tumor microenvironments must be elucidated.

The upregulated CA 19-9 levels are reported to be an independent prognostic factor for LN metastasis. Preoperative chemotherapy can improve the prognosis of patients with PDAC exhibiting LN metastasis (20,21). Therefore, the prediction of LN metastasis is important to develop an efficient therapeutic strategy for patients with PDAC. Contrast-enhanced computed tomography (CECT) is commonly used for the preoperative diagnosis of LN metastasis in patients with PDAC. A previous prospective study has demonstrated that the diagnostic accuracy of LN metastasis through CECT is low (73%) in PDAC (22). The significance of enlarged LNs in PDAC is not well defined because the LNs can enlarge due to local inflammation or biliary obstruction. Additionally, LN metastasis is not correlated with this enlargement (23). A recent study reported that a six-microRNA risk prediction model could distinguish patients with PDAC patients exhibiting LN metastases from those with PDAC not exhibiting LN metastases (AUC of 0.84 and 0.73 in the training and validation sets, respectively) (24). In this study, the upregulated levels of Fuc-C4BPA were positively correlated with LN metastasis. The AUC of Fuc-C4BPA (0.703) was significantly higher than that of CA19-9 (0.500). This indicated that a single serum biomarker, Fuc-C4BPA, is a good indicator of LN metastasis and can aid in determining the treatment strategy for patients with PDAC.
There are several limitations associated with the clinical application of serum Fuc-C4BPA level as a diagnostic marker for PDAC. The diagnostic window (e.g. the detection limit) of the lectin ELISA for measuring the Fuc-C4BPA level is narrow. Additionally, the serum Fuc-C4BPA level, but not the serum total C4BPA level, in patients with CP was significantly higher than that in HVs. This may be due to the correlation between inflammation and carcinogenesis, which are critical for the activation of various common molecules. The identification and measurement of specific target sites of fucosylation, which are directly involved in PDAC progression, may resolve these issues. Moreover, this is a retrospective study involving a small sample size. Further validation in an independent and prospective large cohort is needed to establish Fuc-C4BPA as a promising serum diagnostic biomarker for PDAC. In conclusion, we established a novel lectin ELISA for the measurement of serum Fuc-C4BPA. Serum Fuc-C4BPA has a powerful diagnostic ability with potential applications for the development of therapeutic strategies for PDAC.

Acknowledgements

Not applicable.

Funding

This work was supported by a Grant-in-Aid for Scientific Research (KAKENHI; grant nos. ‘KIBAN’ C:19K07947, 19K09113, 20K09073 and ‘KIBAN’ B:19H03725).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

KaS, SY, ST, FN, and MO were involved in the study design, data analysis and development of the study. KoS, YM, HT KF, TT, and SK collected and analyzed the clinical data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Chiba University, Graduate School of Medicine (approval no. #2155) and Kashiwado Hospital (approval no. #007), respectively. Informed consent was obtained from all participants and patients for the acquisition of clinical and pathological information and for the use of serum samples.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Takano S, Yoshitomi H, Togawa A, Sogawa K, Shida T, Kimura F, Shimizu H, Tomonaga T, Nomura F and Miyazaki M: Apolipoprotein C-1 maintains cell survival by preventing from apoptosis in pancreatic cancer cells. Oncogene 27: 2810-2822, 2008.
2. Moulder R, Bhosalde SD, Gooddlett DR and Ladesma R: Analysis of the plasma proteome using iTRAQ and TMT-based Isobaric labeling. Mass Spectrom Rev 37: 583-606, 2018.
3. Bhawal R, Oberg AL, Zhang S and Kohli M: Challenges and opportunities in clinical applications of blood-based proteomics in cancer. Cancers (Basel) 12: 2428, 2020.
4. Sogawa K, Takano S, Iida F, Satoh M, Tsuchida S, Kawashima Y, Yoshitomi H, Sanda A, Kodera Y, Takizawa H, et al: Identification of a novel serum biomarker for pancreatic cancer, C4b-binding protein α-chain (C4BPA) by quantitative proteomic analysis using tandem mass tags. Br J Cancer 115: 949-956, 2016.
5. Mikami M, Tanabe K, Matsuou K, Miyazaki Y, Miyazawa M, Hayashi M, Asai S, Ikeda M, Shida M, Hirasawa T, et al: Fully-sialylated alpha-chain of complement 4-binding protein: Diagnostic utility for ovarian clear cell carcinoma. Gynecol Oncol 139: 520-529, 2019.
6. Liu YS, Luo XY, Li QR, Li H, Li C, Ni H, Li RX, Wang R, Hu HC, Pan YJ, et al: Shotgun and targeted proteomics reveal that pre-surgery serum levels of LRG1, SAA, and C4BP may refine prognosis of resected squamous cell lung cancer. J Mol Cell Biol 4: 344-347, 2012.
7. Jia L, Zhang J, Ma T, Guo Y, Yu Y and Cui J: The function of fucosylation in progression of lung cancer. Front Oncol 8: 565, 2018.
8. Li D and Satomura S: Biomarkers for hepatocellular carcinoma (HCC): An update. Adv Exp Med Biol 867: 179-193, 2015.
9. Jelski W and Mroczko B: Biochemical diagnostics of pancreatic cancer-Present and future. Clin Chim Acta 498: 47-51, 2019.
10. Nishihara S, Sugano K, Okura H, Fujita S and Hirohashi S: Lewis and secretor gene dosages affect CA19-9 and DU-PAN-2 serum levels in normal individuals and colorectal cancer patients. Cancer Res 58: 512-518, 1998.
11. Kawa S, Oguchi H, Kobayashi T, Tokoo M, Furuta S, Kanai M and Homma T: Elevated serum levels of D upan-2 in pancreatic cancer patients negative for Lewis blood group phenotype. Br J Cancer 64: 899-902, 1991.
12. Stanczyk FZ and Clarke NJ: Advantages and challenges of mass spectrometry assays for steroid hormones. J Steroid Biochem Mol Biol 121: 491-495, 2010.
13. Farrell CJ, Martin S, McWhinney B, Straub I, Williams P and Herrmann M: State-of-the-art vitamin D assays: A comparison of automated immunoassays with liquid chromatography-tandem mass spectrometry methods. Clin Chem 58: 531-542, 2012.
14. Soldin SJ, Soukhoova N, Janicke N, Konlaas J and Soldin OP: The measurement of free thyroxine by isotope dilution tandem mass spectrometry. Clin Chim Acta 406: 7-18, 2009.
15. Li D and Satomura S: Biomarkers for hepatocellular carcinoma (HCC): An update. Adv Exp Med Biol 867: 179-193, 2015.
16. Nomura F and Miyazaki M: Fully-sialylated alpha-chain of complement 4-binding protein: Biomed Chromatogr 32: e4180, 2018.
17. Jia L, Zhang J, Ma T, Guo Y, Yu Y and Cui J: The function of fucosylation in progression of lung cancer. Front Oncol 8: 565, 2018.
18. Li D and Satomura S: Biomarkers for hepatocellular carcinoma (HCC): An update. Adv Exp Med Biol 867: 179-193, 2015.
19. Jelski W and Mroczko B: Biochemical diagnostics of pancreatic cancer-Present and future. Clin Chim Acta 498: 47-51, 2019.
20. Nishihara S, Sugano K, Okura H, Fujita S and Hirohashi S: Lewis and secretor gene dosages affect CA19-9 and DU-PAN-2 serum levels in normal individuals and colorectal cancer patients. Cancer Res 58: 512-518, 1998.
21. Kawa S, Oguchi H, Kobayashi T, Tokoo M, Furuta S, Kanai M and Homma T: Elevated serum levels of D upan-2 in pancreatic cancer patients negative for Lewis blood group phenotype. Br J Cancer 64: 899-902, 1991.
21. Tran Cao HS, Zhang Q, Sada YH, Silberfein EJ, Hsu C, Van Buren G II, Chai C, Katz MHG, Fisher WE and Massarweh NN: Value of lymph node positivity in treatment planning for early stage pancreatic cancer. Surgery 162: 557-567, 2017.

22. Roche CJ, Hughes ML, Garvey CJ, Campbell F, White DA, Jones L and Neoptolemos JP: CT and pathologic assessment of prospective nodal staging in patients with ductal adenocarcinoma of the head of the pancreas. AJR Am J Roentgenol 180: 475-480, 2003.

23. Tseng DS, van Santvoort HC, Fegrachi S, Besselink MG, Zuithoff NP, Borel Rinkes IH, van Leeuwen MS and Molenaar IQ: Diagnostic accuracy of CT in assessing extra-regional lymphadenopathy in pancreatic and peri-ampullary cancer: A systematic review and meta-analysis. Surg Oncol 23: 229-235, 2014.

24. Nishiwada S, Sho M, Banwait JK, Yamamura K, Akahori T, Nakamura K, Baba H and Goel A: A microRNA signature identifies pancreatic ductal adenocarcinoma patients at risk for lymph node metastases. Gastroenterology 159: 562-574, 2020.