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Effectiveness of Photodynamic Screening Using 5-Aminolevulinic Acid for the Diagnosis of Pancreatic Cancer

TSUKASA IKEURA[1], YUICHI HORI[1], TOSHIYUKI MITSUYAMA[1], HIDEAKI MIYOSHI[1], MASAAKI SHIMATANI[1], KAZUSHIGE UCHIDA[2], MAKOTO TAKAOKA[1], URARA OTA[3], ATSUKO KAMIYA[3], KIWAMU TAKAHASHI[3], MASAHIRO ISHIZUKA[3], MASAKI KAIBORI[4] and KAZUICHI OKAZAKI[1]

[1]*The Third Department of Internal Medicine, Kansai Medical University, Osaka, Japan;*
[2]*Department of Gastroenterology and Hepatology, Kochi Medical School, Kochi University, Kochi, Japan;*
[3]*SBI Pharmaceuticals Co. Ltd., Tokyo, Japan;*
[4]*Department of Surgery, Kansai Medical University, Hirakata, Japan*

Abstract. *Background/Aim: We evaluated urinary levels of porphyrin metabolites, such as uroporphyrin (UP) and coproporphyrin (CP), after 5-Aminolevulinic acid (ALA) administration in patients with or without pancreatic cancer (PaC). Patients and Methods: Sixty-seven subjects with PaC, 11 with pancreatitis, and 9 with normal pancreas (NP) were enrolled. Urine samples from all subjects were collected prior to ALA administration and at more than 4 hours after ALA administration. We measured the urinary levels of UP and CP by high-performance liquid chromatography analysis. Results: The PaC group showed significantly higher UP levels compared to NP groups (104.9 nmol/g Cre vs. 53.4 nmol/g Cre, p=0.014). Moreover, PaC patients with long-term survival had significantly lower urinary levels of UP at diagnosis (98.8 nmol/gCre) than the short-term survival group (125.2 nmol/gCre) (p=0.042). Conclusion: The urinary levels of UP after ALA administration might serve as a promising biomarker for diagnosis and prognosis prediction of PaC.*

In Japan, the incidence and mortality of pancreatic cancer (PaC) have been increasing in both genders, and PaC is the fourth leading cause of cancer death (1). Although the prognosis of many other types of cancers has improved, the estimated 5-year survival rate of patients with PaC is low, at <10% (2). One of the reasons for such a poor prognosis is the absence of a suitable biomarker for the diagnosis of PaC. Although CA19-9 is frequently used as a serum biomarker for the diagnosis of PaC, the diagnostic sensitivity, specificity, and accuracy are not satisfactory (3). Therefore, an easy-to-use and reliable biomarker is required to improve the poor prognosis of PaC.

5-Aminolevulinic acid (ALA) is an intrinsic amino acid in both plants and animals and is involved with heme synthesis (4). When ALA is exogenously administered, it is imported into cells, where it is subsequently metabolized to heme through heme precursors, such as uroporphyrinogen I and III, coproporphyrinogen I and III, and protoporphyrin IX (PpIX) (Figure 1). In patients with cancer, exogenous administration of ALA results in the accumulation of PpIX in cancer cells owing to alterations in the expression of membrane transporters and enzymatic activities in the heme synthesis pathway (5-7). This cancer-specific feature is used in "photodynamic diagnosis", thereby enabling the visual identification of cancer cells, as PpIX is a photosensitizing porphyrin that emits a typical red fluorescence under illumination with blue-violet light. Accordingly, in clinical practice, photodynamic diagnosis has been used for the intraoperative visualization of malignant glioma (8), bladder cancer (9), and peritoneal metastasis from gastric cancer (10). In addition, patients with cancer showed an increase in uroporphyrin (UP) and coproporphyrin (CP) levels in body fluids owing to their efflux from cancer cells (11, 12). In fact, when patients with bladder cancer and colorectal cancer were premedicated with ALA, their urinary levels of UP and CP were higher compared to non-cancer subjects, suggesting that the measurement of urinary porphyrin metabolites after ALA administration can be useful for "photodynamic screening" of these cancers (13, 14).

Therefore, in the current study, we compared the urinary levels of UP and CP after ALA administration between patients with PaC and those with a normal pancreas or with pancreatitis. Furthermore, we examined the feasibility of

Correspondence to: Tsukasa Ikeura, The Third Department of Internal Medicine, Kansai Medical University, 2-3-1, Shinmachi, Hirakata, Osaka, 573-1191 Japan. Tel: +81 728040101, Fax: +81 728042524, e-mail: ikeurat@hirakata.kmu.ac.jp

Key Words: Pancreatic cancer, 5-aminolevulinic acid, photodynamic screening, biomarker.

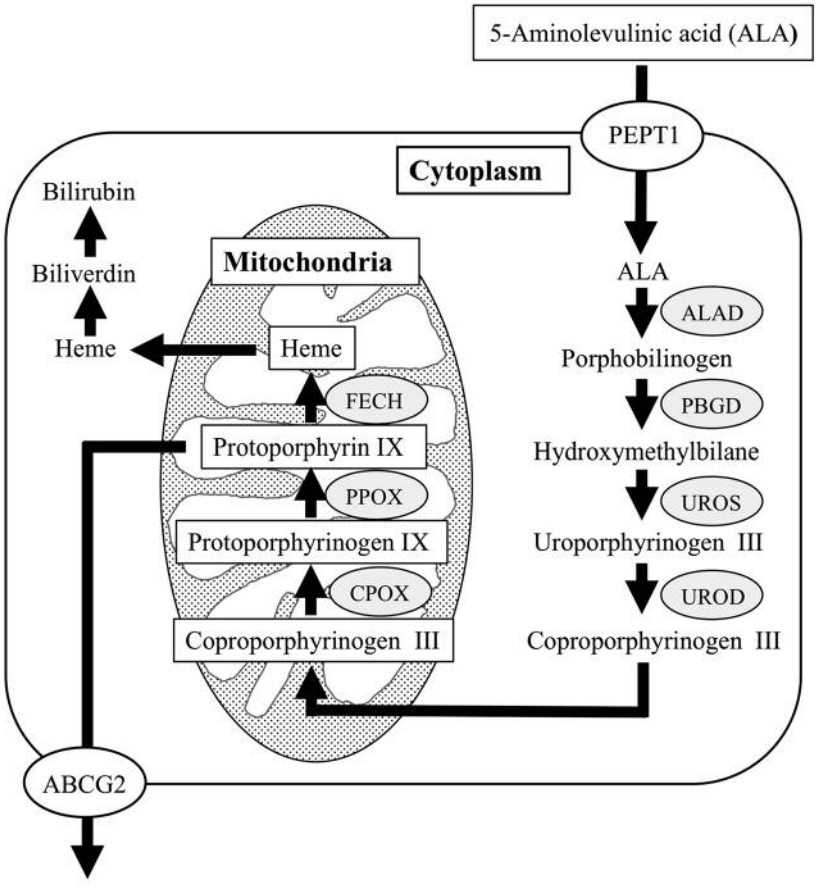

Figure 1. *Metabolism of 5-aminolevulinic acid. PEPT1: Peptide transporter 1; ABCG2: ATP-binding cassette transporter G2; ALAD: 5-aminolevulinate dehydratase; PBGD: porphobilinogen deaminase; UROS: uroporphyrinogen III synthase; UROD: uroporphyrinogen decarboxylase; CPOX coproporphyrinogen oxidase; PPOX: protoporphyrinogen oxidase; FECH: ferrochelatase.*

prognosis prediction of PaC by measuring the urinary levels of UP and CP after ALA administration.

Patients and Methods

Study design. This study was approved by the Kansai Medical University's ethics committee (1509).

All subjects were orally administered ALA phosphate at a concentration of 450 mg. Urine was collected before the administration of ALA for use as the control sample and more than 4 hours after ALA administration (mean time from ALA administration to urine collection 6.2±1.3 h). Immediately after collection, urine samples were stored in the dark at –30˚C until the urinary levels of ALA metabolites were measured. Urinary levels of UP I, UP III, CP I and CP III were determined by using high-performance liquid chromatography (HPLC) analysis, which were corrected for urine creatinine levels to appropriately perform statistical analysis (13). For the purpose of this study, the levels of porphyrin metabolites were expressed as the total UP (UP I plus UP III) and CP (CP I plus CP III) levels.

Subjects diagnosed with PaC based on the pathological examination of samples obtained by fine needle aspiration guided by endoscopic ultrasound and/or surgical resection were classified in the PaC group. Subjects who were diagnosed with chronic pancreatitis and autoimmune pancreatitis according to the Japanese clinical diagnostic criteria were classified in the pancreatitis group (15, 16). Subjects who did not have any serological and morphological abnormality in the pancreas were classified in the normal pancreas (NP) group.

The following clinicopathological characteristics of patients were prospectively assessed: gender, age at diagnosis, laboratory data [aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (T-Bil), C-reactive protein (CRP), hemoglobin (Hb), carcinoembryonic antigen (CEA), and carbohydrate antigen 19-9 (CA19-9) levels], treatment (chemotherapy and resection), prognosis, and histological findings of the resected specimen.

Statistical analysis. All statistical analyses were performed using STATA version 14 (Stata Corporation, College Station, TX, USA). The results are expressed as the median and range. Comparisons of categorical variables were performed using the Fisher exact tests. Comparisons between two groups were performed using the Wilcoxon test or Mann–Whitney *U*-test. *p*-Values less than 0.05 were considered significant. To evaluate the diagnostic ability of

Table I. *Demographic characteristics and laboratory data in normal pancreas (NP), pancreatitis, and pancreatic cancer (PaC) groups.*

	NP N=9	Pancreatitis N=11	PaC N=67	*p*-Value		
				NP *vs*. Pancreatitis	Pancreatitis *vs*. PC	NP *vs*. PaC
Age	69 (64-76)	67 (61-75)	72 (68-77)	0.542	0.137	0.314
Male	9 (100%)	8 (73%)	48 (72%)	0.089	0.941	0.065
Diabetes	2 (22%)	3 (27%)	22 (33%)	0.795	0.714	0.520
Laboratory data						
AST	29 (15-40)	19 (18-55)	24 (16-39)	0.761	0.724	0.840
ALT	29 (13-55)	22 (12-75)	22 (12-53)	0.790	0.661	0.841
T-Bil	1.4 (0.9-2.2)	0.7 (0.6-1.0)	0.9 (0.7-1.6)	0.027	0.170	0.249
CRP	0.2 (0.1-0.3)	0.5 (0.3-0.8)	0.4 (0.1-1.1)	0.040	0.118	0.942
Hb	14.3 (13.4-14.6)	12.5 (12.0-13.5)	13.1 (11.9-13.9)	0.080	0.646	0.030
CEA	2.2 (1.7-3.4)	3.2 (2.5-4.8)	5.0 (2.5-9.0)	0.221	0.175	0.125
CA19-9	40.6 (16.7-47.3)	6.6 (2.7-14.5)	266 (46-941)	0.026	<0.001	0.020

biomarkers for the diagnosis of PaC, receiver-operating characteristic (ROC) curves were prepared.

Results

Patient characteristics. Sixty-seven subjects were classified in the PaC group, 7 in the pancreatitis group, and 9 in the NP group. Thirteen of the 67 subjects with PaC underwent pancreatic resection. No subject experienced adverse events associated with the administration of ALA.

Table I shows the comparison between patient characteristics and laboratory data among the three groups. There was no significant difference in age and gender between the groups. Serum CA19-9 levels were significantly higher in the PaC group than in the NP group (p=0.020) and in the pancreatitis group (p<0.001).

Analysis of urinary levels of UP and CP in subjects with NP, pancreatitis, and PaC. Figure 2 shows the urinary UP and CP levels before and after the administration of ALA. In each group, urine UP and CP levels were significantly increased after ALA administration compared to those before ALA administration.

Figure 3 demonstrates the comparison of urinary UP and CP levels after administration of ALA among the 3 groups. Notably, the PaC group showed significantly higher UP levels compared to the NP group (104.9 nmol/gCre *vs*. 53.4 nmol/gCre, p=0.014). The urinary levels of CP were higher in the PaC group than in the NP and pancreatitis groups; however, the difference was not significant. These data indicate that urinary UP levels after ALA administration could be a novel biomarker for the detection of PaC.

Diagnostic ability of CA19-9, UP, and CP levels, and their combination for PaC detection. We calculated the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and efficiency of CA19-9, UP, and CP levels based on the best cut-off values to diagnose PaC using the ROC curve (Table II). The sensitivity, specificity, PPV, NPV, and efficiency of CA19-9 levels (cut-off value > 49.7 U/ml) were 74.6%, 83.3%, 94.3%, 46.9%, and 76.5%, respectively. The combination of CA19-9 and CP levels (cut-off value >2609.3 nmol/gCre) was the most efficient for PaC detection, which was improved compared to that of CA19-9 levels alone.

Predictive ability of urinary UP and CP levels after ALA administration for the prognosis of PaC. To evaluate whether urinary UP and CP levels after ALA administration were correlated with PaC prognosis, we compared the levels of these porphyrin metabolites between patients who showed long-term survival (≥12 months) and those who showed short-term survival (<12 months). This analysis included 55 patients with PaC who died due to PaC or who were followed-up for more than 12 months after the diagnosis of PaC. The number of patients with long-term survival and those with short-term survival was 28 and 27, respectively. PaC patients with long-term survival had significantly lower UP urinary levels at diagnosis (98.8 nmol/gCre) compared to those who showed short-term survival (125.2 nmol/gCre; p=0.042; Figure 4). However, the serum CA19-9 levels were not associated with long-term survival (244 U/ml in the short survival group *vs*. 540 U/ml in the long survival group, respectively; p=0.381).

Discussion

In this prospective study, we assessed the levels of porphyrin metabolites after ALA administration as a potential screening tool for the detection and prognosis prediction of PaC.

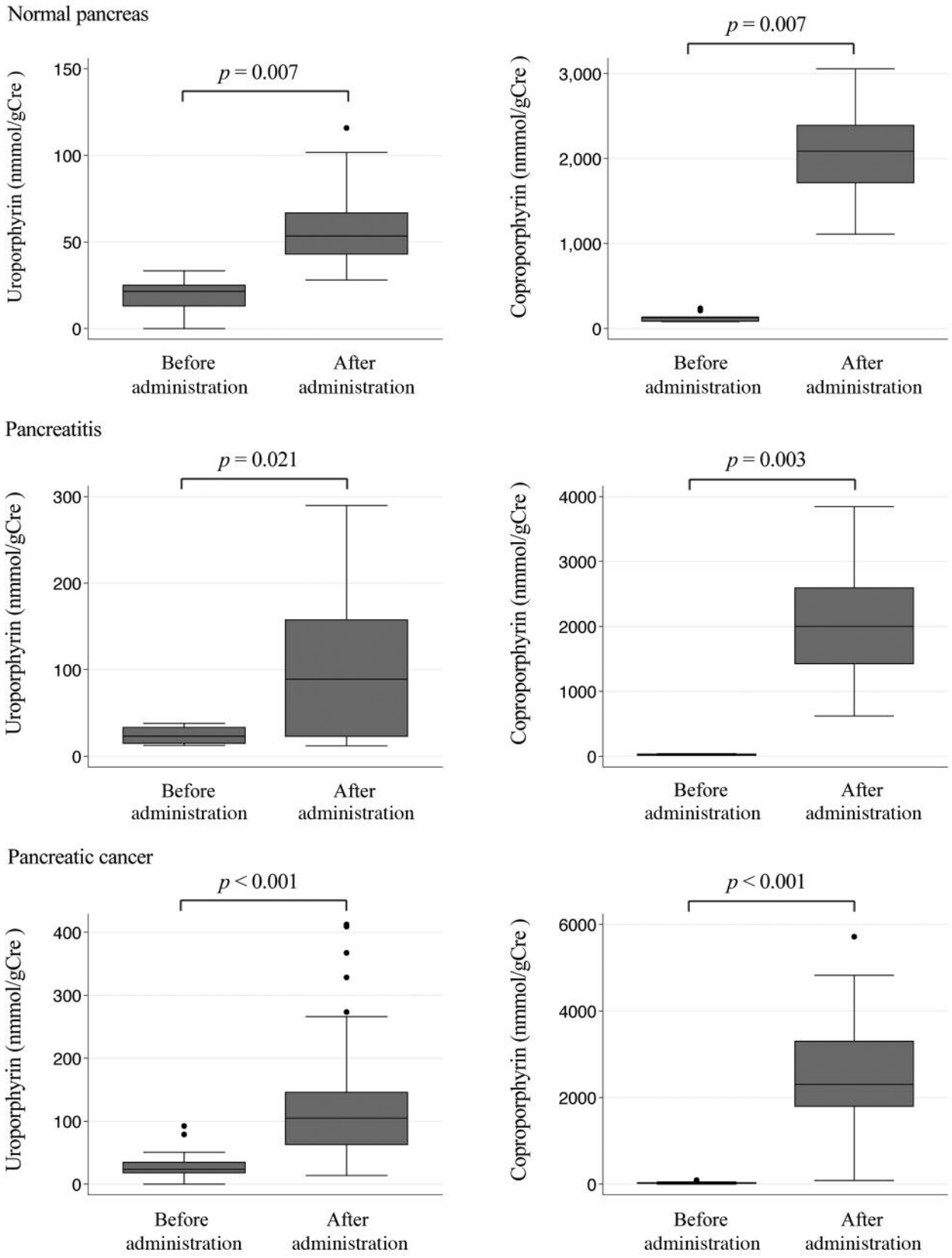

Figure 2. *Comparison between the urinary levels of uroporphyrin and coproporphyrin before and after administration of 5-aminolevulinic acid*.

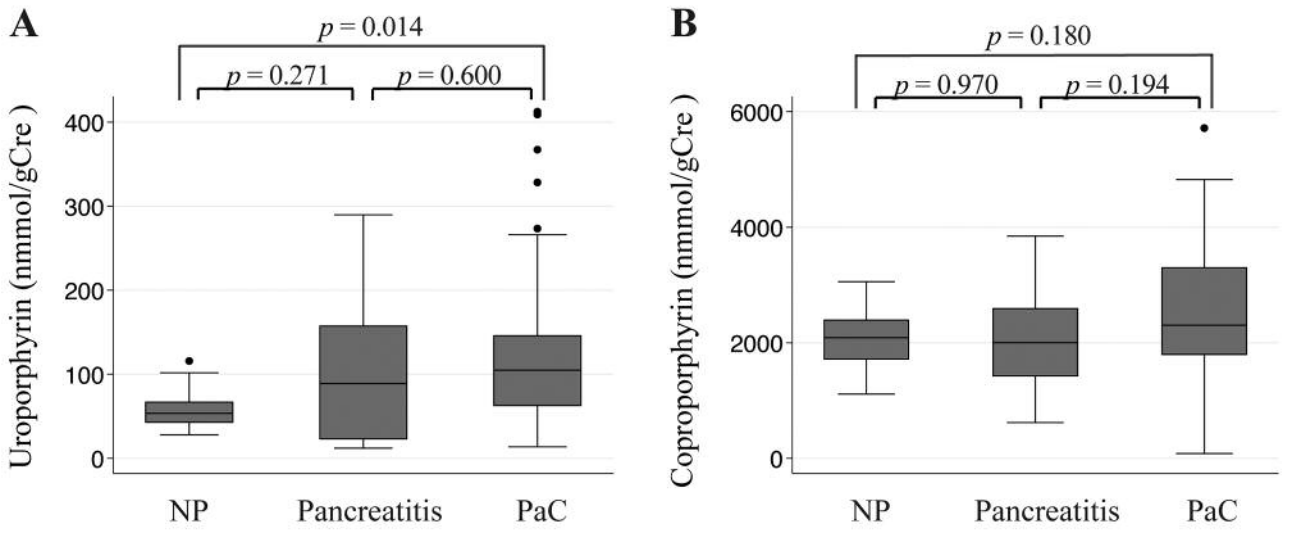

Figure 3. *Comparison between the urinary levels of uroporphyrin and coproporphyrin among the three groups. NP: Normal pancreas; PaC: pancreatic cancer.*

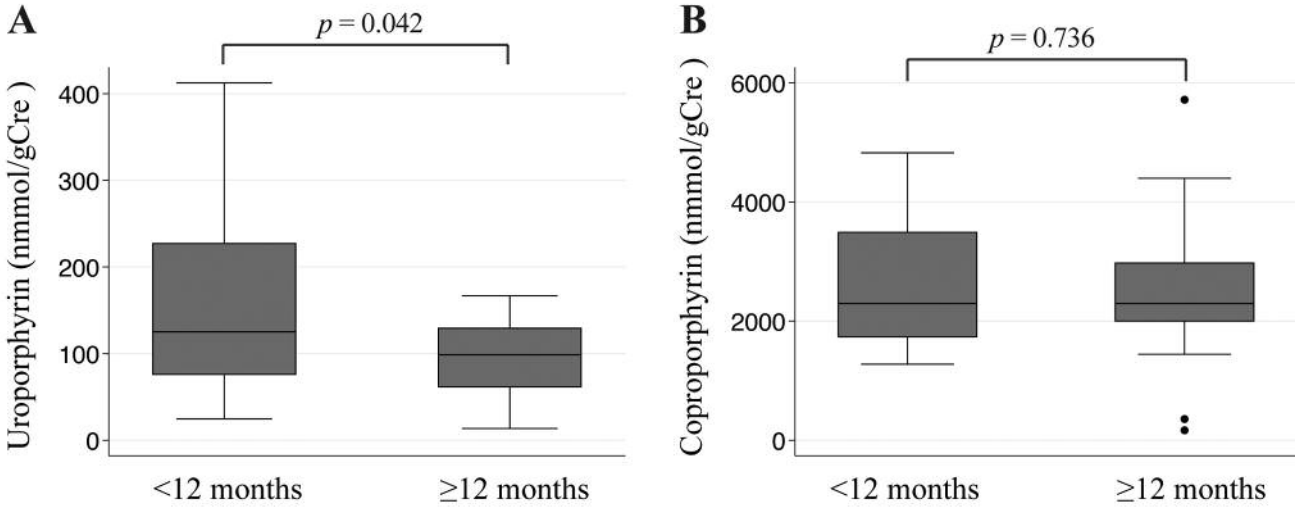

Figure 4. *Comparison of urinary levels of uroporphyrin and coproporphyrin between short-(<12 months) and long-term survival groups (≥12 months).*

Urinary UP and CP levels after ALA administration were higher in the PaC group than in the non-PaC groups, and particularly, a significant difference was found in the UP levels between the PaC and NP groups. Regarding the detection of PaC, the diagnostic efficiency of CA19-9 was improved when combined with urinary CP. Moreover, urinary UP levels were significantly higher in patients with PaC who showed short-term survival compared to those who showed long-term survival.

PaC has a poor prognosis because most patients have surgically unresectable disease; 80-85% of PaC patients

Table II. *Sensitivity, specificity, and diagnostic accuracy of CA19-9, uroporphyrin (UP), and coproporphyrin (CP) for the detection of pancreatic cancer.*

	Sensitivity	Specificity	Efficiency
CA19-9 (cut-off value >49.7 U/ml)	74.6%	83.3%	76.5%
UP (cut-off value >102.1 nmol/gCr)	55.2%	70.0%	58.6%
CP (cut-off value >2609.3 nmol/gCr)	41.8%	85.0%	51.7%
CA19-9+UP	83.6%	57.9%	77.9%
CA19-9+CP	86.6%	68.4%	82.6%
CA19-9+UP+CP	89.6%	52.6%	81.4%

present with locally advanced or distance metastatic disease at diagnosis (17, 18). Therefore, early diagnosis of PaC is important to reduce the mortality rate. In current clinical practice, CA19-9 is the only diagnostic biomarker for PaC diagnosis, approved by the U.S. Food and Drug Administration (FDA) (19); however, its diagnostic potential is limited owing to its restricted sensitivity and specificity, supporting the urgent need for novel biomarkers. Furthermore, a prognostic biomarker might provide essential information regarding personalized treatment decisions for individual patients. This is the first report showing that analysis of UP and CP levels in urine after ALA administration, i.e. photodynamic screening, can serve as a novel potential diagnostic and prognosis biomarker for PaC.

When ALA is exogenously administered, PpIX, one of the porphyrins derived from ALA, selectively accumulates in cancer cells. UP and CP are also porphyrin metabolites of ALA, and urinary UP and CP levels are elevated after ALA administration in tumor-bearing mice and patients with cancer (11, 13, 14). Although the molecular mechanism of such a cancer-specific phenomenon is not well understood, a possible role of membrane transporters has been reported. In gastric and bladder cancer cells, increased expression of peptide transporter 1 (PEPT1) was involved in ALA influx into cells and decreased expression of the ATP-binding cassette transporter G2 (ABCG2) was involved in PpIX efflux out of the cells (5, 6). Furthermore, the ATP-binding cassette transporter B6 (ABCB6) in the plasma membrane exports CP III extracellularly, and increased expression of ABCB6 during hypoxia resulted in increased extracellular levels of CP III (20). No data are available about membrane transporters of porphyrins in PaC cells, which seem to overexpress PEPT1 and ABCG2 (21, 22). Our previous study on the photodynamic diagnosis of PaC demonstrated that pancreatic tissue obtained from PaC patients premedicated with ALA showed increased red fluorescence induced by the photosensitized emission of PpIX, indicating that PaC cells accumulate ALA-induced porphyrins (23). Accordingly, further investigations on ALA metabolites depending on the cancer type are required to enhance the diagnostic ability of photodynamic screening and diagnosis.

So far, tumor-bearing mice and patients with cancer are known to have higher levels of UP, CP, and PpIX after ALA administration compared to normal controls (11-14). The first clinical evidence of elevated urinary levels of porphyrin metabolites after ALA administration was shown in patients with bladder cancer. In that study, the sensitivity and specificity of urinary UP I and CP I levels for bladder cancer detection were high, ranging from 91.6% to 100% (13). Moreover, Kamada et al. have evaluated urinary porphyrin metabolites in patients with colorectal cancer and revealed urinary UP I and CP III levels were significantly higher in patients with colorectal cancer than in the control group (14). In that study, regarding the comparison between urinary porphyrin metabolites and conventional serum tumor markers for the diagnostic ability for colorectal cancer, the area under the curve (AUC) for the ROC curve of CP I (0.84) was higher compared to that of serum CEA (0.64). In addition, no severe adverse events were observed in these studies. All these results including ours highlight that the urinary porphyrin assay after ALA administration is useful and safe for cancer diagnosis.

In conclusion, although a large variety of diagnostic biomarkers for PaC, including circulating DNA, microRNAs, and methylated DNA, have been reported, photodynamic screening using ALA might be a promising biomarker for the diagnosis and prognosis prediction of PaC.

Conflicts of Interest

The Authors declare that there are no conflicts of interest regarding this study.

Authors' Contributions

Conception and design of the study: Tsukasa Ikeura, Urara Ota, Atsuko Kamiya, Kiwamu Takahashi, and Masahiro Ishizuka; Collection of subjects: Tsukasa Ikeura, Yuichi Hori, Toshiyuki Mitsuyama, Hideaki Miyoshi, Masaaki Shimatani, Kazushige Uchida, and Makoto Takaoka; Drafting: Tsukasa Ikeura; Revision of the manuscript; Urara Ota, Atsuko Kamiya, Kiwamu Takahashi, Masahiro Ishizuka, and Masaki Kaibori; Approval of the final version of the manuscript: Kazuichi Okazaki.

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