Cyst Fluid Interleukin-1β (IL1β) Levels Predict the Risk of Carcinoma in Intraductal Papillary Mucinous Neoplasms of the Pancreas

Ajay V. Maker¹, Nora Katabi², Li-Xuan Qin³, David S. Klimstra³, Mark Schattner⁵, Murray F. Brennan², William R. Jarnagin⁴, and Peter J. Allen²

Abstract

**Purpose:** Biomarkers for high-grade dysplasia in patients with radiographically identified intraductal papillary mucinous neoplasms (IPMN) have not been described. We hypothesized that dysplasia in IPMN invokes an immunogenic/proinflammatory microenvironment that can be identified by cyst fluid cytokine levels.

**Experimental Design:** Pancreatic cyst fluid aspirates were collected at resection (2005–2009). Samples were grouped into low-risk [low-grade (n = 6) or moderate dysplasia (n = 15)] and high-risk groups [high-grade dysplasia (n = 13) or carcinoma (n = 6)]. Cytokine expression was determined using a multiplex sandwich immunossay. Differences in cytokine expression were evaluated using the 2-sample t test. Sample classification was performed using a logistic regression adjusting for sample covariates.

**Results:** IL5 and IL8 concentrations were higher in the cyst fluid from patients in the high-risk group than the low-risk group. Interleukin (IL)-1β concentrations were also higher in the cyst fluid from patients with high-grade dysplasia or cancer (n = 19) than those with low- or moderate-grade dysplasia (n = 21, 539 ± 255 pg/mL vs. 0.2 ± 0.1 pg/mL; P < 0.0001). IL1β remained a significant predictor of high-risk cysts after multivariate analysis. There was no significant difference in levels of IL2, IL4, IL10, IL12, IL13, TNF-α, or IFN-γ between the groups. That IL1β levels identified cysts at a high risk of malignancy was confirmed in an independent validation set.

**Conclusions:** Cyst fluid levels of IL1β can differentiate low- from high-risk IPMN. This study introduces IL1β as a potential biomarker for validation in larger clinical studies. *Clin Cancer Res; 17(6); 1502–8.* ©2011 AACR.
Materials and Methods

Between 2005 and 2009, 147 patients underwent pancreatic resection for IPMN at Memorial Sloan-Kettering Cancer Center (MSKCC). Patients were preoperatively consented to an IRB (Institutional Review Board) tissue collection protocol and a waiver of authorization was obtained from the IRB prior to data review. Pancreatic cyst fluid aspirates were collected at the time of resection from 40 of these patients. All 40 samples were included in this study. Correlative studies on preoperative cyst fluid CEA and FNA cytology were performed on a subset of these patients. All 40 samples were included in this study. Resected specimens were immediately transported to the MSKCC tumor procurement facility in the Department of Pathology where cyst fluid was aspirated with an 18 to 21 gauge needle, divided into 500 μL aliquots, and stored at −80°C. Aspiration was performed by a surgeon, pathologist, or technician. All analyses were performed on samples with no prior freeze-thaw cycles. Samples were run neat without dilution except for 11 highly viscous samples, which were serially diluted in PBS.

IPMN were classified as gastric, intestinal, pancreatobiliary, or oncocytic based on their histopathologic characteristics. In some cases, the cyst contained 2 dominant histologic subtypes, in which case they were classified as a mix of the 2 pathologies. The most severe degree of cellular atypia identified in the cyst determined the grade of dysplasia as being low, moderate, or high. Invasive carcinoma in IPMN was characterized as tubular or colloid on the basis of cellular morphology and mucin content. Samples were divided into low-risk (low-grade and moderate dysplasia) and high-risk groups (high-grade dysplasia and invasive carcinoma). IPMNs were distinguished from mucinous cystic neoplasms by the absence of ovarian-type stroma in the cyst wall. Histopathology was independently reviewed and assessed by a single dedicated gastrointestinal pathologist who was blinded to the cytokine assay results (N.K.).

Cyst fluid was assessed for cytokine expression using an ultrasensitive multiplex sandwich immunoassay (Meso Scale Discovery). Standard curves were performed for individual cytokines and verified. All samples were run according to manufacturer’s guidelines. Six cyst fluid aspirates from serous cystadenomas were included as benign, non-IPMN pancreatic cyst control specimens.

Cytokine expression levels were log2 transformed to improve the normality of the data. Differential expression analysis of data sets were assessed utilizing the 2-sample t test. Sample classification was performed using a logistic regression adjusting for sample covariates including cyst size and duct type. Cytokine values are expressed as the mean ± SEM. A receiver operating characteristic curve (ROC) was created for IL-1β. ROC analysis was utilized to determine the area under the curve and diagnostic cutoff points.

Results

Patient demographics and cyst characteristics

Cyst fluid was available from 40 patients with IPMN (13 men and 27 women). There were 19 “high-risk” cysts, consisting of 5 with invasive carcinoma and 14 with high-grade dysplasia, and 21 “low-risk” cysts, consisting of 15 with moderate dysplasia and 6 with low-grade dysplasia. Cyst size ranged from 1.2 to 23 cm with a mean diameter of 4.2 cm. There were 12 main duct lesions, 19 branch duct lesions, and 9 mixed (branch and main duct) cysts. Six high-risk cysts and 1 low-risk cyst contained a solid component or nodularity whereas the majority of cysts (33) were void of any concerning radiographic features.

Histopathologic subtype and dysplasia

Of the 19 high-risk cysts, 8 were of the intestinal subtype, 7 were pancreatobiliary, 2 were oncocytic, and 2 were gastric-type IPMN. All of the low-risk cysts were of the gastric subtype.

Degree of cyst dysplasia and cytokine expression

IFN-γ and interleukin (IL) 4 expression was very low (<1 pg/mL) in the cyst fluid across all groups of IPMN and there was no correlation between these cytokine levels and the degree of cyst dysplasia. IL-2, IL-10, IL-12, IL-13, and TNF-α expression was much higher than IFN and IL4 across all IPMN groups, but there was no association between the level of dysplasia and cytokine expression (Table 1).
In a univariate analysis, IL1β, IL5, and IL8 had higher levels of expression in the presence of high-grade dysplasia or invasive carcinoma. High-risk cysts (n = 19) had increased expression of IL1β (539 ± 255 vs. 0.2 ± 0.1 pg/mL; P < 0.0001) and IL5 (0.2 ± 0.5 vs. 0.02 ± 0.05 ng/mL; P = 0.03) than low-risk cysts (n = 21). IL8 levels were the highest of all cytokines measured and were different between high- and low-risk cysts (8,088 ± 2,288 vs. 2,758 ± 847 pg/mL; P = 0.03), though this difference did not maintain significance when the sample variance was normalized (Wilcoxon rank-sum test, P = 0.07; Fig. 1). IL1β levels measured in nonmucinous serous cystadenomas were barely detectable (0.1 ± 0.1 pg/mL; n = 6).

IL1β levels alone remained a significant predictor of high- from low-risk cysts (343 ± 298 pg/mL, n = 8 vs. 0.15 ± 0.09 pg/mL, n = 7; P = 0.05). On the basis of the previously determined ROC calculated value of 1.26 pg/mL to distinguish high- from low-risk cysts, IL1β levels had a positive predictive value (PPV) of 71% for correctly identifying high-risk cysts with high-grade dysplasia or invasive cancer and a negative predictive value (NPV) of 75% for correctly identifying low-risk cysts that contained low or moderate dysplasia.

Cyst fluid FNA cytology and CEA
Preoperative fine-needle aspirates were available from the cysts of 29 patients: 14 high-risk patients and 15 low-risk patients (Table 2). Cytology was read as suspicious or atypical in 6 of 15 low-risk and 10 of 14 high-risk samples. The findings of suspicious or atypical cells on FNA did not differentiate the level of cyst dysplasia or determine high- from low-risk cysts (P = 0.14). Cytology was read as negative in 6 of 15 low-risk and 3 of 14 high-risk samples and was unsatisfactory or nondiagnostic in 3 of 15 low-risk samples and 1 of 14 high-risk samples.

Eight of the 10 cysts with branch duct pathology, no nodularity or solid component, and larger than 3 cm had

Table 1. Mean cytokine concentration in IPMN cyst fluid (pg/mL)

<table>
<thead>
<tr>
<th></th>
<th>IL1β</th>
<th>IL2</th>
<th>IL4</th>
<th>IL5</th>
<th>IL8</th>
<th>IL10</th>
<th>IL12</th>
<th>IL13</th>
<th>TNF-α</th>
<th>IFN-γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>High risk</td>
<td>539</td>
<td>1.8</td>
<td>&lt;0.1</td>
<td>0.2</td>
<td>8,088</td>
<td>2.5</td>
<td>2.2</td>
<td>3</td>
<td>31.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Low risk</td>
<td>0.2</td>
<td>0.2</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>2,758</td>
<td>0.6</td>
<td>0.6</td>
<td>0.4</td>
<td>2.2</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Serous cystadenoma</td>
<td>0.1</td>
<td>1.8</td>
<td>&lt;0.1</td>
<td>6.7</td>
<td>6,576</td>
<td>2.1</td>
<td>0.2</td>
<td>1.3</td>
<td>3.4</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

* P < 0.0001.

Figure 1. Levels of IL1β, IL5, and IL8 were higher in high-risk cysts [high-grade dysplasia (HGD) or invasive cancer (Ca), n = 19] than in low-risk cysts [low-grade dysplasia (low) or moderate dysplasia (mod), n = 21]. *, P < 0.05. In a multivariate predictive analysis model, only IL1β remained a significant predictor of dysplasia (P = 0.0003).
FNA cytology available for analysis. Six cysts were branch duct lesions (2 high-risk lesions and 4 low risk) and 4 cysts were mixed branch + main duct lesions (1 high-risk lesion and 3 low risk). The corresponding cytology and IL1β levels are displayed in Table 3.

Fine-needle aspirate cyst fluid CEA values were available for 15 low-risk lesions and 9 high-risk lesions. CEA values did not differentiate between low- and high-risk cysts (4.423 ± 2.523 pg/mL vs. 3.079 ± 2.910 pg/mL; P = 0.12) whereas IL1β levels did differentiate the groups (0.15 ± 0.09 pg/mL vs. 343 ± 298; P = 0.0007). There was no correlation between cyst fluid CEA levels and IL1β levels in high- (P = 0.61) or low-risk lesions (P = 0.91), and the combination of IL1β and CEA levels together did not distinguish high- from low-risk cysts.

Discussion

The management of IPMN has evolved in the last decade due to a better understanding of the disease process and an increase in patients followed with small (<3 cm), incidentally discovered branch duct IPMN. Current treatment recommendations are based primarily on radiographic findings. Approximately, 50% of lesions involving the main pancreatic duct harbor high-grade dysplasia or microinvasive disease and resection is generally recommended (15, 16). Branch duct lesions (cystic dilation without dilation of the main pancreatic duct) rarely contain invasive cancer and are benign in greater than 95% of IPMN less than 3 cm in diameter and without a solid component. These branch duct lesions can be followed radiographically in selected patients (17, 18). Many patients, however, present without a dilated main duct, solid component or nodularity, or present with a large branch duct lesion. The recommendations for treatment in these patients are controversial. In our data set, many of the lesions were radiographically equivocal and without a solid component or nodularity. Identification of markers highly predictive of dysplasia would spare patients with low-risk lesions the morbidity/mortality of pancreatectomy and allow patients with high-risk lesions to undergo resection prior to the development of invasive cancer.

Pancreatic cyst fluid biochemistry has been useful in distinguishing serous from mucinous lesions. An elevated cyst fluid CEA greater than 192 ng/dL has approximately 80% accuracy for the diagnosis of mucinous cysts; but, the degree of CEA elevation has not been found to correlate with the degree of dysplasia (12, 13, 19). That cyst fluid CEA levels do not correlate with the presence or absence of malignancy was confirmed in the large-scale, prospective multicenter PANDA trial (20). Therefore, for patients with IPMN and equivocal radiographic findings, the present or future risk of malignant cyst transformation cannot be determined by CEA levels. This was again confirmed in the current study where cyst fluid CEA levels were elevated in the cysts, corresponding with diagnosis of an IPMN, but did not correlate with the level of cyst dysplasia. Cyst fluid CEA levels alone, or in combination with IL1β, did not identify high-risk lesions in this subset, whereas IL1β levels alone did confidently identify high-risk lesions (P = 0.0007).

Most IPMN show variable amounts of dysplasia throughout the cyst, therefore preoperative sampling of IPMN by FNA has not accurately reflected the degree of dysplasia in the cyst as a whole. Since the sample represents only a small representation of the lesion and does not provide tissue architecture, cyst FNA cytology is most often described to contain nondiagnostic, normal, atypical, or suspicious cells, and pathologists cannot comment on the level of dysplasia (N. K./D. K.). We, and others, have shown that even these descriptions are often incorrect and lead to the wrong clinical conclusion, with a sensitivity of only 28% for correctly identifying premalignant or malignant mucinous pancreatic lesions (8). As a result, when cyst fluid is aspirated at our institution, it is no longer a clinical practice to submit that sample for cytology due to confusing and often unreliable findings. When we examined FNAs from the current patient samples, suspicious or atypical cells were found in 40% of low-risk cysts and 71% of high-risk cysts, and cytology did not differentiate the level of cyst dysplasia (P = 0.14). Furthermore, suspicious or atypical cells were identified by the pathologist as possibly representing a

Table 2. FNA cytology in IPMN

<table>
<thead>
<tr>
<th>Low risk</th>
<th>High risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>14</td>
</tr>
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mucinous cystic neoplasm and could not differentiate between low- and high-grade dysplasia. In addition, 21% of high-risk cysts containing high-grade dysplasia or invasive cancer on final pathology were FNA cytology negative preoperatively for evidence of cyst atypia or malignancy, which reinforces the need for a better marker of dysplasia than cyst fluid cytology. Furthermore, in a subset analysis of branch and branch + main duct lesions greater than 3 cm in size without a solid component or mural nodule, 2 branch and branch + main duct cysts had negative FNA cytology. The IL1β levels in the fluid of both of these cysts was 0 pg/mL and both were low-risk lesions. However, there was one branch + main duct lesion greater than 3 cm in size without a solid component or mural nodule that had negative FNA cytology and an IL1β level of 275 pg/mL. This was in a high-risk lesion. It is exactly in these instances that a biomarker of dysplasia would be very clinically useful, and in this case, IL1β levels in the cyst fluid could identify a false-negative FNA cytology result.

We are interested in identifying biomarkers of malignancy in these cysts and have recently evaluated cyst fluid mucins for their ability to differentiate low- and high-risk IPMN (14). In the current study, we have explored further the utility of cyst fluid for measuring markers of dysplasia. We found that cyst fluid, which can be obtained preoperatively by endoscopic ultrasound FNA, also contains measurable levels of cytokines reflective of a Th1 and Th2 immune response. Specifically, IL1β levels correlated with the degree of cyst dysplasia and were highly predictive of high-risk lesions. Using a highly sensitive immunoassay, lesions with IL1β concentrations greater than 0.98 pg/mL were 8.3 times more likely to be high-risk cysts. At 1.26 pg/mL, IL1β levels had a high sensitivity and specificity with a likelihood ratio of 17x to distinguish low- from high-risk cysts. Low-risk lesions contained essentially undetectable concentrations of IL1β (<1.4 pg/mL) and high-risk lesions expressed IL1β with a mean concentration of 539 pg/mL. We also prospectively collected cyst fluid samples to analyze for IL1β levels in a validation set, and IL1β did differentiate high- from low-risk cysts, confirming our initial observations. On the basis of the previously determined ROC calculated value of 1.26 pg/mL to distinguish high- from low-risk cysts, IL1β levels had a PPV of 71% for correctly identifying high-risk cysts with HGD or invasive cancer and an NPV of 75% for correctly identifying low-risk cysts that contained low or moderate dysplasia. At the current time, there is no reliable marker in IPMN cyst fluid to aid the surgeon in clinical decision making or to inform the patient that they may have a high-risk IPMN that should be resected, therefore to identify a single test with a validated sensitivity and PPV greater than 70% is very promising and will encourage validation studies in a larger set of patients. As these samples are difficult to obtain in large numbers with correlative surgical pathology, this is an ongoing multi-institutional effort. IL1 is synthesized primarily by mononuclear phagocytes that are stimulated in immunogenic and proinflammatory environments. Whereas IL1α is mostly intracellular or membrane bound, IL1β is secreted into the extracellular space, which makes it an appropriate cytokine to measure in pancreatic cyst fluid (21). Plasma concentrations of IL1β are usually undetectable in the absence of inflammation, severe autoimmune disease, or organ rejection; therefore, IL1β levels in simple serous cysts should also be very low (22, 23). These data are consistent with our finding of near undetectable levels of IL1β in the cyst fluid of low-grade IPMN and serous cystadenomas. The presence of IL1β in dysplastic cysts may reflect an inflammatory microenvironment in the cyst.

Dysplasia is known to arise in a background of chronic inflammation. IL1-mediated inflammation has been shown to be associated with the pathogenesis of colon cancer, where tissue concentrations of IL1 are increased in chronic inflammatory bowel disease, and in gastric cancer, where IL1β production is upregulated in chronic gastritis, initiating the inflammatory response and exacerbating mucosal damage that leads to neoplasia (24–27). Similar to the cancers associated with inflammatory bowel disease and gastritis, chronic inflammation in pancreatic IPMN may lead to severe dysplasia and be reflected in IL1β cyst fluid levels. IL1β expression in tumor specimens has also been found to be a determinant of cancer risk and survival in many other neoplasms, including ovarian, cervical, and breast cancer, and is a mediator of pancreatic cancer cell invasion (21, 25, 28–31).

### Table 3. IL1β and FNA cytology in branch duct IPMN greater than 3 cm without nodularity

<table>
<thead>
<tr>
<th>IPMN duct type</th>
<th>Low risk</th>
<th>High risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N/A</td>
<td>Nondiagnostic</td>
</tr>
<tr>
<td>Branch duct</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>FNA cytology</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>IL1β levels, pg/mL</td>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td>Branch + main duct</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>FNA cytology</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

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*Clinical Cancer Research*
Many cancers present tumor antigens for recognition by host immune cells, and the antitumor immune response can be measured through cytokine levels in the tumors. Cell-mediated and humoral immune responses occur through Th1 and Th2 CD4+ T lymphocytes and their activity can be measured through quantification of the cytokines IL1, IL2, IL4, IL5, IL8, IL10, IL12, IL13, IFN-γ, and TNF-α (11). IL1β is a key mediator of the inflammatory response and is central to cell proliferation, differentiation, and apoptosis. IL1β signaling also activates CD4 T cells in an antigen-dependent fashion, a cascade that may be triggered when low-grade IPMN transform into highly dysplastic IPMN (32). IL5 is produced by Th2 and mast cells. Among other functions, it stimulates B-cell growth and increases immunoglobulin secretion. Increased serum levels of IL5 have been found in patients with pancreatic adenocarcinoma compared with chronic pancreatitis, and this finding supports a systemic Th2 response in patients with pancreas cancer (11). However, the absolute levels of IL5 expressed in this study were too low to be clinically relevant, and many of the samples, both high and low risk, contained concentrations below the detectable limits of our assay. IL8 is a major mediator of the inflammatory response and functions as a chemoattractant and an angiogenic factor. IL8 has been shown to be overexpressed in the duodenal juice of patients with pancreatic carcinoma and here was found in high concentrations in high-risk IPMN (10). However, the large variance in concentrations and low sample size limit its utility as a biomarker, and it was not found to be a significant factor on multivariate predictive analysis. The increased IL8 levels observed in high-risk IPMN may, however, be explained by IL1-stimulated production of IL8. Furthermore, cyst concentrations of IL5 and IL8 were not significantly lower in serous cystadenomas compared with high-risk IPMN, as they were in the case of IL1β.

In conclusion, high-risk IPMN were associated with elevated levels of cytokines reflective of a Th1 and Th2 immunologic response. The presence of IL1β in particular was highly predictive of malignancy, even when factors, such as main pancreatic duct involvement and cyst size, were adjusted for. This study has identified and validated a marker of IPMN dysplasia for further validation studies and suggests that cyst fluid IL1β levels may inform surgeons to more appropriately select patients for operative resection.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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