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ARTICLE

Proteomic Mucin Profiling for the Identification of Cystic Precursors of Pancreatic Cancer

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Background Pancreatic cystic lesions (PCLs) are increasingly frequent radiological incidentalomas, with a considerable proportion representing precursors of pancreatic cancer. Better diagnostic tools are required for patients to benefit from this development.

Methods To evaluate whether cyst fluid mucin expression could predict malignant potential and/or transformation in PCLs, a proteomic method was devised and prospectively evaluated in consecutive patients referred to our tertiary center for endoscopic ultrasound-guided aspiration of cystic lesions from May 2007 through November 2008 (discovery cohort) and from December 2008 through October 2012 (validation cohort). Cytology and cyst fluid carcinoembryonic antigen (CEA; premalignancy > 192 ng/mL, malignancy > 1000 ng/mL) were routinely analyzed, and samples were further processed as follows: one-dimensional gel electrophoresis, excision of high-mass areas, tryptic digestion and nano-liquid chromatography–tandem mass spectrometry, with peptide identification by Mascot software and an in-house mucin database. All diagnostic evaluations were blinded to proteomics results. Histology was required to confirm the presence/absence of malignant transformation. All statistical tests were two-sided.

Results Proteomic mucin profiling proved statistically significantly more accurate (97.5%; 95% confidence interval [CI] = 90.3% to 99.6%) than cytology (71.4%; 95% CI = 59.8% to 80.9%; $P < .001$) and cyst fluid CEA (78.0%; 95% CI = 65.0% to 87.3%; $P < .001$) in identifying the 37 (out of 79; 46.8%) lesions with malignant potential (ie, premalignant or malignant tumors). The accuracy of proteomics was nearly identical (96.6% vs 98.0%) between the discovery (n = 29) and validation (n = 50) cohorts. Furthermore, mucin profiling predicted malignant transformation, present in 16 out of 29 (discovery cohort: 9, validation cohort: 20) lesions with available histology, with 89.7% accuracy (95% CI = 71.5% to 97.3%) (for the validation cohort only: 95.0%; 95% CI = 73.1% to 99.7%). This markedly exceeded corresponding results for cytology (51.7%; 95% CI = 32.9% to 70.1%; $P = .003$) and CEA (57.1%; 95% CI = 34.4% to 77.4%; $P = .02$).

Conclusions Proteomic cyst fluid mucin profiling robustly discriminates benign, premalignant, and malignant PCLs. Consequently, it may improve pancreatic cancer prevention and reduce the morbidity burden of unwarranted pancreatic surgery.

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Pancreatic cystic lesions (PCLs) are increasingly common unexpected findings on imaging, identifiable on up to 20% of abdominal magnetic resonance imaging examinations (1). Because PCLs almost invariably reflect an underlying inflammatory or neoplastic condition, they rank among the most important incidentalomas to have emerged with radiological advances. Above all, this development has offered a unique opportunity for preventive intervention against pancreatic cancer (ductal adenocarcinoma) because a substantial proportion of cystic tumors can be considered precursor lesions of this devastating disease (2–5).

PCLs fall into two categories: lesions with or without malignant potential. Serous cystic neoplasms are the only pancreatic cystic tumors that can safely be regarded as benign (5,6). PCLs with malignant potential include rare neuroendocrine tumors or ductal adenocarcinomas with cystic degeneration, solid pseudopapillary neoplasms, and the much more prevalent mucinous cystic tumors (5–9). The latter are subclassified as mucinous cystic neoplasms or intraductal papillary mucinous neoplasms (IPMNs). Although generally indolent, they are considered forerunners of pancreatic cancer and may follow a similar disease course once malignant transformation has occurred (10–13).

Presently, even the critical distinction between intrinsically benign, premalignant, and malignant cystic lesions remains problematic. Radiology rarely provides sufficient information for this assessment (14). Endoscopic ultrasound (EUS) with fine-needle aspiration (FNA) may result in cytological diagnosis, but the yield

is often scant (15). Cyst fluid carcinoembryonic antigen (CEA) is considered the best indicator of a mucinous tumor, with a diagnostic accuracy of 79% (16,17). However, CEA levels do not correlate with the degree of dysplasia (16). DNA analysis may provide additional information, but its accuracy does not exceed that of CEA (18,19).

The overall aim of this study was to address this unmet clinical need by exploring the potential of cyst fluid mucin expression analysis (mucin profiling) as a diagnostic tool for the evaluation of PCLs.

Mucins are a family of large, membrane-bound or secreted, densely O-glycosylated glycoproteins, which are important for normal epithelial cell barrier function but are also de novo expressed or overexpressed in various cancer types, notably adenocarcinomas (20,21). Postulated roles of membrane-bound mucins in carcinogenesis include the promotion of epithelial growth factor receptor signalling and constitutive activation of cell survival pathways, such as Wnt and NFκB (20–23). Aberrant expression of secreted mucins in (pre)neoplastic lesions may result from epigenetic signalling and has been suggested to provide protection against antitumor immunity (3,20,24,25).

Previous research has demonstrated aberrant expression of both membrane-bound and secreted mucins in ductal adenocarcinomas, pancreatic intraepithelial neoplasia, IPMNs and mucinous cystic neoplasms (3,26–34). Most of these studies have used immunohistochemistry. However, antibody-based detection may lead to under-recognition because the heavy glycosylation of mucins masks their protein identity. Furthermore, the mucin glycosylation pattern is altered in cancer (20,21). To avoid glycosylation-related omissions and obtain complete mucin profiles, we decided to target peptide-protein identification through proteomics.

Proteomics refers to the study of the proteome (ie, the entire set of proteins found in a system in physiological or pathological conditions). Proteomic studies have previously been performed on pancreatic cyst fluid in only a few instances. Two groups used surface-enhanced laser desorption/ionization time-of-flight mass spectrometry to obtain cyst content protein profiles (35,36).This methodology, however, does not permit direct identification of the differentially expressed proteins. Cuoghi et al. assessed aspirates from eight patients by liquid chromatography–tandem mass spectrometry and identified olfactomedin-4 expression as unique to the three mucinous tumors in the study (37). Ke and colleagues applied three different proteomic methods on fluid from 20 lesions and correlated results with histology or CEA levels (38). Several candidate biomarkers were identified, including amylase, mucins, carcinoembryonic antigen-related cell adhesion molecules (CEACAMs), and S100 homologs. The analyses required 8 months of mass spectrometer time.

By contrast, the aim of our study was to specifically evaluate the potential clinical utility of mucins as markers for pre-/cancerous PCLs. Thus, a targeted, potentially high-throughput, proteomic approach was selected. Taking previous findings of MUC6 in serous cystic neoplasms (39,40) and reports on aberrant MUC1 expression in ductal adenocarcinomas (26–29) into account, we formulated two primary hypotheses: 1) proteomic evidence of cyst fluid content of any mucin except MUC6 discriminates PCLs with malignant potential from intrinsically benign lesions; and

Table 1. Study summary*

Study hypotheses	Study population	Discovery cohort	Validation cohort	Women, No. (%)	Age, median (IQR)	Proteomics; definition positive result	Cytology; definition positive result	Cyst fluid CEA; definition positive result	Diagnostic standard	Diagnostic standard; definition positive result
Proteomic mucin profiling can identify pre-/malignant (malignant potential) PCLs with high diagnostic accuracy, as compared with conventional methods.	78	28	50	42 (53.8)	64 (56–70)	Cyst fluid expression of any mucin except MUC6	Mucin; mucinous epithelium; cell atypia	>192 ng/mL	Histology or clinical assessment	Mucinous cystic tumor (MCN or IPMN) Ductal adenocarcinoma
Proteomic mucin profiling is more accurate than conventional tests, in predicting manifest malignancy in PCLs.	29†	9	20	19 (65.5)	64 (56–67)	Cyst fluid MUC1 expression	Severe cell atypia	>1000 ng/mL	Histology or metastasis of pancreatic cancer	Mucinous cystic tumor (MCN or IPMN) with high-grade dysplasia or invasive growth Ductal adenocarcinoma

* CEA, carcinoembryonic antigen; IPMN, intraductal papillary mucinous neoplasm; IQR, interquartile range; MCN, mucinous cystic neoplasm; PCLs, pancreatic cystic lesions.

† The study population of 29 used to assess the diagnostic performance of MUC1/standard tests in predicting malignant transformation represents the subset of the entire study population (n = 78) with supportive pathology or confirmed metastasis.

2) MUC1 expression indicates malignant transformation (ie, at least high-grade dysplasia).

Methods

Study Design and Recruitment of Patients

A prospective study of diagnostic accuracy was designed. Approval was granted by the Vastra-Gotaland Ethics Committee. Study hypotheses and outcome measures were outlined a priori and evaluated at a planned interim analysis after 18 months. The target sample size was also revised at this point. Patients aged 18 years or above referred to Sahlgrenska Hospital, a tertiary center (catchment area = 1.6 million), for EUS-FNA of PCLs between May 2007 and October 2012 were consecutively included. Written informed consent was obtained from all subjects. Pre-established exclusion criteria were 1) solid-pseudopapillary neoplasm and 2) neuroendocrine tumor. These neoplasms are extremely rare (5,6,8,9,41), were deemed unlikely to express mucins, and possess highly characteristic cytological and immunocytochemical features that allow for a relatively straightforward identification (41–44).

EUS Examination, Cytology, and Cyst Fluid CEA Quantification

Lesions were accessed by the transgastric/transduodenal route using a linear echoendoscope (Pentax EG3830UT (Tokyo, Japan)/ Olympus GF-UCT140 (Tokyo, Japan)) with a 19, 22, or 25 gauge needle (Wilson-Cook, Limerick, Ireland/Olympus, Aomori, Japan/ Boston Scientific, Spencer, IN). A cytopathology technician was present on site. Cytological examination with periodic acid-Schiff diastase staining for mucus and cyst fluid CEA quantification by immunochemoluminescent technology were routinely performed and always prioritized over proteomic analysis. However, a sample volume less than 500 µL precludes CEA quantification by the method used at our hospital. Fluid intended for mucin profiling was stored at –80°C.

Gel Electrophoresis and Protein Digestion

Samples (approximately 20 µL) were reduced by 100 mM dithiothreitol (Sigma-Aldrich, St Louis, MO), heated to 95°C for 30 minutes, subsequently loaded onto a 5% Laemmli polyacrylamide gel with a 3% stacking gel, and separated on a one-dimensional

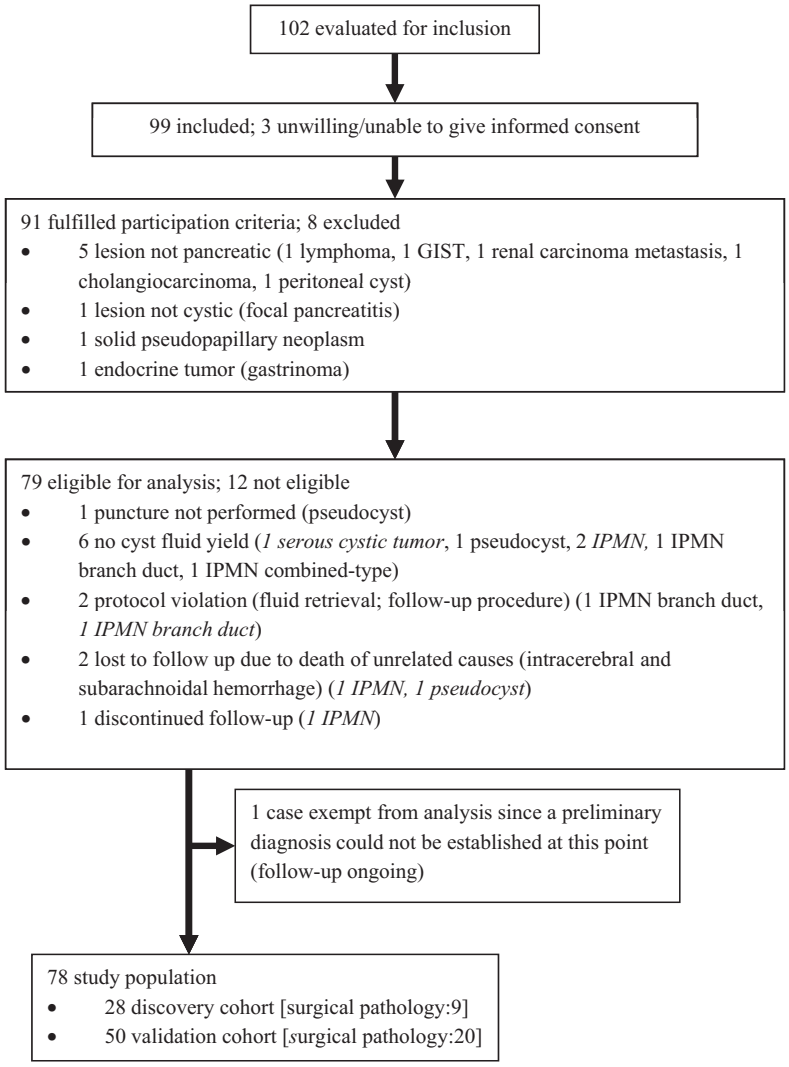

Figure 1. Flow chart of patients in the study. Diagnoses provided in **italics** are tentative. GIST, gastrointestinal stromal tumor; IPMN, intraductal papillary mucinous neoplasm.

sodium dodecyl sulfate polyacrylamide gel electrophoresis system. Precision protein standards (Bio-Rad, Hercules, CA) were used as molecular mass markers. Gels were stained with Imperial (Thermo Scientific, Rockford, IL). The high molecular mass area (>100 kDa) of the separation gel was, together with the stacking gel, excised for each lane. Destaining of the gel pieces, protein digestion by trypsin, and peptide extraction were performed as previously described (45).

Mass Spectrometry and Protein Identification

Peptides were separated on a 50-µm C18 reversed column coupled to a hybrid linear ion trap-Fourier transform ion cyclotron resonance mass spectrometry instrument equipped with a 7-tesla magnet (LTQ-FT; Thermo Electron, Bremen, Germany) and operated in a data-dependent mode to automatically switch between mass spectrometry and tandem mass spectrometry acquisition. Search parameters were set to the following: mass spectrometry accuracy of 5 ppm; tandem mass spectrometry accuracy of 0.5 Da; one missed cleavage allowed. For the searches, propionamide (from acrylamide) on cysteine was set as a fixed modification, and oxidation of methionine as a variable modification. Results were evaluated using Mascot software and an in-house mucin database (http://www.medkem.gu.se/mucinbiology/databases/). Peptides were also screened against the nonredundant protein sequence database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). A Mowse score of 45 or greater, including at least one unique peptide at the 99% statistical significance level (ion score >40) or two unique peptides at the 95% significance level (ion score >28), was accepted for protein identification. To test reproducibility, different aspirate portions were prepared and analyzed two to four times for 12 patients (15.4%).

Endpoints

For the assessment of malignant potential, in addition to histology, clinical follow-up was accepted as a reference standard to minimize selection bias and allow inclusion of pseudocysts as negative controls. Case subjects with remaining diagnostic ambiguities after the completion of follow-up (usually including evaluation by a multidisciplinary board) were reviewed by an experienced pancreatic surgeon (A.G. Hyltander). Patients lost to follow-up before a diagnosis could be established were excluded from analysis.

To confirm/exclude malignant transformation, histology was required as an endpoint unless there was evidence of metastasis.

Diagnostic assessments were blinded to proteomics results. Conversely, proteomics results were interpreted (K.S. Jabbar) without knowledge of the diagnostic standard. Histology of surgical specimens was reviewed by the study pathologist (C. Verbeke).

Interim Analysis and Validation

After 18 months an interim analysis was performed. The series then consisted of 29 lesions, denoted the discovery cohort (DC). No alterations of the primary study hypotheses or methodology were deemed necessary.

Interim analysis indicated that for the evaluation of the first study hypothesis, a confidence interval (CI) of maximum ±15% for diagnostic accuracy should preclude overlap between proteomics' results and the 79% accuracy previously reported for cyst fluid CEA by a large multicenter study (16). At a confidence level of 95%, this equals a validation cohort (VC) of 43 individuals. Regarding the second

Table 2. Study population; distribution of diagnoses, and baseline characteristics for each diagnosis group*

Demographic and clinical characteristics	All	Pseudocysts/ chronic pancreatitis	All cystic tumors†	Pseudocysts vs cystic tumors‡	Serous cystic neoplasms	Mucinous cystic neoplasms	IPMN branch duct	IPMN combined type	IPMN main duct	Ductal adenocarcinomas
Number of patients	78	37	41 (42)‖	N/A	5	4	13 (14)‖	8	3	8
Women, No. (%)	42 (53.8)	18 (48.6)	24 (58.5)	.50	3 (60.0)	3 (75.0)	3 (23.1)	6 (75.0)	2 (66.7)	7 (87.5)
Age, median (IQR)	64 (56–70)	59 (46–65)	68 (62–76)	**<.001**	75 (56–77)	64 (63–65)	69 (66–74)	66 (59–72)	79 (78–79)	66 (58–71)
Maximum diameter in mm, median (IQR)	26 (18–70)	70 (25–110)	20 (15–35)	**<.001**	56 (40–60)	50 (44–50)	19 (15–20)	19 (15–33)	23 (17–29)	21 (20–26)
History of acute/chronic pancreatitis, No. (%)	34 (43.6)	28 (75.7)	6 (14.6)	**<.001**	0	0	1 (7.7)	2 (25.0)	1 (33.3)	2 (25.0)
Incidental finding, No. (%)	17 (21.8)	3 (8.1)	14 (34.1)	**.006**	2 (40.0)	1 (25.0)	6 (46.2)	4 (50.0)	0	1 (12.5)
Samples assessed as viscous by examiner, No. (%)	37 (46.8)	17 (45.9)	20 (47.6)	1.00	0	2 (50.0)	8 (57.1)	4 (50.0)	1 (33.3)	5 (62.5)

* IPMN, intraductal papillary mucinous neoplasm; IQR, interquartile range. Viscosity assessments were blinded to patient identity and lesion morphology.

† This is the sum of the different subcategories of cystic tumors that are individually listed in the six rightmost columns.

‡ Two-sided *P* values for the comparison of subjects with cystic tumors vs subjects with pseudocysts. Mann–Whitney *U* test and Fisher exact test were used for continuous and categorical data, respectively. $P < .05$ are noted in bold.

‖ For one patient, two lesions were separately analyzed: both were diagnosed as branch-duct IPMN.

primary hypothesis, the accuracy of MUC1 considerably exceeded that of conventional methods in the DC. Hence, here a population of 20 was predicted to suffice to detect differences in performance.

Furthermore, based on results from the DC, a further, secondary hypothesis was generated: that a panel of MUC5AC, MUC2, and MUC1 may suffice to stratify benign, premalignant, and malignant PCLs.

Statistics

The diagnostic accuracy of mucin profiling was compared with corresponding results for standard tests (ie, cyst fluid CEA and cytology). Proteomic evidence of any mucin except MUC6 was regarded as indicative of malignant potential; MUC1 expression was considered predictive of malignancy. For CEA, previously suggested cutoffs of 192 ng/mL for premalignancy (16) and 1000 ng/mL for malignancy (46) were used. CEA was not quantifiable for 19 lesions, which were excluded from the assessment of its performance. Cytology samples were evaluated by trained cytopathologists as per standard operating protocol. EUS morphology and cyst size [cutoff of 30mm (5,6)] were additionally appraised as malignancy predictors. Sex-specific analyses were not performed.

Fisher exact test and Mann–Whitney U-test were used for the comparison of categorical and continuous data, respectively. All *P* values are two-tailed, and the statistical significance level was set to less than .05. The Holm–Bonferroni multiple comparisons correction was applied to the primary comparisons of the accuracy of mucin profiling vs conventional methods. Statistical significance levels for other comparisons were adjusted by the Bonferroni procedure whenever appropriate.

Table 1 summarizes how the primary study hypotheses were evaluated.

Results

Study Population

The inclusion/exclusion process that resulted in a series of 78 patients (42 women; median age = 64 years), comprising a DC of 28 patients (n = 29 lesions) and a VC of 50 patients, is outlined in Figure 1. Histology or evidence of metastasis (n = 1 patient) was available for 29 patients (19 women; median age = 64 years; DC = 9 patients, VC = 20 patients). Diagnoses and baseline characteristics of the study population are provided in Table 2. Patients with cystic tumors tended to be older than those with pseudocysts and to have smaller lesions (median size = 20 vs 70 mm). The distribution of diagnoses was similar between the DC and the VC, as shown in Figure 2, a and b.

The endpoints for the evaluation of the first study hypothesis were 1) histology or evidence of metastasis (n = 29; 37.2%); 2) (near-)complete resolution (n = 18; 23.1%); 3) follow-up with imaging (median = 19 months; interquartile range [IQR] = 10–35; n=26; 33.3%); or 4) clinical assessment, in case of diagnostic ambiguity by a multidisciplinary conference (n = 5; 6.4%). Endpoints are summarized in more detail in Supplementary Figure 1 (available

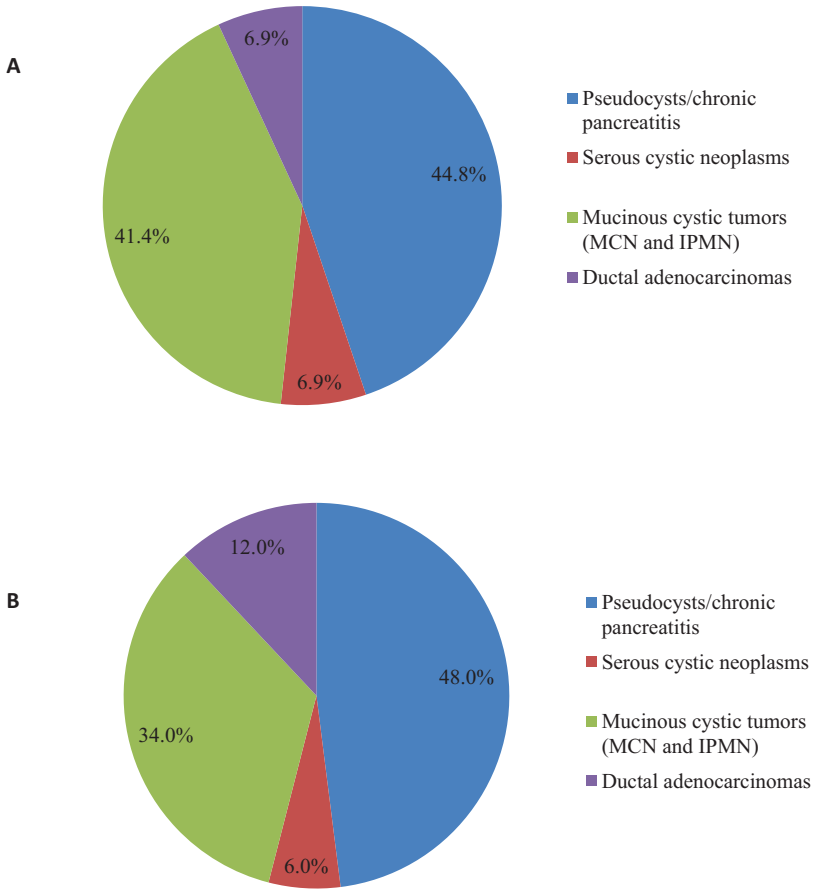

Figure 2. Distribution of diagnoses for the discovery cohort (n = 29) (**A**) and validation cohort (n = 50) (**B**). IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm.

online) and are provided for each patient in Supplementary Tables 1 and 2 (available online). Surgery was performed shortly after EUS-FNA (median interval = 2 months; IQR = 1–4).

Cyst Fluid Mucin Profiling

Table 3 summarizes the mucin profiles of the different lesions; individual results are compiled in Supplementary Tables 1 and 2 (available online). Although mucins were not detected in pseudocyst aspirates (with two exceptions), MUC6 was identified in two of five serous cystic tumors, corroborating previous reports (39).

Repeated analysis (n = 1–3 times) with up to a 2-year interval of samples from 12 patients (15.4%) showed results to be reproducible (Supplementary Table 3, available online).

Identifying Cystic Lesions With Malignant Potential

As demonstrated in Figure 3, proteomic mucin profiling discriminated PCLs with malignant potential (n = 37 of 79; 46.8%) from benign lesions with 97.5% accuracy (95% CI = 90.3% to 99.6%). This statistically significantly exceeded corresponding values for cytology (71.4%; 95% CI = 59.8% to 80.9%; $P < .001$), and cyst fluid CEA (78.0%; 95% CI = 65.0% to 87.3%; $P < .001$). Only one false negative result was recorded, from a grossly hemorrhagic aspirate. The accuracy of mucin profiling was nearly identical between the DC and VC (96.6% vs 98.0%). Statistically significant differences in accuracy compared with conventional methods, were independently observed in the VC. Full performance characteristics for the evaluated analyses, including likelihood ratios, are provided in Supplementary Tables 4 and 5 (available online).

Of note, cyst fluid requirements for CEA quantification (500 µL) precluded CEA analysis in 19 patients (24.4%), 15 of whom had neoplastic PCLs.

At the point of interim analysis, a diagnostic algorithm for the assessment of PCLs, comprising a panel of three mucins (MUC5AC, MUC2 and MUC1), was tentatively introduced (Figure 4). In the VC, all lesions with malignant potential (n = 23) expressed at least one of these mucins; 19 of 23 (82.6%) contained MUC5AC. Also provided in Figure 4 are the most abundant unique peptides observed for each of these three mucins, information that could potentially be used to facilitate their individual identification. (Basic information on each detected mucin, including a full peptide hit list is compiled in Supplementary Tables 6 and 7, available online.)

Detecting Manifest Malignancy

For lesions with procurable histology or confirmed metastasis (n = 29), MUC1 expression was separately compared with current state-of-the-art malignancy indicators for PCLs: severe cellular atypia on cytological examination, cyst fluid CEA > 1000 ng/mL (46), and EUS morphology suggesting malignant transformation (Figure 5; Supplementary Tables 8 and 9, available online). Malignancy, defined as minimum high-grade dysplasia, was present in 16 of 29 (55.2%) lesions according to the reference standard.

As shown in Figure 5, the accuracy of MUC1 for the identification of malignant PCLs was, at 89.7% (95% CI = 71.5% to 97.3%), markedly higher than corresponding results for cytology, EUS morphology, and cyst fluid CEA (51.7%, 95% CI = 32.9% to 70.1%; 58.6%, 95% CI = 39.1% to 75.9%; and 57.1%, 95% CI = 34.4% to 77.4%; $P = .003$, .01, and .02, respectively). Conventional methods

Table 3. Cyst fluid mucin expression profiles for the different types and subtypes of pancreatic cystic lesions*

Mucin expression	Pseudocysts (n = 37)	Serous cystic neoplasms (n = 5)	IPMN branch duct (n = 14)	IPMN combined (n = 8)	IPMN main duct (n = 3)	Mucinous cystic neoplasms (n = 4)	All mucinous cystic tumors (n = 29), No. (%)	Ductal adenocarcinomas (n = 8)	All cystic tumors with malignant potential (n = 37), No. (%)
No. of lesions expressing MUC1	1	0	7	5	1	3	16 (55.2)	7	23 (62.2)
No. of lesions expressing MUC2	0	0	3	3	1	1	8 (27.6)	1	9 (24.3)
No. of lesions expressing MUC5AC	1	0	11	7	3	4	25 (86.2)	6	31 (83.8)
No. of lesions expressing MUC5B	0	0	3	3	1	2	9 (31.0)	5	14 (37.8)
No. of lesions expressing MUC6	1	2	7	3	1	1	12 (41.4)	3	15 (40.5)
No. of lesions expressing MUC16	0	0	0	0	0	1	1 (3.4)	1	2 (5.4)
No. (%) of lesions expressing any mucin	1 (2.7)	2 (40.0)	14 (100.0)	8 (100.0)	3 (100.0)	4 (100.0)	29 (100.0)	7 (87.5)	36 (97.3)

* IPMN, intraductal papillary mucinous neoplasm. Numbers denote the lesions in each diagnosis group expressing a particular (or, in the last row, any) mucin. For one patient, two lesions were separately assessed; thus a total of 79 lesions were analyzed.

attained an optimum accuracy of 61.9% (95% CI = 38.7% to 81.0%) when evaluated in combination ($P = .04$ for the comparison with MUC1). These differences in performance remained statistically significant after Holm–Bonferroni correction for multiple (n = 4) comparisons. In the VC (n = 20), the accuracy of MUC1 was even higher (95.0%; 95% CI = 73.1% to 99.7%).

A size greater than 30 mm for a cystic tumor is considered indicative of a substantial risk of malignant transformation (5–7). In our material (excluding three benign lesions that were resected for purely symptomatic reasons), the size criterion demonstrated 37.5% sensitivity and 80.0% specificity for malignancy.

Cyst fluid MUC1 expression was furthermore detected in eight conservatively treated patients who were not included in this analysis. The first three of these subjects died during the course of the study, likely because of metastasized malignancy where no other primary neoplasm has been identified (autopsy not performed). The remaining five were recently sampled and are currently undergoing follow-up; two of them also had CEA levels greater than 1000 ng/mL (Supplementary Table 1, available online).

Discussion

Mucinous cystic tumors, which in the recent past were the topic of case reports, are now known to account for a considerable proportion of PCLs. The prevalence of PCLs, in turn, is presently estimated at 10% to 24% in the elderly population (47,48). At the same time, emerging evidence that mucinous tumors should be considered forerunners of pancreatic cancer has offered a visible target for the prevention of this devastating disease. Unfortunately, the lack of reliable instruments for the differential diagnosis of PCLs could exclude many patients from timely intervention and expose others to the risk of unnecessary pancreatic surgery (49–51)

In this study, we have shown that mucin profiling by a proteomic approach identified cystic forms/precursor lesions of pancreatic cancer with an accuracy of 97.5%. Results from an independent validation cohort were nearly identical. This compares very favorably with cyst fluid CEA (78.0%; $P < .001$), which is currently considered the most useful diagnostic test for PCLs (16). The performance of conventional methods in this study are in accordance with previous reports (16,17), as is the observation that cyst fluid yield precluded CEA quantification in nearly every fourth (n = 19 of 78) patient and every second (n = 14 of 29) mucinous tumor (52). By contrast, 25 times less material was required for proteomics, allowing for the analysis of all aspirates.

Mucinous tumors are frequent incidental findings, most commonly affecting the elderly (5–7,53,54). Progression to malignancy may take several years (10,11,55). Consequently, although resection is generally life-saving once malignant transformation has occurred, many patients with premalignant tumors would likely benefit greatly from being spared surgery. Currently, however, conventional methods for the prediction of malignancy in PCLs are not sufficiently reliable to safely guide treatment recommendations (16,17). MUC1 expression by contrast, identified malignancy in PCLs with 89.7% accuracy and could thus add a new dimension to the evaluation of these lesions. Further studies are required to validate these promising findings.

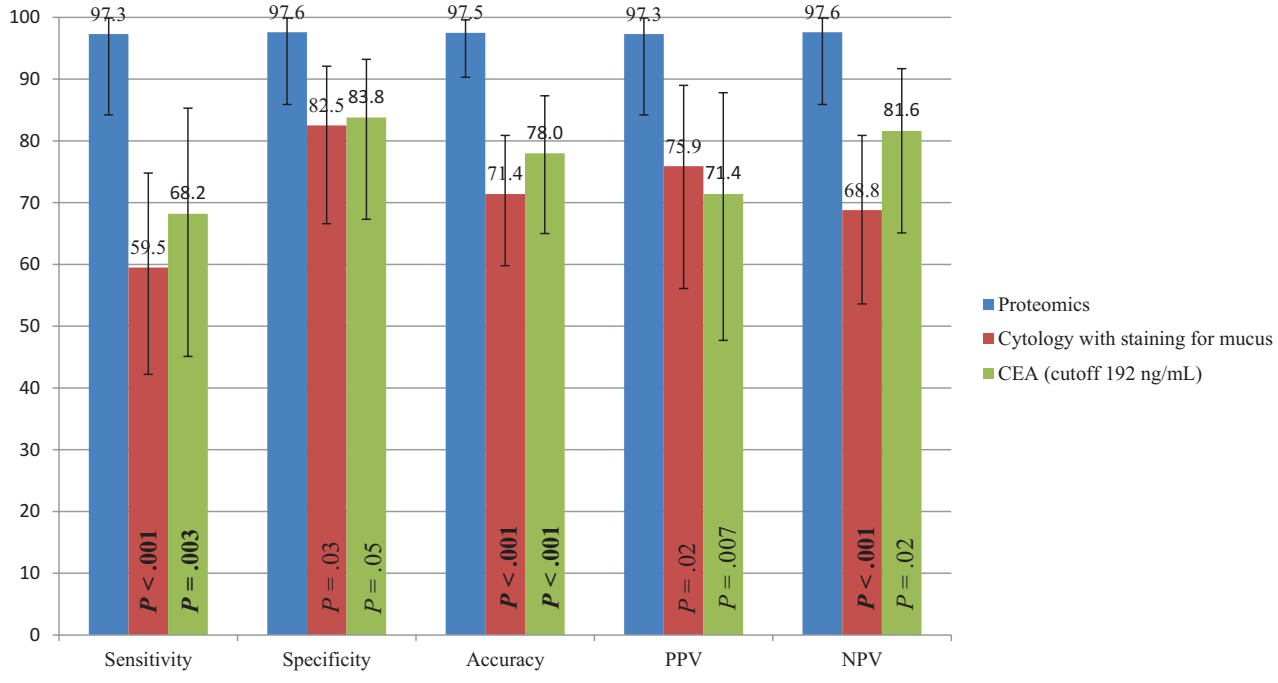

Figure 3. Identification of pancreatic cystic lesions with malignant potential: performance of proteomics, cytology, and cyst fluid CEA (192 ng/mL). **The vertically oriented text** on the columns representing cytology and CEA refers to two-sided *P* values for the comparison with mucin profiling (proteomics; Fisher exact test). Error bars illustrate the 95% confidence interval (Wilson score method with continuity correction). *P* values < .005 (statistical significance threshold after Bonferroni correction) are in **bold** text. CEA, carcinoembryonic antigen; NPV, negative predictive value; PPV, positive predictive value.

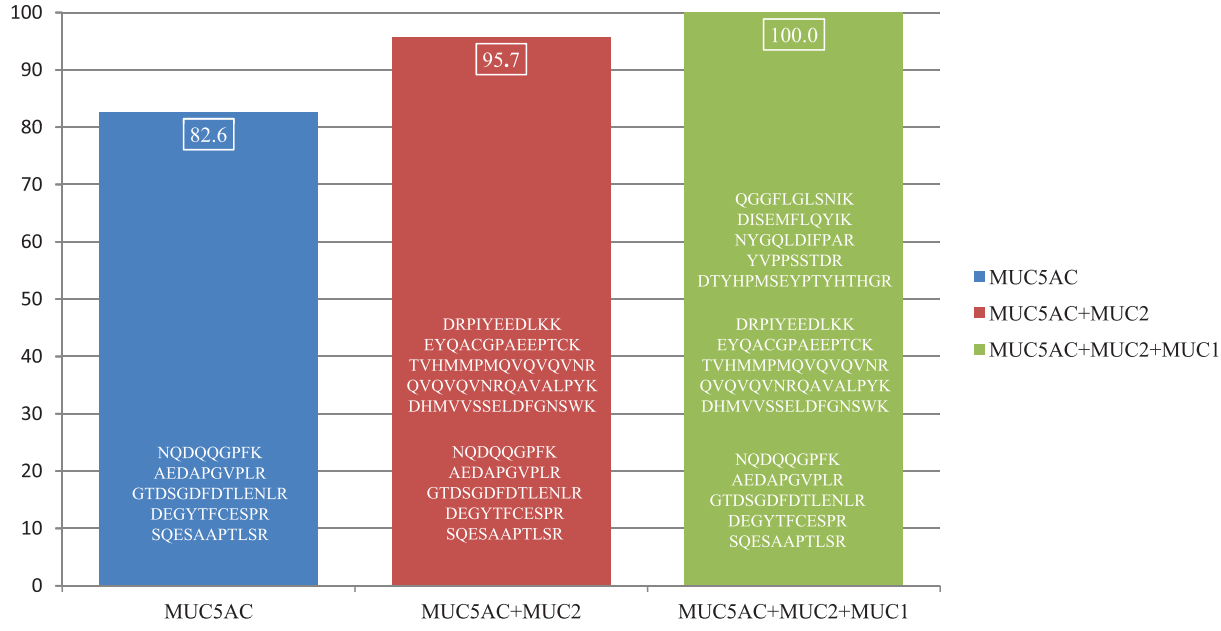

Figure 4. Step-wise approach to the assessment of pancreatic cystic lesions with regard to malignant potential. **Columns** represent sensitivity values for the identification of pre-/malignant lesions for MUC5AC (**left**), MUC5AC+MUC2 (**middle**), and the optimal combination of MUC5AC+MUC2+MUC1 (**right**). Results are from the validation cohort. **Text within columns** list the five most abundant unique peptides observed for each mucin.

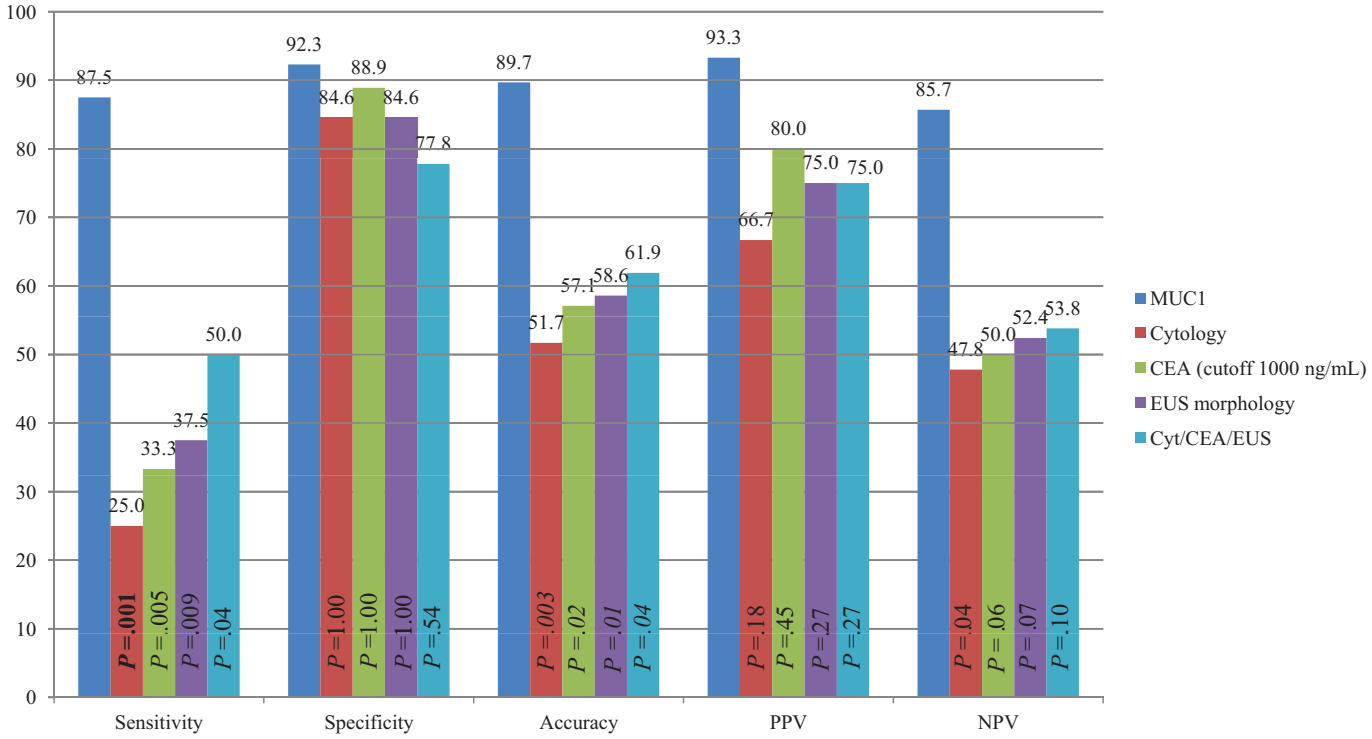

Figure 5. Detecting manifestly malignant pancreatic cystic lesions: performance of *MUC1*, cytology, cyst fluid CEA (1000 ng/mL), and endoscopic ultrasound (EUS) morphology. The rightmost (**light blue**) columns refer to a combination of standard analyses, with at least one positive result considered indicative of malignancy. **Vertical text in columns** representing traditional methods show two-sided *P* values for the comparison with MUC1 expression (Fisher exact test). *P* values <.0025 (statistical significance threshold after Bonferroni correction) are in **bold** text. For the primary comparison of the accuracy of MUC1 expression vs conventional methods, the Holm–Bonferroni correction was separately applied (statistically significant results shown in **italics**). CEA, carcinoembryonic antigen; NPV, negative predictive value; PPV, positive predictive value.

Some limitations of the study merit further consideration. First, to avoid bias from underrepresentation of benign lesions, the analysis was not restricted to cases with supportive histology. The follow-up period for conservatively treated lesions was defined by what was considered clinically motivated for each patient and, whenever warranted, supported by review by a multidisciplinary board/senior expert surgeon. Second, the rare solid pseudopapillary neoplasms and cystic neuroendocrine tumors were not considered

in this study because they can be readily identified by cytology/immunocytochemistry (41–44). It should therefore be emphasized that mucin profiling is not intended to replace routine cytological assessment. These two analyses can easily be performed in parallel.

Furthermore, for one of the four histological subtypes of IPMNs, the so-called intestinal type, previous reports on MUC1 expression after malignant transformation are conflicting (28,29,56). Evaluation of the performance of MUC1 specifically for the detection of malignancy in this tumor type would require a considerably larger patient series. However, intestinal-type IPMNs typically involve the main pancreatic duct, which is in itself an absolute indication for surgery, making the detection of malignant transformation less critical (5,7,33,34). In addition, intestinal-type IPMNs should be identifiable by mucin profiling given their characteristic predominant MUC2 expression, which could be used to mitigate this possible pitfall (28,29,31,33,34).

The protein and peptide identifications reported in Figure 4 could potentially inform the design of simplified and/or quantitative assays. Possible problems with contamination by gastric [MUC5AC, MUC6 (40,57)] and/or intestinal [MUC2 (57)] epithelium could, for instance, motivate quantitation, although this study did not identify this as a concern. Although antibody-based tests could provide a straightforward solution, the extensive and variable glycosylation of mucins might hamper their performance (20,21). A targeted, quantitative, high-throughput proteomic technique, such as selected reaction monitoring, could be a clinically feasible alternative (58). Meanwhile, mucin profiling, as described here, appears well suited for clinical use. Importantly, results could be obtained for lesions smaller than 1 cm and delivered within a working week.

PCLs, although very common, may act as harbingers of one of the deadliest cancer forms known. Thus, accurate diagnostic tools for their assessment are urgently required. We conclude that the proteomic approach evaluated in this study shows great promise in this regard. If further studies can corroborate these results, this methodology should be considered for clinical recruitment.

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Notes

K.S. Jabbar and R. Sadik had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. K. S. Jabbar, G.C. Hansson, and R. Sadik were responsible for the study concept and design. K. S. Jabbar, C. Verbeke, A.G. Hyltander and R. Sadik were responsible for acquisition of data. K. S. Jabbar, C. Verbeke, A.G. Hyltander, H. Sjövall, G.C. Hansson, and R. Sadik were responsible for analysis and interpretation of data. K.S. Jabbar was responsible for drafting of the manuscript. K. S. Jabbar, C. Verbeke, A.G. Hyltander, H. Sjövall, G.C. Hansson, and R. Sadik were responsible for critical revision of the manuscript for important intellectual content. K.S. Jabbar was responsible for statistical analysis. G.C. Hansson and R. Sadik obtained funding. H. Sjövall, G.C. Hansson, and R. Sadik were responsible for study supervision.

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