JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2013, 64, 1, 109-114
www.jpp.krakow.pl

A. BUDZYNSKA, E. NOWAKOWSKA-DULAWA, T. MAREK, H. BOLDYS, A. NOWAK, M. HARTLEB

DIFFERENTIATION OF PANCREATOBILIARY CANCER FROM BENIGN BILIARY STRICTURES USING NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN

Department of Gastroenterology and Hepatology, Medical University of Silesia, Katowice, Poland

Aim of the study was to investigate the value of serum and bile neutrophil gelatinase-associated lipocalin (NGAL) for distinguishing malignant strictures caused by cholangiocarcinoma (CCA) or pancreatic cancer from benign biliary strictures. The study was performed prospectively on patients admitted for endoscopic or radiologic biliary decompression. Forty patients with dilated biliary ducts, including 16 cases of CCA, 6 cases of pancreatic cancer, and 18 cases of benign biliary stricture were enrolled. Their sera and bile were collected to measure NGAL. Routine biochemistry including measurement of serum levels of carbohydrate antigens (CA) 19-9 and carcinoembryonic antigen (CEA) was also performed. The serum CA19-9, serum CEA, and bile NGAL levels were significantly increased in patients with malignant strictures as compared with patients with benign biliary diseases. Serum NGAL had no significant value for discriminating between malignant and benign biliary strictures. Bile NGAL levels had a receiver characteristic area under the curve of 0.74, sensitivity 77.3, and specificity 72.2% for discriminating between pancreatobiliary cancer and benign biliary diseases. Bile NGAL and serum CA19-9 were independent parameters and their combined use improved diagnostic accuracy (sensitivity 91%, negative predictive value 85.7%). We conclude that measurement of biliary, but not serum NGAL, may differentiate malignant pancreatobiliary from benign biliary strictures, serving as a complementary biomarker for serum CA19-9.

Key words: *benign biliary strictures, neutrophil gelatinase-associated lipocalin, cholangiocarcinoma, pancreatic cancer*

INTRODUCTION

The incidence rate of cholangiocarcinoma (CCA) has significantly increased over the last few decades (1-3). Biliary cancer is divided into two types, intrahepatic and the more common extrahepatic type, which in the advanced stage presents as obstructive jaundice. CCA typically grows longitudinally along the bile ducts without forming a mass, therefore imaging techniques are generally not useful for visualizing this neoplasm (4). Currently, the diagnosis of CCA is based mainly on magnetic resonance imaging and endoscopic cholangiography. Tissue sampling from biliary duct strictures can be performed by brush smear or biopsy forceps, but cytology and histopathology examinations have limited sensitivity, generally not exceeding 60% (5). Introduction of new diagnostic modalities such as digital imaging analysis and fluorescence *in situ* hybridization have improved differentiation between benign and malignant biliary strictures (6, 7). Widely used serum markers, such as carbohydrate antigen 19-9 (CA 19-9) and carcinoembryonic antigen (CEA), are not specific for CCA and have unsatisfactory diagnostic sensitivity.

A definitive diagnosis is difficult to obtain in CCA patients, as there are many causes of benign biliary stenoses having an inflammatory (autoimmune or infective), genetic, vascular, or iatrogenic background. Moreover, the malignant strictures can also be of pancreatic or metastatic origin. Diagnostic uncertainty is responsible for the considerable delay in a definitive diagnosis and treatment. Surgery is the only potentially curative treatment, but it can be offered to only a minority of patients (8, 9). Therefore, markers to discriminate between benign and early stage malignant disease of extrahepatic biliary ducts are urgently needed.

The bile seems to be an attractive medium for diagnostic purposes as it is potentially rich in husking epithelium and tumor-derived products. Considering that obstructive jaundice is usually an indication for endoscopic intervention, the bile is easily accessible.

Recent studies indicated that bile may be a source of diagnostically valuable biomarkers (10-13). Neutrophil gelatinase-associated lipocalin (NGAL) is a low molecular weight protein released from activated neutrophils. This protein can bind and transport different hydrophobic substances into the cell cytoplasm, where it forms a complex with the gelatinase, matrix metalloproteinase-9 (14). High NGAL expression was observed in many tumors (15), including tissues of esophageal cancer (16), gastric cancer (17), pancreatic cancer (18) and cell lines of colon cancer (19) and CCA (20). Elevated serum levels of NGAL have been reported in patients with gastric (17, 21), pancreatic (18), and hepatocellular cancer (22). Only a couple studies, however, have examined both serum and bile levels of NGAL in patients with CCA, and these studies have provided ambiguous results (13, 23).

The aim of the present study was to determine the diagnostic accuracy of serum and bile concentrations of NGAL as biomarkers useful for differentiating strictures caused by CCA or pancreatic cancer infiltrating the common bile duct from benign biliary stenoses.

MATERIAL AND METHODS

Study design

Forty patients (17 women, 23 men) with cholestasis due to bile flow obstruction at the level of the extrahepatic biliary tract demonstrated by magnetic resonance imaging or CT were prospectively enrolled into the study. The study was performed from July 2010 to December 2011 among patients admitted to Department of Gastroenterology and Hepatology of Medical University of Silesia, Katowice, Poland. All patients were qualified for drainage procedures by endoscopic stenting or percutaneous transhepatic biliary decompression. Exclusion criteria were gall stone disease, chronic pancreatitis, malignancy other than pancreatobiliary cancer, and unsuccessful catheterization of the common bile duct during endoscopic retrograde cholangiopancreatography (ERCP) or no access to the biliary tree by percutaneous transhepatic cholangiography (PTCA). The study was approved by local ethics committee (nr KNW/0022/KB1/98/10) and informed consent was received from each patient.

Serum and bile samples

Fasting peripheral venous blood samples were collected on the day of ERCP or PTCA. Blood morphology and serum biochemistry, including alkaline phosphatase (ALP), γ-glutamyltranspeptidase (GGTP), alanine transaminase (ALT), aspartate transaminase (AST), bilirubin and C-reactive protein (CRP) were determined by flow cytometry (Sysmex XT-1800i) and colorymetry (Olympus AU680), respectively. Serum levels of CA19-9 and CEA were measured by immunoassay (CMIA Architect i2000SR). All automated methods are routinely used in the hospital's central laboratory. Additionally, 5 ml of blood and 10 ml of bile were obtained, centrifuged for 15 min at 1500 g and immediately stored in small aliquots at –80°C. Bile samples were aspirated after common bile duct cannulation, immediately before contrast administration. Bile and serum levels of NGAL were measured using a commercially available ELISA kit (BioVendor R&D Systems, Czech Republic) according to each manufacturer's protocol. NGAL levels were calculated from the standard calibration curve.

Statistical analysis

Statistical analysis was performed using Statistica 10 software (StatSoft, Polska). The Mann-Whitney U test or Student's t-test were used for analysis of quantitative variables as appropriate. Data are presented as mean ±S.D. The Kruskal-Wallis analysis of variance was used for comparisons between more than two groups. The Pearson Chi-squared test was used for comparisons of independent groups. A P value of less than 0.05 was considered significant. Receiver operating characteristic (ROC) analyses for serum Ca19-9, serum CEA, and bile NGAL levels were performed and the area under the curve (AUC-ROC) was obtained to compare the significance of these variables for the differentiation of benign and malignant strictures. The optimal cut-off points, and sensitivity, specificity, and positive and negative predictive values were estimated from the ROC curve analysis. Correlation analysis between serum CA19-9, serum CEA, bile NGAL, and serum routine laboratory parameters was performed using the Spearman rank test.

RESULTS

Patient characteristics

Forty patients (17 women, 23 men) with biliary duct strictures were enrolled into the study. ERCP was successful in 36 of the patients and 4 patients were referred to PTCA. The biliary stricture was benign in 18, and malignant in 22. CCA was diagnosed in 16 patients and pancreatic cancer in 6 patients. The CCAs were located at the level of perihilar duct branching (Klatskin tumor) in 10 cases, and in the middle or distal portions of the common bile duct in 2 and 4 cases, respectively. The diagnosis of pancreatobiliary cancer was based on cytologic or histologic examination during ERCP in 7 of 22 cases. However, most diagnoses were based on the results of ultrasound, CT/MRI, MRCP and ERCP (*Table 1*) and later confirmed by follow-up data. Benign biliary strictures included 5 cases of primary

Table 1. Diagnostic procedures and follow-up data of patients with malignant and benign common bile duct strictures.

Diagnostic procedure /treatment/follow-up	Malignant stricture N = 22	Benign stricture N= 18
MRCP	9 (41%)	9 (50%)
Spiral CT	21 (96%)	12 (67%)
EUS + FNAB	1 (4.6%)	1 (6%)
Histology/cytology (ERCP)	6 (27%)[a]	0
Histology (surgery)	7 (32%)	6 (33%)
Chemo/radiotherapy	6 (27%)[b]	0
Death	18 (82%)	5 (28%)[c]
Follow-up (months± SD)	10.7±8.7	17.3±6.5

MRI - magnetic resonance imaging; MRCP - magnetic resonance cholangiopancreatography; CT - computed tomography; EUS - endoscopic ultrasonography; FNAB - fine needle biopsy; ERCP - endoscopic retrograde cholangiography.
[a]CCA in 5 cases, [b]CCA in all cases, [c]2 deaths due to postransplant complications, 3 deaths related to co-morbidities.

sclerosing cholangitis, 12 cases of iatrogenic postoperative stenosis and 1 case of adenoma of the papilla Vater. Biliary drainage was performed in all 22 patients with malignant disease and 15 of 18 patients with benign stricture. The patients' clinical and laboratory characteristics are shown in *Table 2*. Patients with malignancies did not differ in sex or body mass index from those with benign strictures, but tended to be older (p=0.06). The prevalence of weight loss, pain, and fever were similar between groups. At the admission jaundice was more frequent in patients with malignant as compared with benign biliary stricture (91% *vs*. 44%, p=0.001). White blood cell count; hemoglobin concentration; and serum CRP, ALT, AST, and ALP levels were not significantly different between patients with pancreatobiliary cancers and benign biliary strictures. However, platelet count and serum levels of GGTP and bilirubin, were significantly higher in patients with malignant than in those with benign biliary disease.

Follow-up data

Follow-up data were collected until January 2013 by reviewing medical records of hospitalized patients or telephone contact. None of the 10 patients with perihilar cholangiocarcinoma was operated due to advanced stage of disease - all but one were classified as type IV of Bismuth classification. Three patients with distal cholangiocarcinoma were operated - for two of them this treatment was curative and in one the tumour appeared to be unresectable. In

Table 2. Clinical and laboratory characteristics of patients with malignant (CCA & pancreatic cancer) and benign biliary tract strictures.

	Malignancy N = 22	Benign disease N = 18	*P* value
Age	63.8±9.4	54.8±19.7	0.06
Female/male	9/13	8/10	0.82
Jaundice	20 (91%)	8 (44%)	0.01
Fever	4 (18%)	5 (28%)	0.61
Pain	7 (32%)	6 (33%)	0.94
Weight loss (> 5kg /6 months)	13 (59%)	8 (44%)	0.43
Hemoglobin (11.5-15.0 g/dl)	12.1±1.5	13.2±1.6	0.05
WBC (4.0-10.0 G/l)	8.3±3.1	7.9±4.2	0.74
Platelets (130-400 G/l)	322±131	214±105	0.007
CRP (< 5 mg/l)	36.1±46.0	25.7±54.1	0.51
ALT (< 34U/l)	124±91.4	75.5±103	0.12
AST (< 31U/l)	134±111	87.5±133	0.23
Alk. phosphatase (38-126U/l)	434±188	312±409	0.22
GGTP (< 38U/l)	624±462	242±295	0.004
Bilirubin (0.3-1.2 mg/dl)	19.1±12.5	3.21±3.42	<0.0001

Parameters presented as frequencies (percent) or mean values ±S.D.
ALT - alanine transaminase; AST - aspartate transaminase; CRP - C-reactive protein; GGTP - γ-glutamyltranspeptidase.

Table 3. Serum CA 19-9 - serum CEA and serum and bile NGAL concentrations in patients with pancreatobiliary malignant and benign bile duct strictures.

	Benign	Malignant	P value benign *vs*. malignant	CCA	P value benign *vs*. CCA	Pancreatic cancer	P value benign *vs*. pancreatic cancer
CA 19-9 (0–37 U/ml)	38.3 (75.4)	5690 (13000)	<0.0001	7267 (15008)	0.0001	1484 (2235)	0.02
CEA (0–5 ng/ml)	1.7 (1.0)	27.7 (65.4)	<0.0001	19.7 (41.2)	0.0001	48.9 (110)	0.01
Serum NGAL ng/ml	95.1 (51.9)	91.9 (57.4)	0.79	97.5 (59.4)	0.94	77.1 (53.8)	0.42
Bile NGAL ng/ml	674 (966)	1244 (1186)	0.01	1379 (1361)	0.02	884 (372)	0.08

CA 19-9 - carbohydrate antigens 19-9; CCA - cholangiocarcinoma; CEA - carcinoembryonic antigen; NGAL - neutrophil gelatinase-associated lipocalin. Mean values ±S.D.

cholangiocarcinoma group only two radically operated patients are still alive. Among 6 patients with pancreatic cancer four were operated, but only in one case the surgery was radical (two patients are alive). All operated patients received a histopathologic confirmation of the cancer. Three patients with cholangiocarcinoma received palliative radiotherapy and the other three received chemotherapy.

Six of 18 patients with benign biliary stricture finally required surgery, including 3 patients with primary sclerosing cholangitis who underwent liver transplantation. Two of them died because of post-transplant complications and other three died of co-morbidities.

Serum levels of CA 19-9, CEA, and neutrophil gelatinase-associated lipocalin in patients with cholangiocarcinoma, pancreatic cancer, and benign biliary strictures

Concentrations of serum CA19-9, CEA, NGAL, and bile NGAL are shown in *Table 2*. Serum CA19-9 levels were significantly higher in patients with malignant than in those with benign biliary stricture (5690±13,000 U/ml *vs*. 38.3±75.0 U/ml; p<0.0001). Similarly, serum CEA concentration was significantly higher in patients with malignancies compared to patients with benign biliary disease (27.7±665.0 ng/ml *vs*. 1.7±1.0 ng/ml; p<0.0001). Serum CA19-9 did not significantly differ between patients with CCA and those with pancreatic cancer (7267±15,007 U/ml *vs*. 1484±2234 U/ml, p=0.5). The CEA concentration also did not differentiate patients with CCA and pancreatic cancer (19.7±41 *vs*. 48.9±109 ng/ml; p=0.9). Serum NGAL concentrations were statistically indistinguishable between patients with CCA (97.4±59 ng/ml), pancreatic cancer (77.0±53 ng/ml), and benign biliary strictures (95.1±51 ng/ml).

Bile neutrophil gelatinase-associated lipocalin levels in patients with cholangiocarcinoma, pancreatic cancer, and benign biliary strictures

Bile NGAL concentration was significantly higher in patients with pancreatobiliary cancers than in those with benign biliary strictures (1244±1185 ng/ml *vs*. 674±965 ng/ml; p=0.01). This difference was also statistically significant for CCA patients (p=0.02), but not for patients with pancreatic cancer (p=0.08; *Table 3*). The difference between bile NGAL concentration in CCA and pancreatic cancer patients was not statistically significant (1379±1361 ng/ml *vs*. 884±372 ng/ml: p=0.94).

Table 4. Spearman's coefficients showing correlations between serum CA 19-9, serum CEA and bile NGAL and routine laboratory data.

	CA 19-9	CEA	Bile NGAL
Hemoglobin	–0.2348	–0.2492	–0.3261*
Leukocytes count	0.1192	0.1030	0.0984
Platelets count	0.3011	0.3827*	0.1270
CRP	0.1816	0.2474	0.4103*
ALT	0.4658*	0.2137	0.0420
AST	0.5458*	0.2505	0.1176
Alk. phosphatase	0.4677*	0.2600	0.1566
GGTP	0.4555*	0.3051	0.1944
Bilirubin	0.6007*	0.4999*	0.3910*
CEA	0.4980*	-	0.2566
CA 19-9	-	0.4980*	0.1889
Bile NGAL	0.1889	0.2566	-

ALT - alanine transaminase; AST - aspartate transaminase; CA 19-9 - carbohydrate antigen 19-9; CEA - carcinoembryonic antigen; CRP - C-reactive protein; GGTP - γ-glutamyltranspeptidase; NGAL - neutrophil gelatinase-associated lipocalin; p<0.05.

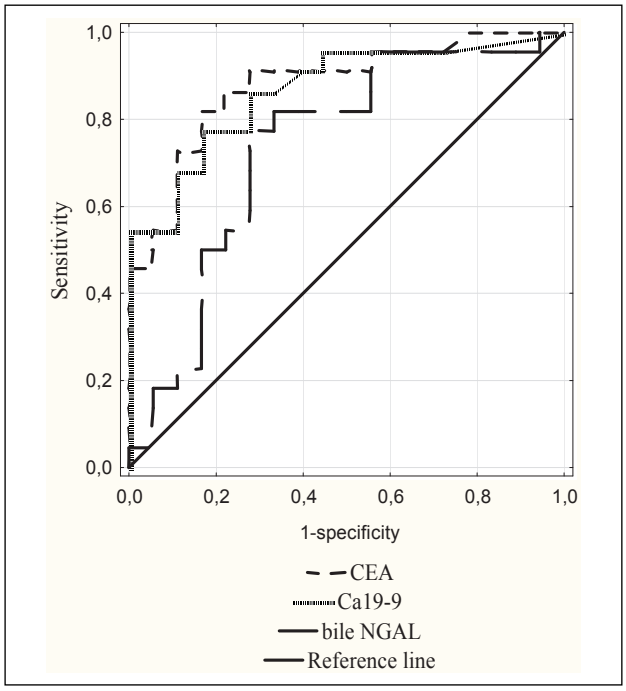

Fig. 1. AUC-ROC of serum CA19-9, CEA, and bile NGAL for diagnoses of malignant bile duct strictures.

Table 5. Diagnostic performance of serum and biliary biomarkers in distinguishing pancreatobiliary cancers from benign biliary strictures.

	Sensitivity %	Specificity %	Accuracy %	PPV %	NPV %
Serum CA 19-9	77.3	83.3	80.0	85.0	75.0
Serum CEA	81.8	83.3	82.5	85.7	78.9
Bile NGAL	77.3	72.2	75.0	77.3	72.2
Serum CA 19-9 & Bile NGAL	90.9	66.7	80.0	76.9	85.7

CEA - carcinoembryonic antigen; CA 19-9 - carbohydrate antigen 19-9; NGAL - neutrophil gelatinase-associated lipocalin; NPV - negative predictive value; PPV - positive predictive value. Cut-off value for CA19-9 was 30.1 U/ml, for CEA was 2.56 ng/ml and for bile NGAL was 459 ng/ml.

Correlations between cancer markers and routine laboratory data

The relationships between tumor markers: CEA, CA19-9, NGAL and routine laboratory parameters are shown in *Table 4*. Serum CA19-9 was significantly correlated with ALT, AST, ALP, GGTP, bilirubin, and CEA levels (*Table 4*). Serum CEA concentrations were significantly correlated with platelet count, serum bilirubin, and CA19-9 levels. Bile NGAL was correlated with hemoglobin, CRP, and bilirubin levels. Nevertheless, the correlation coefficients for all these parameters were low (<0.7).

Diagnostic accuracy of cancer markers in discriminating between pancreatobiliary cancers and benign biliary strictures

The AUC-ROCs for serum CA19-9, serum CEA, and bile NGAL were 0.869, 0.878, and 0.735, respectively (*Fig. 1*). The estimated cut-off values for serum CA19-9, serum CEA, and bile NGAL were 30.1 U/ml, 2.56 ng/ml, and 459 ng/ml, respectively. The AUC-ROC values for serum CA19-9, serum CEA, and bile NGAL were not significantly different. The sensitivity, specificity, accuracy, and positive and negative predictive values for diagnosis of malignant biliary duct strictures for the established cut-off levels are given in *Table 5*. The combined use of serum CA19-9 and bile NGAL had a diagnostic sensitivity of 90.9%.

DISCUSSION

NGAL is a small molecular weight glycoprotein comprising more than 50 molecules that belongs to the lipocalin family (15, 24). This glycoprotein creates complexes with matrix metalloproteinase-9 (14) and plays an important role in several stages of carcinogenesis, as well as in apoptosis and inflammation (15, 25, 26). Moreover, NGAL probably protects matrix metalloproteinase-9 from degradation (15, 20). Higher levels of matrix metalloproteinase-9 were found in pancreatic cancer and were associated with tumor invasiveness and lymph node metastases (15, 20, 27).

Increased NGAL expression is observed in early epithelial lesions that can lead to invasive carcinomas, namely ovarian borderline tumors (28), esophageal dysplasia (16), intraductal papillary mucinous neoplasm (29), and pancreatic intraepithelial neoplasia (19). Further, NGAL expression is positively correlated with late-stage and poor prognosis of breast (30) and gastric cancers (17).

High NGAL expression in CCA cell lines and tissues was observed (13, 20, 32). Two studies investigating the diagnostic performance of serum or bile NGAL in patients with pancreatobiliary cancer were recently published (13, 23). In the first, Leelawat *et al.* (23) showed similar usefulness of serum measurements of NGAL and CA19-9 in the discrimination between CCA and benign bile duct obstruction. However, in that study the bile NGAL was not measured. In another elegant study performed on training and validation subsets of patients, the bile NGAL differentiated pancreatobiliary cancer from benign biliary disease with 94% sensitivity (13). An important limitation of that study was small number of patients with CCA, while it is known that CA19-9 and CT are much more efficient for diagnosing pancreatic tumors than CCA. Moreover, the benign biliary group was heterogenous involving patients with gall stone disease, sphincter of Oddi dysfunction and pancreas divisum, diseases usually recognizable without serological markers.

In the present study, serum NGAL had no diagnostic value for pancreatobiliary cancer. Analogous results were obtained by Zabron *et al.* (13) who reported comparable serum NGAL levels in patients with malignant and benign biliary diseases. The discrepancies might result from differences in patient recruitment, particularly in regard to the stage of cancer. A recent study demonstrated that NGAL mRNA expression in pancreatic cancer correlates with the differentiation of the neoplasm, achieving high levels in early dysplastic lesions and declining to low values in poorly differentiated cancer (19). The near normal serum NGAL level in patients with cancer in our study could indicate an advanced stage of neoplasm as only one of patients with pancreatic cancer had resectable tumour. If bile NGAL is truly a marker of dysplastic lesions in the biliary tree, it would be important to study this protein in patients with primary sclerosing cholangitis.

In contrast to serum NGAL, bile NGAL was elevated in patients with malignant biliary strictures in our study and unlike pancreatic cancer, the difference between patients with CCA and benign biliary disease was statistically significant. Diagnostic performance of bile NGAL in distinguishing malignant from benign biliary strictures was characterized by an AUC-ROC of 0.735, having similar efficacy to that of CA19-9 and CEA. The cut-off value for bile NGAL was estimated to be 459 ng/ml with reasonable sensitivity and specificity to detect pancreatobiliary malignancy (77.3% and 72.2%, respectively). We assume that NGAL is an independent biomarker of biliary cancer, as it did not show any relationship with CA19-9 or CEA. This may be important as it is known that serum CA19-9 levels may be considerably altered by bacterial cholangitis, which is a frequent complication of biliary strictures, irrespective of their etiology. The combined use of bile NGAL and serum CA19-9 improved the diagnostic sensitivity to 91% at a cost of reduced specificity. The bile NGAL level correlated with hemoglobin, bilirubin, and serum CRP levels. The relationship with CRP might reflect the "stress protein" behavior of NGAL, associated with cancer (15). In a study by Moniaux *et al.* (19), the serum NGAL level was elevated in pancreatic cancer to the same degree as in acute pancreatitis.

In conclusion, our findings indicated that NGAL concentration in bile is elevated in patients with malignant biliary strictures, especially in CCA. This protein may differentiate malignant from benign biliary disease, but cannot discriminate between biliary and pancreatic cancer. The mechanisms of NGAL biliary overexpression seem to differ from that of CA19-9, and therefore these biomarkers may have complementary diagnostic significance. Further studies are needed to clarify the role of NGAL in tumorigenesis, its relationship with the biliary cancer stage, and the effect on clinical outcome.

Acknowledgements: We thank Dr. Roman Kaczor for collecting bile during ERCP and Mrs. Maria Wikarek for assistance with the laboratory analysis. This work was supported by the grant KNW 1-104/10 from the Medical University of Silesia, Katowice, Poland.

Conflict of interests: None declared.

REFERENCES

1. Welzel TM, McGlynn KA, Hsing AW, O'Brien TR, Pfeiffer RM. Impact of classification of hilar cholangiocarcinomas (Klatskin tumors) on the incidence of intra- and extrahepatic cholangiocarcinoma in the United States. *J Natl Cancer Inst* 2006; 98: 873-875.
2. Khan SA, Emadossadaty S, Ladep N, Thomas HC, *et al.* Rising trends in cholangiocarcinoma: is the ICD classification system misleading us? *J Hepatology* 2012; 56: 848-854.

3. Khan SA, Toledano MB, Taylor-Robinson SD. Epidemiology, risk factors and pathogenesis of cholangiocarcinoma. *HPB* (*Oxford*) 2008; 10: 77-82.
4. Weber A, Schmid RM, Prinz C. Diagnostic approaches for cholangiocarcinoma. *World J Gastroenterol* 2008; 14: 4131-4136.
5. Weber A, von Weyhern C, Fend F, *et al.* Endoscopic transpapillary brush cytology and forceps biopsy in patients with hilar cholangiocarcinoma. *World J Gastroenterol* 2008; 14: 1097-1101.
6. Baron TH, Harewood GC, Rumalla A, *et al.* A prospective comparison of digital image analysis and routine cytology for the identification of malignancy in biliary tract strictures. *Clin Gastroenterol Hepatol* 2004; 2: 214-219.
7. Smoczynski M, Jablonska A, Matyskiel A, *et al.* Routine brush cytology and fluorescence in situ hybridization for assessment of pancreatobiliary strictures. *Gastrointest Endosc* 2012; 75: 65-73.
8. Seehofer D, Kamphues C, Neuhaus P. Management of bile duct tumors. *Expert Opin Pharmacother* 2008; 9: 2843-2856.
9. Khan SA, Davidson BR, Goldin RD, Heaton N, *et al.* Guidelines for the diagnosis and treatment of cholangiocarcinoma: an update. *Gut* 2012; 61: 1657-1669.
10. Alvaro D, Macarri G, Mancino MG, *et al.* Serum and biliary insulin-like growth factor I and vascular endothelial growth factor in determining the cause of obstructive cholestasis. *Ann Intern Med* 2007; 147: 451-459.
11. Korner T, Kropf J, Hackler R, Brenzel A, Gressner AM. Fibronectin in human bile fluid for diagnosis of malignant biliary diseases. *Hepatology* 1996; 23: 423-428.
12. Alvaro D. Serum and bile biomarkers for cholangiocarcinoma. *Curr Opin Gastroenterol* 2009; 25: 279-284.
13. Zabron AA, Horneffer-van der Sluis VM, Wadsworth CA, *et al*. Elevated levels of neutrophil gelatinase-associated lipocalin in bile from patients with malignant pancreatobiliary disease. *Am J Gastroenterol* 2011; 106: 1711-1717.
14. Kjeldsen L, Johnsen AH, Sengelov H, Borregaard N. Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. *J Biol Chem* 1993; 268: 10425-10432.
15. Bolignano D, Donato V, Lacquaniti A, *et al.* Neutrophil gelatinase-associated lipocalin (NGAL) in human neoplasias: a new protein enters the scene. *Cancer Lett* 2010; 288: 10-16.
16. Zhang H, Xu L, Xiao D, *et al.* Upregulation of neutrophil gelatinase-associated lipocalin in oesophageal squamous cell carcinoma: significant correlation with cell differentiation and tumour invasion. *J Clin Pathol* 2007; 60: 555-561.
17. Wang HJ, He XJ, Ma YY, *et al.* Expressions of neutrophil gelatinase-associatedlipocalin in gastric cancer: a potential biomarker for prognosis and an ancillarydiagnostic test. *Anat Rec* (*Hoboken*) 2010; 293: 1855-1863.18.
18. Moniaux N, Chakraborty S, Yalniz M, *et al.* Early diagnosis of pancreatic cancer: neutrophil gelatinase-associated lipocalin as a marker of pancreatic intraepithelial neoplasia. *Br J Cancer* 2008; 98: 1540-1547.
19. Hu L, Hittelman W, Lu T, *et al.* NGAL decreases E-cadherin-mediated cell-cell adhesion and increases cell motility and invasion through Rac1 in colon carcinoma cells. *Lab Invest* 2009; 89: 531-548.
20. Nuntagowat C, Leelawat K, Tohtong R. NGAL knockdown by siRNA in human cholangiocarcinoma cells suppressed invasion by reducing NGAL/MMP-9 complex formation. *Clin Exp Metastasis* 2010; 27: 295-305.
21. Chan AOO, Chu K-M, Lam SK, *et al.* Early prediction of tumor recurrence after curative resection of gastric carcinoma by measuring soluble E-cadherin. *Cancer* 2005; 104: 740-746.
22. Soyama A, Eguchi S, Takatsuki M, *et al.* Significance of the serum level of soluble E-cadherin in patients with HCC. *Hepatogastroenterology* 2008; 55: 1390-1393.
23. Leelawat K, Narong S, Wannaprasert J, Leelawat S. Serum NGAL to clinically distinguish cholangiocarcinoma from benign biliary tract diseases. *Int J Hepatol* 2011; 2011: 873548. DOI: 10.4061/2011/873548.
24. Xu S, Venge P. Lipocalins as biochemical markers of disease. *Biochim Biophys Acta* 2000; 1482: 298-307.
25. Zerega B, Cermelli S, Michelis B, Cancedda R, Cancedda FD. Expression of NRL/NGAL (neu-related lipocalin/neutrophil gelatinase-associated lipocalin) during mammalian embryonic development and in inflammation. *Eur J Cell Biol* 2000; 79: 165-172.
26. Jayaraman A, Roberts KA, Yoon J, *et al.* Identification of neutrophil gelatinase-associated lipocalin (NGAL) as a discriminatory marker of the hepatocyte-secreted protein response to IL-1beta: a proteomic analysis. *Biotechnol Bioeng* 2005; 91: 502-515.
27. Lekstan A, Lampe P, Lewin-Kowalik J, *et al.* Concentrations and activities of metalloproteinases 2 and 9 and their inhibitors (TIMPS) In chronic pancreatitis and pancreatic adenocarcinoma. *J Physiol Pharmacol* 2012; 63: 589-599.
28. Lim R, Ahmed N, Borregaard N, *et al.* Neutrophil gelatinase-associated lipocalin (NGAL) an early-screening biomarker for ovarian cancer: NGAL is associated with epidermal growth factor-induced epithelio-mesenchymal transition. *Int J Cancer* 2007; 120: 2426-2434.
29. Terris B, Blaveri E, Crnogorac-Jurcevic T, *et al.* Characterization of gene expression profiles in intraductal papillary-mucinous tumors of the pancreas. *Am J Pathol* 2002; 160: 1745-1754.
30. Bauer M, Eickhoff JC, Gould MN, Mundhenke C, Maass N, Friedl A. Neutrophil gelatinase-associated lipocalin (NGAL) is a predictor of poor prognosis in human primary breast cancer. *Breast Cancer Res Treat* 2008; 108: 389-397.
31. Furutani M, Arii S, Mizumoto M, Kato M, Imamura M. Identification of a neutrophil gelatinase-associated lipocalin mRNA in human pancreatic cancers using a modified signal sequence trap method. *Cancer Lett* 1998; 122: 209-214.
32. Han H, Bearss DJ, Browne LW, Calaluce R, Nagle RB, Von Hoff DD. Identification of differentially expressed genes in pancreatic cancer cells using cDNA microarray. *Cancer Res* 2002; 62: 2890-2896.

Received: September 25, 2012
Accepted: February 25, 2013

Author's address: Dr. Agnieszka Budzynska, Department of Gastroenterology and Hepatology, Medical University of Silesia, 14 Medykow Street, 40-752 Katowice, Poland.
E-mail: budzynskaagnieszka@poczta.onet.pl