RESEARCH LETTER

Increased protein degradation as well as lactate and malate dehydrogenase activity in sterile and infected walled-off pancreatic necrosis

Łukasz Rojek[1], Marian Smoczyński[1], Magdalena Stojek[1], Tomasz Śledziński[2], Ryszard T. Smoleński[3], Krystian Adrych[1]

1 Department of Gastroenterology and Hepatology, Medical University of Gdańsk, Gdańsk, Poland
2 Department of Pharmaceutical Biochemistry, Medical University of Gdańsk, Gdańsk, Poland
3 Department of Biochemistry, Medical University of Gdańsk, Gdańsk, Poland

Introduction Postinflammatory pancreatic fluid collections (PFCs) are abnormal deposits of liquid and sometimes solid material that may or may not be clearly demarcated from the surrounding tissues by a discrete wall. In the past, all peri-PFCs were referred to as "pseudocysts".[1] The pancreatic "pseudocyst" fluid was found to be a mixture of plasma and pancreatic juice with a high proteolytic activity.[2] Mönkemüller et al[3] reported that total protein, albumin, and lactate dehydrogenase (LDH) in PFCs were significantly increased in patients with infected fluid collections compared with those with sterile fluid collections.

Currently, PFCs are classified into 4 main types: 1) acute peri-PFC; 2) pancreatic pseudocyst; 3) acute necrotic collection, and 4) walled-off pancreatic necrosis (WOPN).[4] The first 2 PFC types usually resolve spontaneously over time, whereas acute necrotic collections and WOPN need aggressive treatment.[1] WOPN is a mature, encapsulated collection of pancreatic or peripancreatic necrosis that has developed a well-defined inflammatory wall and usually occurs 4 weeks after the onset of disease.[4] It can be sterile or infected.[4] The finding of infected WOPN is an important indication for therapeutic intervention; however, the diagnosis of infection is difficult and challenging.[5] The presence of infection can be presumed when there is extraluminal gas in the pancreatic or peripancreatic tissue on computed tomography or when percutaneous, image-guided, fine-needle aspiration biopsy sample is positive for bacteria or fungi.[4]

In 2013, the International Association of Pancreatology and American Pancreatic Association recommended image-guided percutaneous (retroperitoneal) or endoscopic transluminal drainage as the optimal intervention for patients with suspected or confirmed infected WOPN.[6] However, late sequelae and safety of nonintervention management in patients with asymptomatic WOPN remain unclear.[7]

To our best knowledge, the biochemical characteristics of WOPN have not been studied so far. To better understand the clinical history and possible treatment options in WOPN, it is necessary to obtain more information on its basic biochemical composition. The aim of the study was to characterize: 1) total protein and albumin concentration; 2) the degree of protein degradation; and 3) LDH and malate dehydrogenase (MDH) activities of pancreatic necrotic fluid (PNF) from patients with sterile and infected WOPN.

Patients and methods The study included 35 adult patients aged from 20 to 85 years (mean age, 51 ±2.5 years) with WOPN, admitted to the Department of Gastroenterology and Hepatology, Medical University of Gdańsk, Gdańsk, Poland. WOPN was classified according to the definitions set forth in the 2012 Revision of the Atlanta classification.[4] The study included patients with clear indications for an endoscopic intervention, such as the clinical suspicion of infected WOPN or documented infected WOPN. The presence of infection was presumed when the patient's condition suggested infection (eg, deterioration of general condition, fever), and there was extraluminal gas in the pancreatic or peripancreatic tissue on contrast-enhanced computed tomography or when image-guided fine-needle aspiration biopsy sample was positive for bacteria or fungi.[4] In the absence of infected WOPN, indications for endotherapy included ongoing gastric outlet, duodenal, or biliary obstruction due to mass effect, persistent symptoms such as intractable pain, or

Correspondence to:
Krystian Adrych, MD, PhD,
Katedra i Klinika Gastroenterologii i Hepatologii, Gdański Uniwersytet Medyczny, ul. Smoluchowskiego 17, 80-214 Gdańsk, Poland,
phone: +48 58 349 36 40,
e-mail: kgastro@gumed.edu.pl
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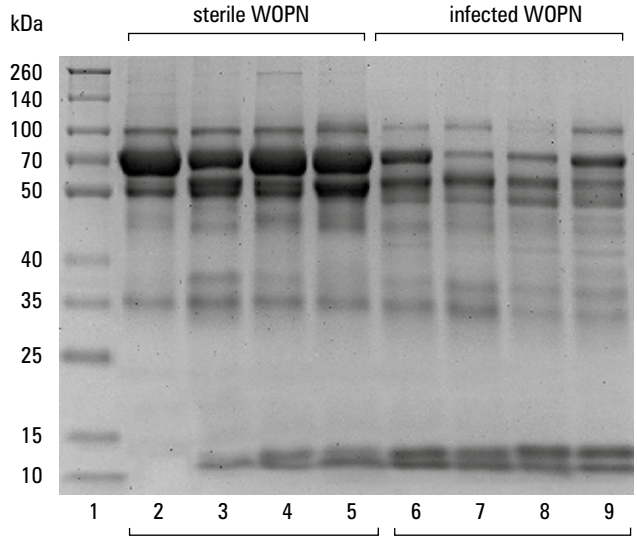

FIGURE 1 Pancreatic necrotic fluid protein electrophoresis; lane 1, standard molecular weight; lane 2–5, necrotic fluid from sterile walled-off pancreatic necrosis (WOPN); lane 6–9, necrotic fluid from infected walled-off pancreatic necrosis. Samples of pancreatic necrotic fluid containing 10-μg protein were separated.

			P value
total protein, g/l	33.8 ±2.2	16.5 ±2.9	0.02
albumin, g/l	12.5 ±2	4.0 ±1.6	0.01
total amino acids, mmol/l	11.7 ±4	26.4 ±3.5	0.0025
lactate dehydrogenase, U/l	1300 ±500	5300 ±2016	0.05
malate dehydrogenase, U/l	470 ±190	1700 ±600	0.04

disconnected duct syndrome (transection of the pancreatic duct in the presence of pancreatic necrosis). Indications for intervention were consistent with the guidelines issued by the International Association of Pancreatology and American Pancreatic Association.[6] The exclusion criteria were as follows: PFCs other than WOPN, asymptomatic sterile WOPN, benign or malignant pancreatic cystic tumors, pregnancy, age below 18 years, and inability to provide informed consent. In our study, a neoplastic etiology of the pancreatic cystic lesions was excluded on the basis of medical history, computed tomography, or magnetic resonance images, cytology, and fluid markers such as carcinoembryonic antigen (CEA) and cancer antigen 19-9 (CA 19-9). In our study, the mean concentrations of CEA and CA 19-9 were below the cut-off value for pancreatic cystic tumors (79.5 ng/ml and 19170 U/ml, respectively).[8]

Endosonography-guided transgastric or transduodenal drainage was performed using a linear array ultrasound video endoscope (Pentax EG 3870UTK, Naka-Ochiai, Shinjuku-ku, Tokyo, Japan / Hitachi Avius, Soto-kanda, Chiyoda-ku, Tokyo, Japan). Fluid samples were obtained during the first stage of endoscopic treatment of WOPN. The study was performed in accordance with the Declaration of Helsinki of the World Medical Association and was approved by the Ethics Committee of the Medical University of Gdańsk (NKBBN/190/2012). All patients signed an informed consent form for this investigation. A detailed description is presented in Supplementary material online.

Total protein in PNF and serum was measured by the Lowry's method. Albumin PNF was measured by the immunoturbidimetric method using an ARCHITEC C8000 autoanalyser (Abbott, Abbott Park, Illinois, United States). LDH and MDH activities in PNF were measured no later than 2 hours after PNF aspiration and centrifugation, as previously described.[9] PNF and serum amino acid concentrations were assayed by ion pairing/reversed phase liquid chromatography--mass spectrometry, as described previously.[10]

Samples of PNF and serum containing 10-μg protein were separated by acrylamide gel electrophoresis with 10% sodium dodecyl sulfate. After electrophoresis gels were stained with Coomassie Brilliant Blue.

Data were expressed as mean (± the standard error of the mean). Depending on the distribution of variables, the statistical significance of differences between means of the biochemical parameters was estimated by using a parametric test (*t* test) or nonparametric tests (Mann–Whitney, Wilcoxon). A statistical analysis was performed using the Statistica software (Statistica 10, Stat Soft, Tulsa, Oklahoma, United States). A *P* value of less than 0.05 was considered statistically significant for all analyses.

Results Infected WOPN was found in 9 of 35 examined patients (about 26%). Pathogens detected in the infected WOPN fluid included *Escherichia coli* (3 of 9 patients), *Streptococcus* (2 of 9 patients), and *Enterococcus* (2 of 9 patients) and single cases of *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Staphylococcus*, and *Candida*. Four of nine patients were found to be infected with multiple pathogens. Most of the patients had necrosis of both pancreatic and peripancreatic tissues (combined-type WOPN) (Supplementary material online, *Figure S1A*). Pancreatic parenchymal necrosis (Supplementary material online, *Figure S1B*) was found in approximately 20% of the patients.

There was a significant difference in total protein and albumin concentrations between the

PNF of patients with sterile and infected WOPN (FIGURE 1). The mean percentage of PNF albumin concentration (compared with total protein) was lower in infected than in sterile WOPN (FIGURE 1). Significant protein degradation (more bands corresponding to protein with lower mass, especially between 10-15 kDa, are present) was observed in the PNF of patients with infected rather than in those with sterile WOPN (FIGURE 1, compare lane 2–5 with lane 6–9). It is possible that some bands observed in infected PNF could originate from bacterial protein degradation. However, this issue requires further research. The total amino acid concentration (sum of all amino acid concentrations) was approximately 2-fold higher in infected than in sterile WOPN (FIGURE 1).

Serum protein electrophoresis showed a thinner band corresponding to serum albumin with signs of some degradation in patients with infected WOPN, as compared with those with sterile WOPN (Supplementary material online, *Figure S2*, compare lanes 2 and 3). In patients with both sterile and infected WOPN, total soluble protein and albumin concentrations were significantly lower in the PNF compared with serum (FIGURE 1 and Supplementary material online, *Figure S2*). Interestingly, the percentage of PNF albumin (compared with total protein) was significantly lower than that in serum (FIGURE 1 and Supplementary material online, *Figure S2*).

The significant differences between LDH and MDH activities in sterile and infected necrotic fluid were also found (FIGURE 1).

Discussion Our results suggest that PNF obtained from patients with WOPN contains plasma-like proteins. However, we observed signs of PNF protein proteolysis, especially with regard to higher molecular-weight proteins, including albumin (FIGURE 1). The protein breakdown is much more intense in infected PNF (FIGURE 1). The proteolysis observed by the electrophoretic analysis was confirmed by greater concentrations of total amino acids (product of protein degradation). This is in contrast to the previously reported results indicating that protein concentrations in PNF aspirated from infected chronic pseudocysts were approximately 3-fold higher compared with those in sterile chronic pseudocysts.[3] The activity of LDH (released from cells) is widely used as a marker of necrosis.[11] In the present study, there were significant differences in LDH and MDH activities between sterile and infected WOPN.

The main advantage of our study is its cognitive value. A biochemical analysis of pancreatic necrosis has extended our understanding of this pathology. Patients with infected pancreatic necrosis do not always have an unequivocal clinical presentation. Similarly, the clinical picture does not always correlate with the results of biochemical and imaging studies. Simple and fast biochemical assays of PNF such as LDH or MDH may allow a quick identification of infections, especially in situations where awaiting microbiologic confirmation can delay proper treatment. Management of infected pancreatic necrosis requires aggressive drainage, often with surgery, and a multi-specialist approach.

A small sample size is an obvious limitation of this study. As such, it does not allow us to draw unequivocal conclusions about the clinical utility of the biochemical PNF analysis for the detection of infected WOPN. Nevertheless, we found significant differences in a number of parameters such as protein breakdown, total amino acid concentrations, and LDH and MDH activities between the PNF of patients with sterile and infected WOPN. To our best knowledge, this is the first attempt at a biochemical characterization of fluid obtained from the well-defined WOPN.

In conclusion, the results presented in this paper indicate that PNF obtained from patients with WOPN contains a mixture of partially degraded plasma proteins. Total protein and albumin concentrations of PNF are lower than those in serum. Moreover, total protein and albumin concentrations of infected WOPN are lower than those in sterile WOPN, whereas the total free amino acid concentration is higher in infected WOPN. LDH and MDH activities are greater in infected WOPN, which may suggest that necrosis is more pronounced in infected WOPN.

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Supplementary material online Supplementary material is available with the online version of the article at www.pamw.pl.

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