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Cytokine signatures in post-endoscopic retrograde cholangiopancreatography pancreatitis: a pilot study

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Abstract

Background Following endoscopic retrograde cholangiopancreatography (ERCP), post-ERCP pancreatitis (PEP) is the most common complication. The host's innate immune response to periprocedural pancreatic injury is the hallmark of its pathogenesis. Investigating cytokine signatures associated with PEP and its risk factors can guide understanding of PEP immunopathogenesis.

Methods We conducted a single-center, prospective, observational pilot study in adults at high-risk for PEP. Seven serum cytokines relevant to early acute pancreatitis pathogenesis, angiopoietin-2, hepatocyte growth factor (HGF), interleukin-6 (IL-6), IL-8, monocyte chemotactic protein-1, resistin, and soluble tumor necrosis factor-α receptor 1, were measured in sera collected 2 h pre- and post-ERCP. Levels were compared among healthy controls and ERCP participants who either did or did not develop PEP. Heat maps were constructed to perform a multidimensional exploratory analysis that aimed to determine the cytokine signatures associated with PEP and its participant-related risk factors (female sex, young age, and obesity).

Results A total of 65 participants were enrolled (36 undergoing ERCP and 29 healthy controls). Eight of the 36 (22.2%) ERCP participants developed PEP. Baseline IL-8 levels measured before ERCP were elevated in participants who developed PEP (7.5 vs. 14.8 pg/mL, P=0.02), and most strongly upregulated in women under 40 years of age. HGF levels post-ERCP were higher in participants with PEP (738.0 vs. 556.6 pg/mL, P=0.04), and most strongly upregulated in obese participants.

Conclusions Pre-ERCP IL-8 and post-ERCP HGF are associated with the development of PEP. Findings from this pilot study can inform the design of translational work in the immunopathogenesis of PEP.

Keywords Acute pancreatitis, endoscopic retrograde cholangiopancreatography, post-ERCP pancreatitis, pathogenesis

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Conflict of Interest: None

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Introduction

Approximately 400,000 individuals per year undergo endoscopic retrograde cholangiopancreatography (ERCP) in the United States (US) [1-3]. Post-ERCP pancreatitis (PEP) is the most common complication, occurring in 10-15% of patients, and requires acute hospitalization, thus imposing a substantial cost on the healthcare system [4]. Despite preventative measures (i.e., rectal non-steroidal anti-inflammatory drugs and pancreatic duct stent placement), PEP has an upward trend in incidence, affecting 55,225 patients undergoing ERCPs in the US during 2011-2017 [5]. This highlights a major knowledge gap in the field: namely, incomplete understanding of the pathophysiology of PEP—and acute pancreatitis (AP) in general [5]. PEP is the

only "human model" of AP for which the time of pancreatic injury—even though not intentional—is precisely known; thus, it offers an excellent opportunity to investigate the early pathophysiologic events in AP [6].

While prior studies in PEP have exclusively focused on the potential clinical utility of cytokines in diagnosing PEP [7], investigating the cytokine signatures associated with PEP can also inform the design of larger and deeper immunophenotyping studies aimed at an understanding of PEP's immunopathogenesis [8,9]. For example, prior studies have not assessed whether participants with risk factors for PEP (e.g., young women, the obesity) exhibit distinct cytokine signatures as surrogates of the status of their baseline immune environment. Such information could guide the identification of potential immune targets to prevent PEP in individuals at risk. In this pilot biomarker study, we investigated selected cytokines with biological relevance for their association with PEP, as well as patient-related risk factors for PEP.

Patients and methods

Study design

This was a pilot, single-center, prospective, observational study conducted at the University of Pittsburgh Medical Center (UPMC), Pittsburgh, PA, USA. Participants were enrolled between December 1st 2012 and June 30th 2016. The study was approved by the institutional review board of the University of Pittsburgh and required patients' informed consent. The study was registered at ClinicalTrials.gov (NCT03075592). All the authors had access to the study data and approved the final manuscript. Enrolled participants signed a written informed consent.

Study population

Participants 18 years of age or older who were scheduled for an outpatient ERCP were assessed for eligibility. Inclusion criteria comprised having an intact major papilla and an elevated baseline risk of post-ERCP pancreatitis. The presence of at least 1 of the following factors was used to define an elevated risk of PEP, as per society guidelines [10]: suspected dysfunction of the sphincter of Oddi (SOD – a condition that causes severe episodic abdominal pain and liver function test elevations, resulting in emergency room visits and hospitalizations), planned pancreatic therapeutic interventions, and a history of recurrent AP. SOD was defined according to the Milwaukee diagnostic criteria, which represent the widely accepted standard definition in the field [11,12]. Participants were excluded if they had active AP, prior sphincterotomy, history of chronic pancreatitis, confirmed or suspected pancreatobiliary malignancy, surgical altered anatomy, or history of organ transplantation.

Procedure-related factors were not used for eligibility, as cohort enrollment occurred prior to ERCP. The procedure interventions were performed by 1 of 5 experienced pancreatobiliary endoscopists. Co-interventions for PEP prevention, such as intravenous fluids, rectal indomethacin and prophylactic pancreatic stents, were implemented at the discretion of the performing endoscopist. A separate cohort of healthy volunteers not undergoing ERCP was enrolled to serve as controls.

Biomarker measurements

Blood samples were collected within 2 h before starting ERCP and within 2 h after finishing the procedure. In participants admitted for management of PEP, 2 additional blood samples were obtained at a median of 50 h (range: 24-75) and 91 h (range: 55-115) after ERCP, respectively. In the cohort of healthy volunteers, blood samples were obtained at baseline, 24 h, and 48 h. Blood samples were used to measure 7 serum cytokines associated with the early pathogenesis of AP: angiopoeitin-2 (Ang-2) [13], interleukin (IL)-6 (IL-6) [14], IL-8 [15], monocyte chemotactic protein-1 (MCP-1) [16], hepatocyte growth factor (HGF) [13], soluble tumor necrosis factor-α receptor 1 (sTNFα-R1) [17], and resistin [18]. These cytokines were selected because of their distinct role in the pathophysiology of AP, including chemotaxis of innate immune cells (IL-8, MCP-1) [19], amplification of the inflammatory response (IL-6, resistin) [20], lipolysis (resistin) [15], vascular leak (Ang-2, IL-8) [21], and regulation of the innate immune response (HGF, sTNFα-R1) [13].

Serum cytokine concentrations were processed in 2 separate batches using a Meso Scale Discovery (MSD) MULTI-SPOT® Assay System (MESO QuickPlex SQ 120 instrument). The 7 cytokines were quantified using 3 different assay kits: (a) V-Plex Human Proinflammatory for IL-6, IL-8, and MCP-1; (b) custom duplex kit combining Ang2 and HGF; and (c) custom duplex kit combining resistin and sTNFα-R1. The manufacturer's protocol was followed to process each sample, as described in prior publications by our group [13,22]. Periprocedural serum lipase and C-reactive protein (CRP) were also measured within 2 h before and 2 h after ERCP from the same samples.

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Outcomes

PEP was defined based on at least 2 of the following criteria: new or worsening characteristic upper abdominal pain; serum lipase >3× upper normal limit; or findings of AP on cross-sectional images [23]. The severity of PEP was categorized as mild, moderately severe or severe, according to the Revised Atlanta Classification [24].

Statistical analysis

Descriptive statistics were reported using proportions for categorical variables and means ± standard deviation or medians (interquartile range) for continuous variables, as appropriate. The associations between categorical baseline variables and PEP were evaluated using chi-squared tests, while independent samples *t*-tests were used to compare the means of continuous baseline characteristics by group (PEP vs. non-PEP). Mann-Whitney *U* tests were used to compare pre-procedure cytokine levels, post-procedure cytokine levels, and the change in levels from before to after ERCP, between patients who developed PEP and those who did not. Heat maps were constructed to allow for a multidimensional understanding of clinical risk factors, cytokine levels and PEP status (control, no PEP, admitted to the hospital with pain but not PEP, and PEP). Clinical risk factors included female sex, young age (<40 years) and obesity (defined as BMI >30 kg/m^2). Analyses were performed using R version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria) and SPSS version 28.0 (IBM SPSS, Armonk, NY). The level of significance for all analyses was α=0.05.

Results

Baseline characteristics

A total of 65 participants (36 high-risk participants undergoing ERCP, 29 healthy controls) were enrolled in the study. For the 36 ERCP participants, the average age was 46.8±14.4 years, 78% were female, and 8 (22.2%) developed PEP. Fourteen participants (38.9%) were admitted to the hospital with new abdominal pain post-ERCP, but did not fulfill the diagnostic criteria for PEP. Most of the participants had a prior history of (but not active/current) AP (77.8%) and clinical suspicion of SOD (52.8%). Forty-two percent of enrolled patients underwent a pancreatic sphincterotomy. The majority of participants received a prophylactic pancreatic duct stent (88.9%) and/or rectal indomethacin (75%). Compared to participants who did not develop PEP, those with PEP were more likely to have preprocedural abdominal pain (100% vs. 31.1%, P<0.001). Otherwise, baseline characteristics were similar between participants with and without PEP (Table 1). All participants with PEP had mild severity, requiring a mean hospital stay of 4.5±1.7 days without intensive care admission or need for pancreatic interventions.

Cytokine signatures associated with patient-related risk factors and PEP

The differences in periprocedural serum cytokines and changes in cytokine levels before and after ERCP between participants who developed PEP and those who did not are presented in Table 2. Heatmaps displaying the signatures of the 7 cytokines for clinical risk factors by ERCP outcome (control, no PEP, admission with pain but not PEP, and PEP) are shown in Fig. 1A,B.

Proinflammatory cytokines and their relation to PEP risk factors and outcomes

IL-8 signature: pre- and post-ERCP

In blood samples collected within 2 h prior to ERCP, median IL-8 levels were significantly higher in participants who developed PEP as compared to those without PEP (14.8 vs. 7.5 pg/mL, P=0.02). IL-8 levels both pre- and post-ERCP were the highest among female participants less than 40 years of age when compared to the other sex/age groups (Fig. 1A,B). While obesity status alone did not impact pre-ERCP IL-8 levels, both obese patients and female participants <40 years of age exhibited the highest post-ERCP IL-8 levels (Fig. 1A,B).

MCP-1 and IL-6

For pre-ERCP MCP-1 and IL-6, there was a trend towards elevated levels among the PEP group (P=0.08 and P=0.07, respectively), and this trend was maintained for MCP-1 post-ERCP levels (P=0.08), but not for IL-6 (P=0.46).

Ang-2 and resistin

Neither Ang-2 nor resistin levels peri-ERCP were significantly different overall between participants who developed PEP and those who did not. Ang-2 levels were the highest among obese participants who developed PEP, for both pre- and post-ERCP values (Fig. 1A,B). There was no recognizable cytokine signature pattern for resistin.

Anti-inflammatory cytokines and their relation to PEP risk factors and outcomes

HGF signature: pre- and post-ERCP

Within 2 h after ERCP completion, the median HGF serum levels were significantly greater in participants who developed PEP as compared to those without PEP (PEP 738 vs. non-PEP 557 pg/mL, P=0.04). Assessment of pre-ERCP HGF levels in participants who developed PEP indicated that obesity appeared to affect its levels (Fig. 1A,B). For example, within the PEP group, obese participants had the highest pre- and post-ERCP HGF values, but such a pattern was not seen among the non-PEP group.

Table 1 Baseline characteristics

Characteristics	Controls	No-PEP (n=28)	PEP (n=8)	P-value
Age – years (mean ± SD)	43.0±12.6	46.9±15.3	46.3±11.6	0.92
Female sex – n (%)	20 (69%)	20 (71.4%)	7 (87.5%)	0.36
Race – n (%)				>0.99
White	25 (86.2%)	27 (94.6%)	8 (100%)	
Black	1 (3.4%)	1 (3.6%)	0 (0%)	
BMI – kg/m² (mean ± SD)	28.6 ± 6.3	26.7±5.7	26.2±6.4	0.83
Current smoking – n (%)		14 (50%)	4 (50%)	>0.99
Current alcohol use – n (%)		7 (25%)	2 (25%)	>0.99
History of cholecystectomy – n (%)		20 (71.4%)	5 (62.5%)	0.63
Prior ERCP – n (%)		15 (53.6 %)	3 (37.5%)	0.42
Pre-procedure abdominal pain – n (%)		9 (32.1%)	8 (100%)	<0.001
Sphincter of Oddi dysfunction – n (%)		13 (46.4%)	6 (75%)	0.15
History of AP – n (%)		23 (82.1%)	5 (62.5%)	0.24
History of recurrent AP – n (%)		5 (17.9%)	2 (25%)	0.65
Procedural interventions – n (%)				
Difficult cannulation		4 (14.3%)	1 (14.3%)	>0.99
Pre-cut sphincterotomy		1 (3.7%)	0 (0%)	>0.99
Pancreatic sphincterotomy		12 (42.9%)	3 (37.5%)	0.79
Balloon dilation		3 (11.1%)	0 (0%)	>0.99
Rectal indomethacin		21 (75%)	6 (75%)	>0.99
Pancreatic stent		25 (89.3%)	7 (87.5%)	0.48
ERCP duration – min (mean ± SD)		45.8±22.8	40.8±20.8	0.58

PEP, post-ERCP pancreatitis; ERCP, endoscopic retrograde cholangiopancreatography; BMI, body mass index; AP, acute pancreatitis; SD, standard deviation

Table 2 Association of periprocedural cytokines with post-ERCP pancreatitis

Cytokines, pg/mL (median±IQR)	Pre-ERCP cytokines *			Post-ERCP cytokines **			Difference of post- and pre-procedure cytokines ***		
	No-PEP (n=28)	PEP (n=8)	P-value	No-PEP (n=28)	PEP (n=8)	P-value	No-PEP (n=28)	PEP (n=8)	P-value
Ang-2	6505.9±3426.2	7634.1±3442.5	0.95	7118.8±4174.3	6588.6±3235.7	0.93	334.1±1797.4	- 461.98±2846.3	0.47
HGF	477.9±208.0	603.9±216.5	0.15	556.5±242,8	738.0±202.9	0.04	37.0±243.8	28.2±515.0	0.93
IL-6	0.8±0.6	1.3±0.7	0.07	1.1±0.9	1.7±0.8	0.46	0.2±0.5	0.1±0.9	0.51
IL-8	7.5±7.8	14.8±7.0	0.02	11.6±21.9	20.5±44.5	0.24	2.6±17.2	4.5±59.9	0.56
MCP-1	303.8±149.1	390.7±231	0.08	288.6±205.0	451.2±213.9	0.08	26.9±125.2	85.0±232.4	0.34
Resistin	1233.1±964	1242.8±499.4	0.81	1176.9±701.3	1541.6±595.0	0.54	10.8±517.2	159.9±302.5	0.15
TNF-α Receptor1	1068.5±397.5	956.2±259.3	0.28	1164.5±562.3	1049.2±214.3	0.44	75.5±366.2	109.4±212.1	0.84

*Blood drawn within 2 h prior to ERCP
**Blood drawn within 2 h after ERCP
*** The difference is defined as post-procedure minus pre-procedure cytokine level
ERCP, endoscopic retrograde cholangiopancreatography; IQR, interquartile range; Ang-2, angiopoietin-2; HGF, hepatocyte growth factor; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; TNF-α Receptor1, soluble tumor necrosis factor-α receptor 1

sTNFα-R1

There were no significant differences in the pre- or post-ERCP levels of the other anti-inflammatory cytokine, sTNFα-R1, between patients who developed PEP and those who did not.

Lipase and CRP

Comparisons of lipase and CRP levels (pre- and post-ERCP) between PEP group and non-PEP group are shown on Table 3. While post-lipase levels were higher in the PEP group compared to the non-PEP group, this difference did not reach

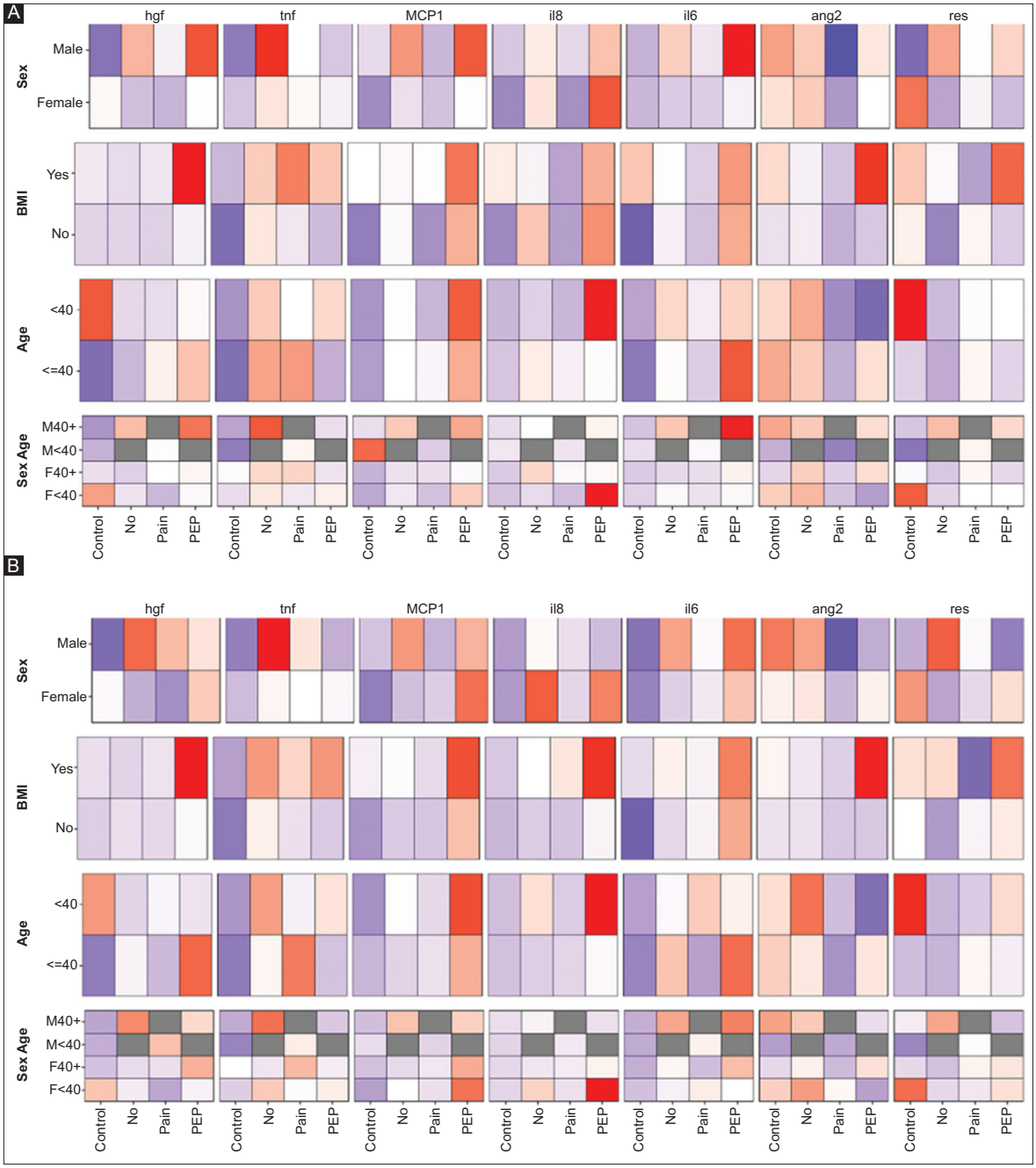

Figure 1 Heatmaps demonstrating cytokine signatures: (A) 2 h before ERCP for clinical risk factors in 3 ERCP outcome groups (no hospital admission, hospital admission with pain but not PEP, PEP); (B) 2 h after ERCP for clinical risk factors in 3 ERCP outcome groups
ERCP, endoscopic retrograde cholangiopancreatography, PEP, post-ERCP pancreatitis

statistical significance. CRP levels were similar pre- and post-ERCP between the 2 groups.

Discussion

In this pilot study, we used prospectively collected data and biosamples to explore changes in biologically relevant cytokines associated with PEP. We identified specific cytokine signatures that are potentially associated with PEP and also specific participant characteristics. Pre-ERCP IL-8 levels were associated with PEP and were the highest among young females—a recognized risk factor for PEP [10,25]. There was also a trend towards a statistically significant association between pre-ERCP levels of IL-6, a proinflammatory cytokine, MCP-1, a chemotactic cytokine, and PEP. When taken together, we propose that a baseline

Table 3 Characteristics of periprocedural lipase and C-reactive protein in the prediction of post-ERCP pancreatitis

Variable	No-PEP (n=28)	PEP (n=8)	P-value
Pre-ERCP lipase	25.0±24.5	33.0±25.0	0.69
Post-ERCP lipase	56.0±91.0	126.5±141.0	0.22
Difference of post- and pre-ERCP lipase	57.5±70.0	67.0±160.0	0.30
Post-ERCP lipase > 3 × ULN	3 (10.7%)	1 (12.5%)	>0.99
Pre-ERCP CRP	0.15±0.25	0.13±0.36	0.73
Post-ERCP CRP	0.14±0.22	0.15±0.33	0.86
Difference of post- and pre-ERCP CRP	-0.005±0.04	-0.003±0.13	0.91

ERCP, endoscopic retrograde cholangiopancreatography, PEP, post-ERCP pancreatitis; CRP, C-reactive protein; ULN, upper limit of normal; CRP value is in mg/L, lipase value is in U/L
Post-ERCP Lipase >3 × ULN is currently a diagnostic criterion for PEP [5]

proinflammatory milieu predisposes to PEP in a sub-cohort of young female patients.

We also found that, at 2 h following ERCP, HGF levels were significantly associated with the development of PEP, being the highest in obese patients. Given the pathologic significance of HGF in reflecting both the extent of various tissue injury and its association with the proinflammatory phenotype of obesity [26-28], we propose that obesity predisposes to PEP by priming the innate immune system for a dysregulated response to pancreatic injury.

Our pilot study is the first to investigate 4 mechanistically relevant cytokines (Ang-2, MCP-1, resistin, and TNF-α R1) in PEP. In the largest study published to date, Concepción-Martín *et al* prospectively analyzed IL-6, IL-10, TNF-α, CRP, amylase and lipase before and 4 h after ERCP in 510 patients (36 patients developed PEP). None of the cytokines were associated with PEP, and no associations between cytokines and risk factors for PEP were examined [29]. In a smaller study with 78 patients, Chen *et al* sought to discover a periprocedural cytokine for early diagnosis of PEP [30]. Serum concentrations of TNF-α, IL-1β, IL-6, IL-8 and IL-10 were measured immediately prior to, and at 1, 4, 8 and 24 h after ERCP. Among the 7 patients who developed PEP, the serum levels of the examined cytokines were elevated at 8 and 24 h, but not at 1 and 4 h. As expected, none of the cytokines (i.e., TNF-α, IL-1β, IL-6, and IL-8) carried sufficient accuracies at 1- and 4-hours post-ERCP to predict PEP. Given that disposition decisions on outpatients who have undergone ERCP need to be made within 2 h of the procedure, the study by Chen *et al* highlighted a need to investigate other biomarkers. Additionally, potential disparities between sex and age in the cytokine values were not studied.

Our observations help generate a testable hypothesis about why some ERCP participants are at increased risk of PEP, independently of intraprocedural events [25,31,32]. Being both young and female has been a consistently identified independent risk factor for PEP across different ethnicities [31-33]. Among our cohort, *pre*-ERCP IL-8 levels were higher among those that developed PEP (no-PEP 7.5 vs. PEP 14.8 pg/mL, P=0.02), but the levels were highest among young women. Such sex and age-associated IL-8 level differences were not observed among those who did not develop PEP. While not statistically significant in this pilot study, there was also trend towards an association between pre-ERCP IL-6 and MCP-1 levels and PEP. MCP-1, a chemokine and IL-6, a proinflammatory cytokine, are released from a wide range of tissue types, including pancreatic, adipose and immune cells [34,35]. MCP-1 acts as a potent chemoattractant and activator of monocytes, macrophages and other leukocytes [36]. Pre-ERCP MCP-1 levels were most upregulated in patients with a history of recurrent AP and PEP. Taken together, we propose that a proinflammatory milieu influenced by sex, age and recurrent AP may predispose patients to PEP.

We also found that the post-procedure HGF levels were associated with development of PEP. Intriguingly, HGF levels were exclusively upregulated among obese patients who developed PEP *both* before and after ERCP, in contrast to obese non-PEP patients. HGF is a pleiotropic cytokine that can dampen the innate immune response (anti-inflammatory function), but it is also highly expressed/released as an inducer of angiogenesis in response to tissue hypoxia in a variety of organs, as well as in adipose tissue, peritoneal macrophages, pancreatic acinar and beta cells, and pancreatic stellate cells [37]. It is also highly expressed and secreted upon tissue injury, thus a surrogate for the extent of tissue injury [37,38].

Interestingly, Ang-2, another potent modulator of angiogenesis that is also known to be correlated with obesity, was upregulated among obese patients who developed PEP, but not among non-PEP obese patients. In mechanistic studies, HGF and Ang-2 reflect pathological remodeling of the adipose tissue that promotes a proinflammatory phenotype, while HGF is mainly secreted by resident macrophages in the adipose tissue in obese participants [27,39-42]. Thus, one can hypothesize that elevated HGF and Ang-2 pre-ERCP levels indicate a proinflammatory phenotype of adipose tissue among obese participants "primed" for an accentuated innate immune response, whereas post-ERCP HGF may indirectly indicate the extent of tissue hypoxia caused by pancreatic injury. In support of this, a high level, rather than a low level of HGF has been consistently associated with severe AP—an observation that would be discordant if HGF levels reflected the magnitude of anti-inflammatory immune activation [43,44]. Given that studies have yielded conflicting results on whether obesity is a risk factor for PEP [45-48], our hypothesis, which stratifies obesity into proinflammatory and non-inflammatory phenotype, using HGF and Ang-2 levels pre-ERCP and HGF level post-ERCP, is testable and also has biological plausibility.

Once post-ERCP HGF levels are validated to be strongly associated with PEP in a larger dataset, they can potentially serve as an intermediate biomarker in pilot randomized clinical trials to allow efficient evaluation of newly developed drugs for PEP prevention, by substantially reducing the sample size requirement [49]. The framework of using an accurate intermediate biomarker of PEP as a trial endpoint to

facilitate resource-friendly clinical trials has been described and supported by a robust statistical analysis of meta-data by Srivastava *et al* [49].

Finally, all patients with PEP reported abdominal pain prior to the ERCP. This is an intriguing observation that leads to interesting questions. One could speculate whether the presence of pre-ERCP pain is a strong predictor of PEP development, and whether it may signify that the baseline immune system is more primed for a dysregulated response in such patients than in others. While we did not observe correlations between pre-ERCP pain and the 7 cytokines measured, further immune profiling is warranted in future translational studies.

Our study has several limitations. This was a single-center study with a small sample size, which can potentially introduce selection bias. For example, there were more patients with pancreatic sphincterotomy and balloon sphincteroplasty in the non-PEP group than in the PEP group. Given the small size of the study, we are unable to control for these as covariates and larger studies are needed for more detailed analyses. Nevertheless, given that the cytokine response occurs *after* periprocedural events, our findings are hypothesis-generating; that the post-ERCP HGF response to periprocedural pancreatic injury can predict PEP development better than periprocedural risk factors alone. The study population was high-risk for PEP and was not intended to reflect the general population undergoing ERCP. Given the low number of total events, the interpretation of heat maps should be viewed as a proof of concept rather than demonstrating a definitive mechanism. Serum cytokines were analyzed in 2 separate batches, which may have theoretically introduced risk for a batch effect, but such an effect was not observed. Most participants had a pancreatic stent and/or rectal indomethacin, which may have altered the predictive abilities of pre-ERCP cytokines for identifying PEP. Similarly, the use of indomethacin for some participants may have altered the post-ERCP cytokine values, specifically muting some of the potential differences in the PEP group. Nevertheless, this is a pilot study, which succeeded in formulating testable hypotheses driven by data from mechanistic studies.

Despite these limitations, our study has several strengths. We used a prospective cohort design and measured 7 mechanistically relevant cytokines, 4 of which have never been studied in PEP before, at 2 predetermined time-points before and after ERCP. Additionally, this pilot study generated biologically plausible and testable hypotheses related to the proinflammatory milieu of at-risk patients and its relation to sex, age and obesity. Data were prospectively collected with case report forms on the same day of the procedure, reducing the risk of recall bias. Finally, we enriched the cytokine analyses with healthy controls to provide baseline cytokine levels in participants without any acute illness.

In conclusion, we demonstrated that specific cytokine signatures are associated with the development of PEP. Furthermore, we discovered unique associations between these cytokines and participant characteristics. Our findings can inform the design of translational work in the immunopathogenesis of PEP and, more broadly, of all AP.

Summary Box

What is already known:

- Pancreatitis is a common and unpredictable complication following endoscopic retrograde cholangiopancreatography (ERCP)
- A disordered host immune response to periprocedural pancreatic injury is believed to be the hallmark of post-ERCP pancreatitis (PEP) pathogenesis
- Despite prophylaxis, PEP still occurs in up to 15% of high-risk subjects

What the new findings are:

- Elevated baseline pre-ERCP interleukin-8 levels, which could represent a proinflammatory milieu in patients undergoing ERCP, may be associated with an increased risk of PEP
- Early post-ERCP levels of hepatocyte growth factor, which is a biomarker for organ injury in kidneys, liver and pancreas, are significantly associated with the development of PEP

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