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The Role of Intestinal Alkaline Phosphatase and Bacterial Lipopolysaccharides in Patients Undergoing Pancreaticoduodenectomy

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Zusammenfassung

Als Bürstensaumenzym des Darms ist intestinale alkalische Phosphatase (IAP) ein wichtiger Regulator der intestinalen Homöostase [1]. Darüber hinaus dephosphoryliert dieses Enzym auch Lipopolysaccharide (LPS) und andere Entzündungsmediatoren, die für Endotoxinämie und Inflammation mitverantwortlich sind [1]. Ein IAP-Mangel wird mit verschiedenen Krankheiten in Verbindung gebracht, z. B. mit dem metabolischen Syndrom, Leberfibrose, ischämischen Herzkrankheiten, Gebrechlichkeit und einer verkürzten Lebenserwartung [2-4]. LPS ist ein Hauptbestandteil der äußeren Membran gramnegativer Bakterien und ein starker Auslöser von Entzündungen [5-7]. Jüngste Studien haben über erhöhte zirkulierende LPS-Spiegel bei Patienten mit Dickdarm-, Leber- und Blasenkrebs berichtet [8-11]. Eine intestinale mikrobielle Dysbiose und eine Störung der Darmbarriere können die Translokation bakterieller Produkte in den systemischen Kreislauf verstärken und den LPS-Spiegel im Serum erhöhen [3, 12-14]. Bei der Pankreatikoduodenektomie (PD) werden der Pankreaskopf, die Gallenblase, der Gallengang und der erste Teil des Dünndarms (Duodenum) mit oder ohne Entfernung des Pylorus und des distalen Magens entfernt werden [15]. Da die IAP zu einem Großteil im Duodenum produziert wird [1], welches bei der PD vollständig entfernt wird [15], haben wir die Hypothese aufgestellt, dass eine PD zu einem signifikanten Abfall der IAP Aktivität im Stuhl der Patienten führt.

Zunächst wurden in unserer Einrichtung prä- und postoperative Stuhl- und Blutproben von Patienten gesammelt, die bereit waren, an der Studie teilzunehmen. Sechsunddreißig Patienten, die sich einer PD unterzogen, dienten als Studienpopulation. Zwölf Patienten, die eine distale Pankreatektomie (DP) erhielten, und 15 Patienten, die sich kleineren allgemeinchirurgischen Eingriffen unterzogen, dienten als Kontrollgruppen. Mit der PNPP-Methode und dem LAL-Assay wurde die IAP-Aktivität im Stuhl bzw. die LPS-Level im Serum untersucht. In der gesamten Kohorte war die präoperative IAP-Aktivität im Stuhl negativ mit dem LPS-Spiegel im Serum und dem Alter korreliert. In der PD-Gruppe nahm die IAP-Aktivität im Stuhl nach der Operation deutlich ab. Darüber hinaus korrelierte das verringerte Ausmaß

der IAP-Aktivität positiv mit der Länge der Entnahmelänge des proximalen Dünndarms. In der Kontrollgruppe der Patienten, die sich einer DP unterzogen, war der Rückgang der IAP-Werte deutlich weniger ausgeprägt. Bei Patienten, die sich einer PD unterzogen, kam es nach dem Eingriff zu einem signifikanten Anstieg der Serum-LPS-Spiegel. Die DP führte ebenfalls zu einem Anstieg der Serum-LPS-Spiegel, jedoch in einem wesentlich geringeren Ausmaß als die PD. Bemerkenswert ist, dass der präoperative LPS-Spiegel im Serum bei Patienten mit Pankreaskarzinom signifikant höher war als bei den Patienten ohne Karzinom. Bei den Patienten mit Pankreaskarzinom korrelierte die IAP-Aktivität im Stuhl negativ mit dem LPS-Spiegel im Serum. Bei den Patienten mit Pankreaskarzinom, die sich einer PD unterzogen, korrelierte die postoperative IAP-Aktivität im Stuhl weiterhin negativ mit dem LPS-Spiegel im Serum. Bei den Patienten mit Pankreaskarzinom, die sich einer PD unterzogen, war die postoperative LPS-Konzentration im Blut mit einer höheren Anzahl an Krankenhaustagen verbunden.

Zusammenfassend lässt sich sagen, dass die IAP-Aktivität im Stuhl nach PD signifikant abnahm und dass der Grad der Abnahme der IAP-Aktivität positiv mit der Entnahmelänge des proximalen Dünndarms korrelierte, was unsere Hypothese stützte, dass die Duodenektomie die IAP-Aktivität im Stuhl direkt beeinflusst. Die DP verursachte ebenfalls eine Verringerung der IAP-Aktivität im Stuhl, jedoch in einem viel geringeren Ausmaß als die PD. Die LPS-Konzentrationen im Serum stiegen nach der PD signifikant an. In der DP-Gruppe stiegen die postoperativen LPS-Konzentrationen im Serum ebenfalls an, aber das Ausmaß des Anstiegs war viel geringer als in der PD-Gruppe. Der Anstieg der LPS-Konzentrationen deutet darauf hin, dass die intestinale Homöostase nach der Operation, insbesondere nach der PD, verändert war [16]. Daher könnten Patienten, die sich einer größeren Operation unterziehen, von einer prä- oder probiotischen Behandlung profitieren, um die mikrobielle Homöostase im Darm und die Barrierefunktion des Darms aufrechtzuerhalten. Da IAP bekanntermaßen die intestinale Homöostase aufrechterhält und LPS entgiftet, könnte es als neuer Ansatz zur Verbesserung des Ergebnisses großer Operationen dienen, insbesondere bei PD.

Patienten mit Bauchspeicheldrüsenkrebs hatten signifikant höhere präoperative LPS-Werte im Serum als Patienten ohne Karzinom. Gleichzeitig waren die präoperativen

LPS-Konzentrationen im Serum bei Patienten mit Bauchspeicheldrüsenkrebs negativ mit der IAP-Aktivität im Stuhl korreliert, und die Korrelation zwischen diesen beiden Indikatoren war bei Krebspatienten stärker ausgeprägt als in der gesamten Studienpopulation.

Aufgrund der funktionellen Rollen der IAP, insbesondere ihrer wichtigen Rolle in der Aufrechterhaltung der Integrität der Darmbarriere und ihrer Fähigkeit, von Bakterien stammende Entzündungsmediatoren zu hemmen, könnte eine Supplementierung von exogenem IAP für Patienten, die sich einer PD unterziehen, von Vorteil sein.

Abstract

As a gut brush border enzyme, intestinal alkaline phosphatase (IAP) is a critical regulator of intestinal homeostasis [1]. In addition, this enzyme also functions to dephosphorylate lipopolysaccharide (LPS) and other inflammatory mediators responsible for endotoxemia [1]. IAP deficiency has been linked to various pathologies such as metabolic syndrome, liver fibrosis, ischemic heart disease, frailty, and a decreased life span [2-4]. LPS is a principal constituent of the outer membrane of Gram-negative bacteria and a potent trigger of inflammation [5-7]. Recent studies have reported elevated circulating LPS levels in patients with colorectal cancer, liver cancer, and bladder cancer [8-11]. Intestinal microbial dysbiosis and gut barrier dysfunction can increase the translocation of bacterial products into the systemic circulation and elevate serum LPS levels [3, 12-14]. Pancreaticoduodenectomy (PD) is a complex and difficult operation that removes the head of the pancreas, the gallbladder, the bile duct, and the first part of the small intestine (duodenum), with or without removal of the pylorus and distal stomach[15]. Since IAP is mainly produced in the duodenum [1], which is partially or completely removed in PD [15], we hypothesized that there would be a marked decrease in stool IAP activity accompanied by a significant increase in serum LPS levels.

Firstly, pre- and postoperative stool and blood samples were collected from patients who were willing to be enrolled in the study in our institution. Thirty-six patients undergoing PD served as the study population. Twelve patients receiving distal pancreatectomy (DP) and 15 patients undergoing smaller general surgery procedures served as control groups. PNPP method and LAL assay were performed to test stool IAP activity and serum LPS level, respectively. In the whole cohort, preoperative stool IAP activity was negatively correlated with serum LPS levels and age. In the PD group, the stool IAP activity significantly decreased after surgery. In addition, the decreased extent of IAP activity positively correlated with the removal length of the proximal small intestine. The control group of patients undergoing DP showed a significant less marked reduction in IAP levels. Patients undergoing PD had a significant increase in serum LPS levels after the procedure. DP also led to an increase in the levels of serum LPS, but to a much lesser extent than PD. Of note, preoperative

serum LPS levels were higher in the pancreatic cancer patients than in the non-cancer patients. Furthermore, stool IAP activity negatively correlated with serum LPS levels in pancreatic cancer patients preoperatively. For pancreatic cancer patients undergoing PD, postoperative stool IAP activity still had a negative correlation with serum LPS levels. Postoperative concentrations of blood LPS were positively correlated with hospital days in pancreatic cancer patients undergoing PD.

In summary, stool IAP activity decreased significantly after PD, and the decreased degree of IAP activity positively correlated with the removal length of the proximal small intestine, which supported our hypothesis that duodenectomy directly affected stool IAP activity levels. DP also caused a reduction in stool IAP activity, but to a much lesser extent than PD. Serum LPS levels significantly increased after PD. In the DP group, postoperative serum LPS concentrations also rose, but the increased extent was much smaller than the PD group. The increase in the levels of LPS indicated that intestinal homeostasis was altered after surgery, especially after PD [16]. Therefore, patients undergoing major surgery might benefit from a pre- or probiotic treatment to maintain intestinal microbial homeostasis and gut barrier function. As IAP is known to maintain intestinal homeostasis and detoxifying LPS, it could serve as a new approach for improving the outcome of major surgery, especially for PD.

Patients with pancreatic cancer had significantly higher preoperative serum LPS levels than patients without cancer, which can further accelerate the progression of pancreatic cancer. Meanwhile, preoperative serum LPS concentrations were negatively correlated with stool IAP activity in the patients with pancreatic cancer, and the correlation between these two indicators was more pronounced in cancer patients than in the whole study population.

Based on the multiple functional roles for IAP, in particular its ability to dephosphorylate bacteria-derived inflammatory mediators and its salutary role on gut barrier integrity, supplementation of exogenous IAP might be beneficial for patients undergoing PD.

List of Abbreviations

AKI	acute kidney injury
ALD	alcoholic liver disease
ALP	alkaline phosphatase
ALT	alanine transaminase
AMI	acute myocardial infarction
AST	aspartate transaminase
ATP	adenosine triphosphate
BCA	bicinchoninic acid
BIAP	bovine intestinal alkaline phosphatase
BMI	body mass index
BSA	bovine serum albumin
CA-199	carbohydrate antigen 199
Ca	calcium
CD	cluster of differentiation
CEA	carcinoembryonic antigen
CPB	cardiopulmonary bypass
CPG-DNA	cytosine-phosphate-guanosine-DNA
CRC	colorectal cancer
CRP	C-reactive protein
DHCA	deep hypothermic circulatory arrest
dl	deciliter
DM	diabetes mellitus
DNA	deoxyribonucleic acid
DP	distal pancreatectomy
et al.	et alia
e.g.	exempli gratia
EUS	endoscopic ultrasound
FBG	fasting blood glucose
FIH	first-in-human

FOLFIRINOX	5-Fluorouracil, irinotecan, oxaliplatin
G	gigaparticle
GCAP	germ cell alkaline phosphatase
GFR	glomerular filtration rate
HCl	hydrochloride
hRESCAP	human recombinant placental alkaline phosphatase
IAP	intestinal alkaline phosphatase
IBD	inflammatory bowel disease
IHD	ischemic heart disease
I/R	ischemia-reperfusion
i.g.	id est
KO	knockout
K	kalium
IL	interleukin
IPMN	intraductal papillary mucinous neoplasm
LAL	limulus amebocyte lysate
LPS	lipopolysaccharide
MDCT	multi-detector computed tomography
$MgCl_2$	magnesium chloride
min	minutes
ml	milliliter
mM	millimole per liter
MODS	multiple organ dysfunction syndrome
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
NEC	necrotizing enterocolitis
NET	neuroendocrine tumor
NF-κB	nuclear factor kappa-B
ng	nanogram
nm	nanometer
ns	not significant

OD	optical density
p	p value
PAMPs	pathogen-associated molecular patterns
PBS	phosphate buffer saline
PD	pancreaticoduodenectomy
PET	positron emission tomography
pH	potential of hydrogen
Pi	phosphate
PLT	platelet
PRRs	pattern recognition receptors
PNPP	para-nitrophenyl phosphate
Postop	postoperative
Preop	preoperative
RA	rheumatoid arthritis
rpm	revolutions per minute
RBC	red blood cell
s.c.	subcutaneous
SD	standard deviation
T	teraparticle
TBil	total bilirubin
TJP	tight junction proteins
TLR	toll-like receptor
TLR4-MD2	toll-like receptor 4 and myeloid differentiation factor 2
TNF-α	tumor necrosis factor-α
TNAP	tissue non-specific alkaline phosphatase
T2DM	type 2 diabetes mellitus
TRPV5/6	transient receptor potential vanilloid type 5 and 6
U	unit
UC	ulcerative colitis
UDP	uridine diphosphate
vs	versus

WBC	white blood cell
ZnCl2	zinc chloride
ZO	occludens
χ2-test	chi-square test
5-FU	5-Fluorouracil
%	percentage
°C	degree Celsius
µg	microgram
µl	microliter
µm	micrometer
µM	micromole per liter

1. Introduction

In recent years, intestinal alkaline phosphatase (IAP) has been brought into the focus of gastrointestinal research due to its various beneficial roles. The most important functions of this enzyme are to dephosphorylate some inflammatory mediators, including lipopolysaccharides (LPS), as well as to maintain gut barrier function and microbial homeostasis [2, 3, 17, 18]. Besides, IAP deficiency is related to a variety of pathologies such as metabolic syndrome, liver fibrosis, ischemic heart disease, frailty, and a decreased life span [2-4, 19]. The supplementation of this naturally occurring enzyme has shown its beneficial role in several translational and clinical studies [20-26]. LPS is the primary component of the cell wall of Gram-negative bacteria. It is a trigger of inflammation and is also related to the development, progression, and prognosis of many diseases [8-10, 27-29]. The intestinal microbiota is an important source of circulating LPS [30], and both intestinal dysbiosis and gut barrier dysfunction can increase LPS concentration in the bloodstream [29, 31]. Pancreaticoduodenectomy (PD) is also known as Whipple procedure, which is a complex and difficult operation that includes the resection of the duodenum [15].

1.1. Multiple Functions and Underlying Mechanisms of IAP

Alkaline phosphatases (ALP) are homodimeric enzymes that belong to a superfamily of ecto-nucleotidases [32, 33]. They are anchored to the outside surface of the plasma membrane and catalyze the hydrolysis of monoesters of phosphoric acid with release of inorganic phosphate at basic pH values [34-36]. These enzymes are ubiquitous in nature, from prokaryotes to higher eukaryotes except some higher plants [37-40], and exert various pivotal functions. In mammals, ALPs are divided into four kinds of isoenzymes, which can be classified as tissue non-specific and tissue-specific types. Three of them, including IAP, germ cell alkaline phosphatase (GCAP), and placental alkaline phosphatase (PLAP), are tissue-specific. Whereas the fourth one, tissue non-specific alkaline phosphatase (TNAP), can be expressed in various tissues [1, 41].

As a naturally occurring enzyme, IAP is mainly produced in the small intestine, especially the duodenum [42, 43]. Although this enzyme is primarily membrane-bound, it is also released into the intestinal lumen and the blood. Therefore, the levels of IAP on the brush border of enterocytes are high [44, 45]. The most important functions of IAP are dephosphorylating LPS and other inflammatory mediators responsible for chronic systemic inflammation, and maintaining intestinal microbial homeostasis and gut barrier function (Fig. 1). Due to its various beneficial roles, IAP has been brought into the focus of research in recent years, and its deficiency has been found to be involved in several pathologies. The role of this gut brush border enzyme has been revealed in many basic, translational, and clinical researches.

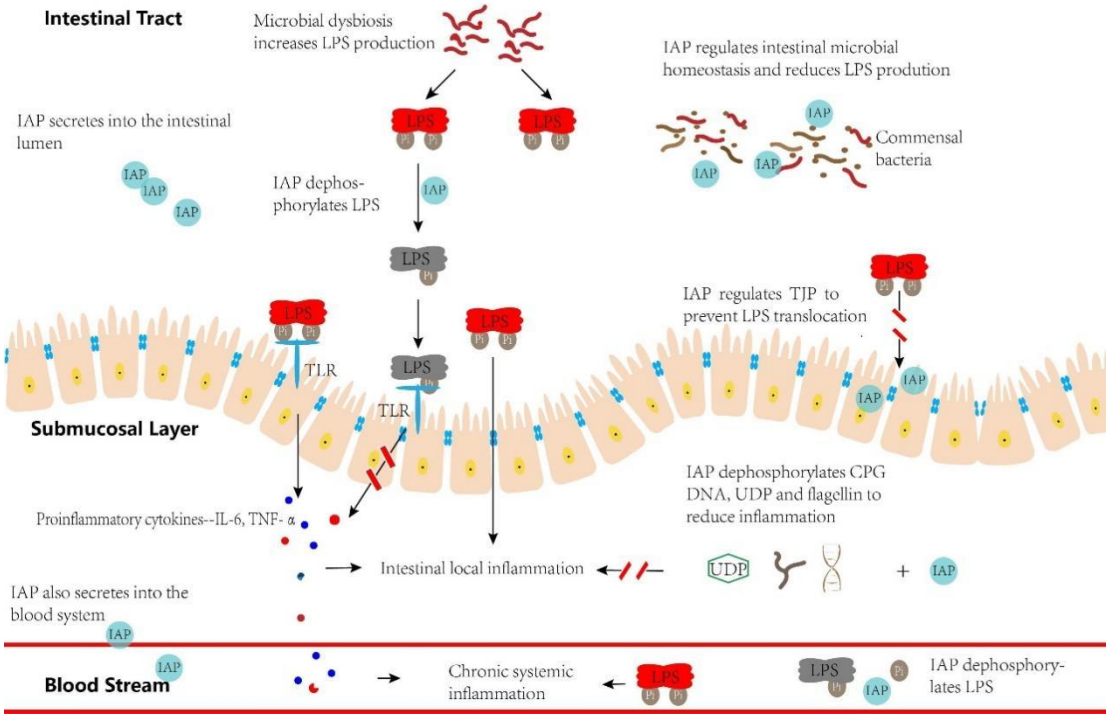


Figure 1. Functions and underlying mechanisms of IAP (adapted from Kühn et al. [46]). IAP regulates intestinal microbial homeostasis and reduces LPS production. Microbial dysbiosis increases the production of LPS, which further activates TLR4 followed by the release of inflammatory mediators; IAP can detoxify LPS, and dephosphorylated LPS can't activate TLR4, thus suppressing the following signaling cascade. Translocated LPS, released cytokines, and inflammatory mediators can cause chronic systemic inflammation and local inflammation in the gut; IAP dephosphorylates these factors and attenuates the inflammation. LPS also transfers into interior milieu through intercellular space; IAP inhibits the translocation of LPS by regulating the

levels and cellular localization of TJP. (Abbreviations: IL-6: interleukin-6; IAP: intestinal alkaline phosphatase; LPS: lipopolysaccharide; TLR: toll-like receptor; TJP: tight junction proteins; TNF-α: tumor necrosis factor-α; CPG-DNA: cytosine-phosphate-guanosine-DNA; UDP: uridine diphosphate)

1.1.1. Dephosphorylation of Inflammatory Mediators

IAP can exert anti-inflammatory effects by dephosphorylating LPS, uridine diphosphate (UDP), cytosine phosphate-guanosine (CpG) DNA and flagellin [17, 47]. Activation of toll-like receptor 4 (TLR4) by LPS can promote nuclear factor kappa-B (NF-κB) to translocate to the nucleus, thereby increasing the production of pro-inflammatory factors including interleukin (IL)-6 and tumor necrosis factor (TNF)-α [48, 49]. IAP can remove one phosphate group from the lipid-A moiety of LPS and significantly decrease its toxicity [50-53]. Detoxified LPS binds to TLR4 primarily as a receptor antagonist, which can't activate the following signaling cascade [50-53]. As we all know, inflammation can stimulate the normal cells to produce nucleotides, such as UDP, and subsequently trigger the release of inflammatory cytokines [54]. UDP can be dephosphorylated by IAP in a dose-dependent mode [54]. CpG DNA and Flagellin stimulate IL-8 secretion from THP-1 cells and HT29 cells in a dose-dependent pattern. [19]. IAP has been demonstrated to dephosphorylate these two inflammatory mediators and cause a >40% decrease in IL-8 release by host cells [47].

1.1.2. Maintaining Intestinal Homeostasis

Previous studies have shown that IAP can regulate the levels and cellular localization of key tight junction proteins, thus promoting intestinal barrier function [17, 18]. It has been proved that IAP gene overexpression leads to a pronounced increase in the mRNA levels of zonula occludens-1 and zonula occludens-2 in T84 and Caco-2 cells, whereas occluding, zonula occludens-1 and zonula occludens-2 levels are lower in IAP knockout (KO) mice [17, 18]. The precise molecular mechanisms underlying the regulatory effects of IAP on tight junction proteins still require further studies to explore. Moreover, supplementation with IAP significantly increased the expression of zonula occludens-1, zonula occludens-2, zonula occludens-3, claudin1, and occludin in starved mice [17, 18]. Besides, IAP deficiency was accompanied by a

pronounced decrease in the intestinal tight junction protein level in mice [3]. IAP also preserved intestinal microbial homeostasis in mice [2, 3]. Previous studies showed that high concentrations of adenosine triphosphate (ATP) resulted in intestinal microbial dysbiosis. Hence, the influence of IAP in commensal bacteria might be achieved by dephosphorylating ATP [55].

1.1.3. Regulation of Intestinal Surface pH

Duodenal enterocytes secret mucus and bicarbonate, creating a pH that is close to neutral on the intestinal surface to protect the mucosa from pepsin and gastric acids [56]. A regulatory feedback loop on the brush border membrane of duodenal enterocytes, involving extracellular ATP, IAP, and G-protein coupled purinergic receptor P2Y1, functions to maintain intestinal surface pH [56]; IAP activity is low in an acidic environment, leading to higher concentrations of extracellular ATP secreted by the enterocytes, which stimulates P2Y receptors to secret more bicarbonate. Subsequently, intestinal surface pH rises progressively, accompanied by higher IAP activity, resulting in lower levels of ATP and bicarbonate [55, 57].

1.1.4. Modulating Intestinal Absorption of Lipid, Ca and Pi

CD36 is widely considered to be related to lipid uptake and transportation, and IAP plays an important role in regulating lipid absorption by acting on CD36 in the intestine [58]. It has been shown that IAP can phosphorylate and dephosphorylate CD36 in mice. Dephosphorylated CD36 is responsible for promoting fat absorption, while phosphorylated CD36 inhibits fatty acid uptake. Therefore IAP interacts with CD36 to optimize fat transport [58]. Intestinal transcellular transportation of Ca^{2+} is associated with the transient receptor potential vanilloid type 5 and 6 (TRPV5/6), which is known to be regulated by the pH on the mucosal surface [59]. Therefore, IAP can control the absorption of Ca^{2+} by affecting intestinal surface pH and TRPV5/6 activity. In animal models, intestinal transcellular Pi transport is related to type II sodium-dependent inorganic phosphate transporter Npt2b on the luminal side of enterocytes [60]. IAP gene deletion decreases the levels of intestinal Npt2b protein, thereby inhibiting Na+-dependent Pi transport in mice [60].

Many major functions and underlying mechanisms of this enzyme have been revealed. IAP has been shown to play important roles across multiple organ systems (Table 1). Some clinical studies have proven the safety and efficacy of applying exogenous IAP, for example, in reducing inflammation and endotoxemia in patients with sepsis and ulcerative colitis (UC) [61, 62]. The route of IAP administration is also an important factor [1]. Previous studies have revealed that oral administration of IAP can reduce systemic inflammation, and also stimulate the production of endogenous IAP [56, 63]. Intraperitoneal or intravenous injection can only decrease systemic inflammation but allow for precise dosing and more efficient absorption [64, 65].

Up to now, the role of IAP in patients undergoing gastrointestinal surgery is still unclear.

Table 1. Organ-specific roles and potential therapeutic implications of IAP

	Exogenous IAP supplementation attenuates the following dysfunctions	IAP deficiency is related to the following dysfunctions
Gut	C. difficile and Salmonella infection [66, 67], UC [26], and NEC [22, 23]	IBD [56] and NEC [68]
Liver	Cirrhosis [69] and ALD [70]	Cirrhosis [69] and Aging-related liver change [3]
Pancreas	Metabolic syndrome [3]	T2DM [71]
Kidney	Renal inflammation [25], damage of I/R-induced AKI [25], and sepsis-induced AKI [21]	
Heart	Myocardial dysfunction [24], complications of AMI [24], and AMI-induced inflammation [72]	IHD [73]
Bone		Lack of IAP increases the volume of intracortical bone and cortical thickness [74]

(Abbreviations: IBD: inflammatory bowel disease; IAP: intestinal alkaline phosphatase; IHD: ischemic heart disease; NEC: Necrotizing enterocolitis; T2DM: type 2 diabetes mellitus; AMI: acute myocardial infarction; LPS: lipopolysaccharide; ALD: alcoholic liver disease; AKI; Acute

kidney injury; I/R：ischemia-reperfusion; UC: ulcerative colitis.)

1.2. Lipopolysaccharide

LPS is the principal component of the outer cell wall of Gram-negative bacteria, forming a barrier against environmental stress such as cationic antimicrobial peptides and antibiotics [6, 7]. LPS is a potent trigger of inflammation and dysregulated host response in sepsis. It is ubiquitous in the environment and can directly modulate the immune response and the host's susceptibility to disease [5, 75]. The classical LPS molecule consists of three moieties: (a) lipid A, the lipophilic portion that anchors LPS to the membrane; (b) oligosaccharide core, which contributes to the integrity of the cell wall together with lipid A; (c) O antigen polysaccharide, which is in direct contact with the external milieu. Lipid A can be recognized by immune cells as a pathogen-associated molecule and is the immunostimulatory portion of LPS [76, 77].

More than 100 years ago, Richard Pfeiffer discovered that cholera bacteria produced another toxin in addition to heat-labile exotoxin [78]. In contrast to the exotoxins released by bacteria, this heat-stable and non-volatile pyrogenic substance was demonstrated to be a component of the bacteria cell wall. Therefore, Pfeiffer termed it endotoxin. Subsequently, this endotoxin was shown to be the main outer membrane constituent of Gram-negative bacteria and played an important role during bacterial infections, sepsis, and shock [27, 79, 80]. Synthesized lipid A molecule was proven to be responsible for most endotoxic activities of LPS [81, 82].

Pattern recognition receptors (PRRs) are considered to be part of the innate immune system, and these proteins are capable of recognizing non-self-molecules found in various pathogens (i.e., pathogen-associated molecular patterns—PAMPs) including LPS [83-85]. Extracellular LPS can elicit inflammation by stimulating Toll-like receptor 4 and myeloid differentiation factor 2 (TLR4-MD2), a PRR mainly present on the surface of dendritic cells, monocytes and macrophages. Activation of TLR4-MD2 by LPS initiates a signaling cascade that induces the production of pro-inflammatory cytokines that help eliminate invading pathogens [49, 86, 87]. However, overexpression of pro-inflammatory cytokines results in various pathophysiological

consequences such as fever, leukopenia, hypotension, disseminated intravascular coagulation, and multiorgan failure [78]. In addition, these overexpressed cytokines can also activate the NF-κβ pathway, which is implicated in DNA damage, cell proliferation and carcinogenesis [31].

Besides the TLR4 pathway, LPS can also activate other recognition systems, such as caspase-4/5/11 pathway and transient receptor potential (TRP) channels. In mice, extracellular LPS stimulates TRP channels present on the neuronal cells mediating acute neurogenic pain and inflammation [88]. Intracellular LPS can be sensed by caspase-11 and caspase-4/5 to induce the production of pro-inflammatory cytokines in innate immune cells [28, 89, 90].

Intestinal dysbiosis and gut barrier dysfunction can increase the level of LPS in circulation, promote chronic systemic inflammation, and enhance the production of pro-inflammatory cytokines [29, 31]. As illustrated before, these cytokines can activate the NF-κβ pathway, which is involved in cell proliferation, DNA damage, and tumor incidence and growth [29, 31]. Increased serum antibody to LPS has been shown to be associated with a greater risk of liver cancer [8, 9]. The circulating levels of LPS were significantly elevated in colorectal cancer (CRC) and bladder cancer groups compared with healthy controls [10, 11]. Moreover, LPS-TLR4 signaling in cancer cells promoted the progression of esophageal squamous cell carcinoma in humans [91]. Pidgeon et al. [92] proved that LPS contributed to the metastatic cancer growth after surgery by directly affecting the cancer cells.

Given these facts, both intestinal dysbiosis and gut barrier dysfunction can increase LPS concentration in the bloodstream [29, 31]. Therefore, it can be considered as a circulating biomarker of intestinal homeostasis. Furthermore, LPS is also related to the development, progression, and prognosis of some types of cancer [8-11, 29, 91, 92], which indicates it is also a therapeutic target and prognostic marker. Until now, the role of serum LPS in pancreatic cancer patients undergoing surgery is still unknown.

1.3. Pancreatic Surgery and Pancreatic Cancer

Pancreatic surgery mainly consists of two different operations: 1. PD for lesions in the head or body of the pancreas; 2. Left-sided or distal pancreatectomy (DP) for

lesions of the pancreatic tail or body [15]. With the improvements in the skills of surgeons, imaging, anesthetic techniques, and critical care, and better antibiotics, pancreatic surgery is demonstrated to be a safe, effective, and critical component in treatment of pancreatic diseases [93]. PD is a complex and difficult operation to remove the head of the pancreas, the first part of the small intestine (duodenum), the gallbladder, and the bile duct, with or without removal of the pylorus and distal stomach [15].

Pancreatic cancer is the main reason for pancreatic surgery [94]. It is widely considered to be one of the most aggressive cancers and one of the most frequent causes of tumor-related death in the world. It ranks twelfth among all cancers in terms of incidence [95]. In 2020, an estimated 495,773 new pancreatic cancer cases and 466,003 related deaths occurred over the world [95]. The occurrence of pancreatic cancer has an explicit geographical distribution, as shown in Figure 2, Europe and North America are the areas with a higher incidence rate. Pancreatic cancer is expected to be the second leading cause of cancer-related deaths by 2030. [96]. The overall 5‐year survival rate of pancreatic cancer is less than 7%; for those patients undergoing surgical resection, the 5‐year survival rate is 15–25% [97].

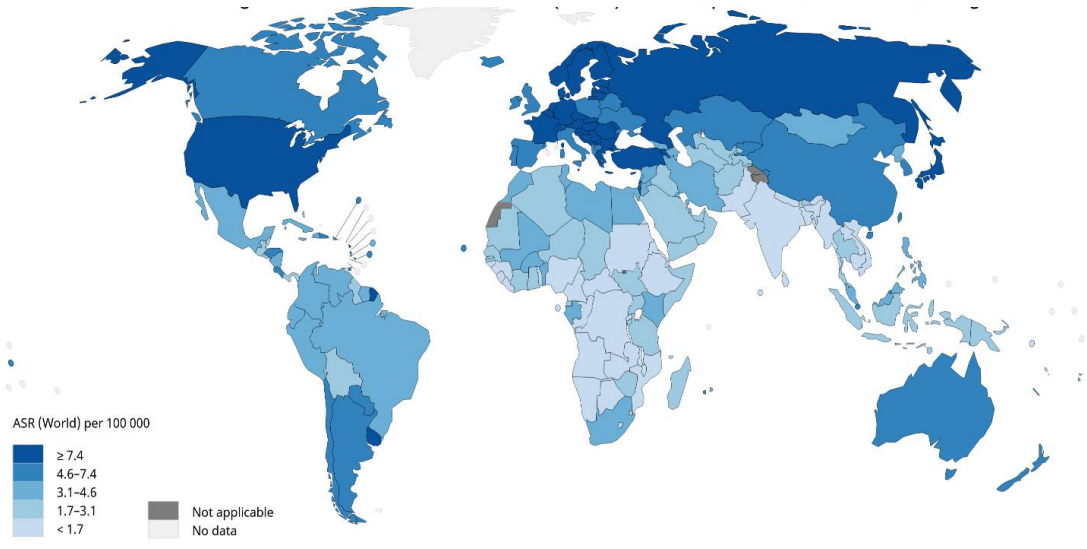


Figure 2. Estimated Age-Standardized Incidence Rates (World) of Pancreatic cancer in 2020, All ages, Both sexes [95].

Known risk factors for this cancer include cigarette smoking, chronic pancreatitis,

diabetes mellitus, male sex, lack of physical activity, advancing age, high body mass index (BMI), high-fat diet, and family history of pancreatic cancer and chronic pancreatitis [98-101]. Although the causes of pancreatic cancer are complex, multifactorial, and insufficiently known, family history and tobacco smoking are dominant. Cigarette smoking causes approximately 20% of pancreatic tumors [102]. These high-risk groups are good targets for screening and early diagnosis programs. It is difficult to diagnose the early-stage pancreatic cancer, which is often because it is clinically silent and difficult to be imaged, and lacks sensitive and specific tumor markers [97]. Currently, the main diagnostic modalities are imaging with multi-detector computed tomography (MDCT), positron emission tomography (PET), magnetic resonance imaging (MRI), and endoscopic ultrasound (EUS) [103-106]. Even though enormous efforts have been made during the past decades, systemic treatment of pancreatic cancer remains a formidable challenge [107-109]. Pancreatic cancer is characterized by a pronounced resistance to most conventional treatment options [97, 110, 111]. Only about 10% of pancreatic cancer patients are diagnosed at an early stage and are thus able to benefit from curative surgical resection [112]. For chemotherapy, gemcitabine was demonstrated to improve the clinical outcomes of pancreatic cancer patients and became the standard first-line treatment for pancreatic cancer in 1992 [113]. In 2003, a phase I trial showed that a combination of 5-Fluorouracil (5-FU), leucovorin, oxaliplatin, and irinotecan (FOLFIRINOX) exhibited good antitumor activity in pancreatic cancer patients [114]. A phase II study confirmed the results in the phase I trial [115]. Subsequently, PRODIGE4/ACCORD 11 was designed as a randomized phase II/III study to assess the efficacy of FOLFIRINOX compared with gemcitabine alone. It demonstrated that FOLFIRINOX had an advantage over gemcitabine monotherapy in terms of response rate, progression-free survival, overall survival, and quality of life [116]. In addition, S-1 (an oral 5-fluorouracil prodrug) and gemcitabine plus nab-paclitaxel also have shown improved survival benefits and are now the most commonly applied first-line treatment options [93, 117]. The role of adjuvant radiotherapy is controversial [118]. Compared with chemotherapy alone, chemoradiation was perhaps harmful and did not increase survival [118]. Endoscopic therapy, immunotherapy, precision medicine, and target therapy also provide promising treatments for pancreatic cancer patients,

even though there are still many obstacles and more researches are needed to explore the potential benefits of these methods [117].

Although surgical resection combined with adjuvant chemotherapy and radiotherapy remains the only curative therapeutic option for pancreatic cancer patients, it is associated with high morbidity and mortality rates [96, 119, 120]. Therefore, it is essential to better understand the impact of pancreatic surgery on human physiological functions and take corresponding measures.

1.4. Aim of this Study

IAP appears to be a positive regulator of microbial homeostasis and gut barrier function, and functions to dephosphorylate LPS and other inflammatory mediators. It is mainly produced in the duodenum [42]. In PD, the duodenum is partially or completely removed, which would theoretically cause a significant decrease in stool IAP level and disturbance of intestinal homeostasis followed by an increase in serum LPS level after surgery.

This study aimed to quantify the stool IAP activity and serum LPS values in patients undergoing PD compared to other general and gastrointestinal surgical procedures, to assess the differences in these markers between groups, and to compare the perioperative changes. As LPS was reported to be associated with an increased risk of liver cancer and colorectal cancer, we also aimed to investigate the roles of IAP and LPS in patients with pancreatic cancer.

2. Materials and Methods

2.1. Materials

2.1.1. Laboratory Equipment

Centrifuge	Eppendorf, Germany
Drying cabinet	Heraeus, Germany
Glassware washer	Miele, Germany
Ice maker	KBS, Germany
Magnetic mixer	GLW, Germany
Microplate reader	Molecular devices, USA
Pipettes	Eppendorf, Germany
Plate heater	VWR, USA
Shaker	Edmund Bühler, Germany
Steam sterilizer	MMM, Germany
Thermomixer	Eppendorf, Germany
Vortex mixer	IKA, China
4°C fridge	Siemens, Germany
-20°C fridge	Siemens, Germany
-80°C fridge	Heraeus, Germany
37°C incubator	Memmert, Germany

2.1.2. Computer and Software

Computer hardware	HUAWEI, China
Prism	Version 8, GraphPad Software, USA
SoftMax Pro	Version 6.5.1, Molecular devices, USA

2.1.3. Consumables

Centrifuge tube 15 ml	91015, TPP, Switzerland
Centrifuge tube 50 ml	91050, TPP, Switzerland
Freezing tubes	122278, Cryo.s, Greiner, Germany
Freezing tubes	122263, Cryo.s, Greiner, Germany
Gloves	ecoSHIELD, USA
Hydrophobic pen	S2002, Dako Pen, Agilent Technologies, USA
Safe-Lock tubes	0030120.094 Eppendorf, Germany
Pipettes reloads	Eppendorf, Germany
Serological pipette 50ml	170358, Thermo Scientific, USA
Serological pipette 25ml	760180, Greiner Bio-one, Germany
Serological pipette 10ml	4488, Costar Stripette, Corning, USA
Serological pipette 5ml	4487, Costar Stripette, Corning, USA
96 well cell culture plates (flat bottom)	83.3924, Sarstedt, Germany

2.1.4. Chemicals

Acetic acid (glacial) 100%	1000562500, Merck, Germany
Albumin Standard	23209, Thermo Scientific, USA
Dulbecco's phosphate-buffered saline (DPBS)	P04-36500, PAN-Biotech, Germany
Glycerin	3783.1, Carl Roth GmbH+Co.KG, Germany
Hydrochloric acid (HCl) 2mol/L	1.09063.1000, Titripur, Merck, Germany
IAP	524572, Sigma-Aldrich, USA
L-Phenylalanine	P2126, Sigma-Aldrich, USA
Magnesiumchlorid Hexahydrat ($MgCl_2.6H_2O$)	63065, Fluka, Switzerland
Para-nitrophenyl phosphate disodium salt (PNPP)	34045, Thermo Scientific, USA

Pierce™ BCA protein assay reagent A	23228, Thermo Scientific, USA
Pierce™ BCA protein assay reagent B	1859078, Thermo Scientific, USA
TRIZMA base	T6066, Sigma-Aldrich, USA
Zinc chloride ($Zncl_2$)	208086, Merck, Germany
80% Ethanol	1004051526001, CLN GmbH Chemikalien Laborbedarf, Germany

2.1.5. Buffers and Solutions

1M MgCl2 solution	
	20.3 g $MgCl_2.6H_2O$
	100 ml Distilled water
10mM $ZnCl_2$ solution	
	0.1364g $ZnCl_2$
	100 ml Distilled water
1M Tris-HCl solution	
	12.11 g TRIZMA base
	100 ml Distilled water
25% Acetic acid	
	25 ml Acetic acid (glacial) 100%
	75 ml Distilled water
PNPP Solution	
	186mg pNPP
	1ml 1M Tris-HCl solution
	100µl 1M $MgCl_2$
	100µl 10mM $ZnCl_2$
	99ml Distilled water
	pH 8.0

2.1.6. Commercial Kits

Pierce™ Chromogenic Endotoxin Quant Kit	A39553, Thermo Scientific, USA

2.2. Methods

2.2.1. Patients and Clinical Data

This project was approved by the ethics commission of the Ludwig - Maximilians University Munich (Project number: 19-233). Stool and blood samples were collected from patients who were willing to be enrolled in the study after signing the informed consent forms. Preoperative and postoperative samples were taken from 36 patients undergoing PD, 12 patients receiving DP, and 15 cases undergoing smaller general surgery procedures (cholecystectomy or hernia repair) from May 2020 to September 2021 in our institution. Exclusion criteria were: acute or chronic infectious diseases, inflammatory bowel disease (IBD), severe liver dysfunction, underwent gastrointestinal surgery and refusal or unexpected discharge. Clinical characteristics including gender, age, BMI, diabetes mellitus (DM), hospital days, operative time and blood loss were recorded. Additionally, routine lab values, including creatinine, hemoglobin, platelet, red blood cell (RBC), white blood cell (WBC), carcinoembryonic antigen (CEA), c-reactive protein (CRP), fasting blood glucose (FBG), aspartate transaminase (AST), bilirubin, glomerular filtration rate (GFR), ALP and blood kalium (K), were collected from our database.

2.2.2. Sample Collection and Storage

Peripheral blood samples were collected from patients undergoing PD or DP one day before surgery and between postoperative day 10-12. For patients receiving smaller general surgery procedures, blood was taken one day before surgery and between postoperative day 2-3. Centrifugation of blood was performed at 2000xg for 10 min at 15°C. The serum layer was extracted and stored in the -80°C fridge. Stool samples were also collected one day before surgery and between postoperative day 10-12 after PD or DP, but not before the patients' second postoperative bowel movement. For patients undergoing smaller general surgery procedures, stool samples were collected one day before surgery and between postoperative day 2-3, but not before the patients' second postoperative bowel movement. All the stool samples were

stored in the -80°C fridge before testing.

2.2.3. Bicinchoninic Acid Assay (BCA assay)

At first, the volume of BCA working reagent required was calculated, then A solution was mixed with B solution at a ratio of 50:1. Standard bovine serum albumin (BSA) solutions were prepared at different concentrations: 31.25, 62.5, 125, 250, 500, 1000, 2000 ug/ml. Next, 25uL of each unknown or standard sample was pipetted in triplicate into the designated microplate well, and distilled water was used as a blank. Then, 200uL of working reagent was added into each well, and the plate was shaken thoroughly on a plate shaker for 30 seconds. The plate was covered and then incubated at 37°C for 30 minutes. After the plate was cooled to room temperature, the absorbance at 562 nm of each well was measured in the spectrophotometer. The average absorbance at 562nm of the blank-corrected triplicates was subtracted from the average absorbance of all the standards and unknown samples. The standard curve was prepared by plotting the average blank-corrected optical density (OD) values for each BSA standard versus its concentration in ug/ml. The protein concentration of each unknown sample was calculated with the standard curve.

2.2.4 Para-Nitrophenyl Phosphate (PNPP) Method

Stool samples were thawed on ice and diluted at a ratio of 1:30 in stool dilution buffer (10mM Tris HCl, pH 8.0; 1mM $MgCl_2$; 10µM $ZnCl_2$), followed by incubation on ice for 30 min. Then the stool solution was homogenized thoroughly on a shaker for 10min. The homogenate was then centrifuged at 10,000g for 10min, and the supernatant was taken for analysis. L-phenylalanine was used as a specific inhibitor of IAP, and it was dissolved in the buffer (10mM Tris HCl, PH 8.0; 1mM MgCl2; 10µM ZnCl2) at a concentration of 10 mM. Standard IAP solutions were prepared at different concentrations: 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625 U/ml. Next, 25uL of each standard solution was pipetted in triplicate into the designated microplate well, and distilled water was used as blank. The same volume of each unknown sample (25uL) was added into the designated microplate well. Six wells were prepared for each sample, then 175 ul of PNPP solution was dispensed into the first 3 wells, and

L-phenylalanine plus PNPP solution was added into the other 3 wells at the same time. The same volume of PNPP solution was pipetted into the standard and control wells. Subsequently, the plate was incubated for 10 minutes at 37 ℃. After the plate was cooled to room temperature, the absorbance of each well was measured at 405 nm in the spectrophotometer. The average absorbance of the blank-corrected triplicates at 405 nm was subtracted from the measurement of all other individual standards and unknown sample triplicates. The standard curve was prepared by plotting the average blank-corrected OD values for each IAP standard versus its activity in U/ml. The formulated standard curve was used to calculate the activity of each unknown sample. At last, the average difference between the L-phenylalanine added and none L-phenylalanine added groups was the specific activity of IAP.

2.2.5. Limulus Amebocyte Lysate (LAL) Assay

All reagents were equilibrated to room temperature before use. The frozen serum samples were taken out from the -80 °C fridge, thawed on ice, vortexed, and then centrifuged (5000 rpm) for 10 minutes at 4°C. The serum was then transferred to another endotoxin-free tube. To remove platelets and other sediments altogether, the serum samples were centrifuged again at 10,000 rpm for 10 minutes at 4°C. Serum supernatant was diluted 50-fold with endotoxin-free water in another tube. The diluted samples were heat-shocked at 70°C for 15 minutes and then put on ice until tested.

Endotoxin standard solutions were prepared with lyophilized E. coli endotoxin and endotoxin-free water in the Pierce™ Chromogenic Endotoxin Quant Kit. The final standard endotoxin concentrations were 0.1, 0.05, 0.025, 0.01 EU/mL, respectively.

The standard solutions were vigorously vortexed for 15 minutes after reconstitution or before subsequent use in case the endotoxin adhered to the container. The amebocyte lysate was reconstituted with endotoxin-free water (1.7 ml for each vial) immediately prior to use and gently swirled to dissolve the powder. The Chromogenic Substrate solution was prepared by adding 3.4 mL of endotoxin-free water into each vial and gently mixing to dissolve the powder. Reconstituted Chromogenic Substrate solution was pre-warmed for the assay to 37°C for no more

than 10 minutes before use. The 96 well plate was pre-equilibrated in a heating block at 37±1°C for 10 minutes. With the plate kept at 37±1°C, 50µL of blank, standard and unknown samples were dispensed in triplicate into the appropriate plate wells. 50µL of endotoxin-free water was used as a blank control. Then 50µL of LAL reagent was pipetted into each well. The moment at which the lysate was added to the first well was the start time. The 96 well plate was removed from the heating block and gently tapped several times to facilitate mixing once the LAL solution had been added into all plate wells. Then the plate was covered with the lid and returned to the heating block to incubate at 37±1°C for T1 indicated on the vial.

Subsequently, 100µL of Chromogenic Substrate solution (prewarmed to 37±1°C) was added into each well. Once the Chromogenic Substrate solution had been pipetted into all wells, the plate was removed from the heater and tapped several times to promote mixing. After being covered with a lid, the plate was incubated at 37±1°C for 6 minutes. After 6 minutes, 50 µL of stop reagent (25% acetic acid) was dispensed into each well in order. At last, the plate was removed from the heating block and gently tapped several times to facilitate mixing after the stop solution was pipetted into all wells. The absorbance at 405nm in the spectrophotometer was measured. The average 405nm absorbance of the blank-corrected triplicates was subtracted from the average absorbance of all individual standards and unknown sample triplicates. A standard curve was prepared by plotting the average blank-corrected OD values for each LPS standard versus its endotoxin concentration in EU/ml. At last, the endotoxin concentration of each unknown sample was determined with the standard curve.

2.2.6. Statistical Analysis

Continuous variables are presented as mean ± SD or as median appropriately. Two and three continuous data sets were compared by the t-test and One-Way ANOVA test, respectively, and $p< 0.05$ was considered statistically significant; multiple comparisons were examined by Fisher's LSD test between the mean of each column and the mean of every other column if $p< 0.05$ in One-Way ANOVA test. Correlations between two continuous data sets were assessed by Pearson's test

and presented as p and r values, and statistical significance was set at $r > 0.3000$ and $p < 0.05$. Relationships between continuous and categorical data were analyzed by Mann-Whitney u test and presented as p values, and statistical significance was set at $p < 0.05$. The χ-square test or Fisher's exact test was adopted to compare contingency variables, $p < 0.05$ was considered statistically significant. All statistics were performed using Prism.

3.Results

3.1. Patients' characteristics

In our study, stool and serum samples from 36 patients undergoing PD and 27 control patients were tested. The demographical characteristics are summarized in Table 2. Twenty-four patients (66.7%) in the PD group were male, the mean age of this group was 66.61±12.60 years old. Twenty-three cases (63.89 %) were diagnosed with pancreatic cancer, 4 patients (11.11 %) had neuroendocrine tumor (NET), and 8 (22.22 %) had intraductal papillary mucinous neoplasm (IPMN) or other pancreatic diseases in the PD group. In the first control group, 12 patients received DP because of pancreatic cancer (3 patients, 25%), NET (4 patients, 33.33%), IPMN (2 patients, 16.67%) or other pancreatic diseases (3 patients, 25%), and 7 (58.33 %) of them were male. Another 15 patients undergoing smaller general surgery procedures (cholecystectomy and hernia repair) were also included as controls and 9 (60 %) of them were male. No severe liver disease was present in the study cohort. There was no significant difference in the preoperative clinical characteristics among the three groups as shown in Table 2.

Table 2. Demographics of Study Population.

Variables (Mean ± SD)	**PD Group** (N=36)	**DP Group** (N=12)	**General Surgery group** (N=15)	**p**
Gender (Male/Female)	24 (66.7%)/12 (33.3%)	7 (58.3%)/5 (41.7%)	9 (60.0 %)/6 (40.0%)	0.8298
Age (Year)	66.61±12.60	67.00±14.58	57.80±12.22	0.0734
BMI	24.36±3.55	26.09±4.11	24.33±3.98	0.3847
CEA (ng/ml)	4.47±5.53	2.62±1.53	_	0.0981
CA-199 (U/ml)	395.90±890.50	127.90±217.80	_	0.1345
AST (U/L)	45.74±44.41	33.08±23.86	34.31±38.99	0.5212
Creatinine (mg/dl)	0.89±0.17	0.93±0.34	0.98±0.22	0.4131
TBil (mg/dl)	1.06±1.73	0.70±0.55	0.52±0.19	0.1341

CRP (mg/dL)	0.91±1.46	0.98±2.60	0.41±0.47	0.1875
WBC (G/L)	7.52±3.82	6.80±2.46	6.73±2.58	0.6710
RBC (T/L)	4.49±0.57	4.54±0.44	4.78±0.73	0.2830
Platelets (G/L)	261.20±55.82	211.30±74.56	241.70±70.53	0.0686
Hemoglobin (g/dl)	13.64±1.25	13.91±1.18	14.65±1.70	0.0642
GFR (ml/min)	82.00±17.93	79.83±20.36	79.60±19.52	0.8907
FBG (mg/dl)	136.40±67.85	109.00±25.19	106.00±12.55	0.0539
ALP (U/L)	245.30±477.90	105.00±77.80	79.00±41.09	0.1004
K (mmol/L)	4.40±0.62	4.26±0.83	4.35±0.58	0.8184

(Abbreviations: ALP: alkaline phosphatase; BMI: body mass index; CEA: carcino-embryonic antigen; CRP: C - reactive protein; PD: pancreaticoduodenectomy; DP: distal pancreatectomy; p: p value; SD: standard deviation; CA-199: carbohydrate antigen 199; AST: aspartate transaminase; TBil: total bilirubin; WBC: white blood cell; RBC: red blood cell; FBG: fasting blood glucose; GFR: glomerular filtration rate; K: blood kalium. All values are expressed as mean ± SD except for gender.)

3.2. Preoperative Stool IAP Activity

Preoperative stool IAP activity showed no significant difference among the 3 groups and decreased with age. Preoperative stool samples were taken from all the patients one day before surgery, and IAP activity was tested. Firstly, we compared the age and IAP levels among the three groups of patients respectively and found no significant differences (Figure 3A and Figure 3B). However, when examining the correlations between IAP and other clinical data (Table 3), we found that preoperative IAP activity declined significantly with age in the whole study population (Figure 3C). No significant associations between preoperative stool IAP levels and other clinical data were detected in the cohort.

Table 3. Correlation between IAP, LPS and Clinical Parameters in the whole cohort.

Clinical Parameters	Preoperative Stool IAP	Preoperative Serum LPS
Gender	p = 0.7973	p = 0.2175
Age	**r = -0.3880, p = 0.0017**	r = 0.0400, p = 0.7890
BMI	r = 0.0300, p = 0.8157	r = -0.0750, p = 0.5634
CEA	r = -0.0410, p = 0.0114	r = 0.0730, p = 0.6440
CA-199	r = -0.1195, p = 0.4627	r = 0.0318, p = 0.8455
AST	r = -0.1395, p = 0.2879	r = 0.1901, p = 0.1456
TBil	r = -0.0914, p = 0.4833	r = 0.1562, p = 0.2293
Creatinine	r = -0.0681, p = 0.5962	r = -0.1704, p = 0.1818
CRP	r = -0.1859, p = 0.1479	r = -0.0426, p = 0.7422
WBC	r = 0.1207, p = 0.3462	r = -0.1368, p = 0.2849
RBC	r = 0.1881, p = 0.1399	r = 0.0185, p = 0.8854
Platelets	r = -0.1869, p = 0.1425	r = 0.1014, p = 0.4290
Hemoglobin	r = 0.2358, p = 0.0628	r = 0.1110, p = 0.3862
GFR	r = 0.2542, p = 0.0444	r = 0.0091, p = 0.9437
FBG	r = -0.2242, p = 0.0798	r = 0.2320, p = 0.0696
ALP	r = 0.1107, p = 0.3955	r = -0.0186, p = 0.8871
K	r = -0.1184, p = 0.3552	r = 0.2121, p = 0.0952

(Abbreviations: IAP: intestinal alkaline phosphatase; LPS: lipopolysaccharide; r: r value; p: p value; BMI: body mass index; CEA: carcino-embryonic antigen; CA-199: carbohydrate antigen 199; AST: aspartate transaminase; TBil: total bilirubin; FBG: fasting blood glucose; CRP: C-reactive protein; WBC: white blood cell; RBC: red blood cell; GFR: glomerular filtration rate; ALP: alkaline phosphatase; K: blood kalium.)

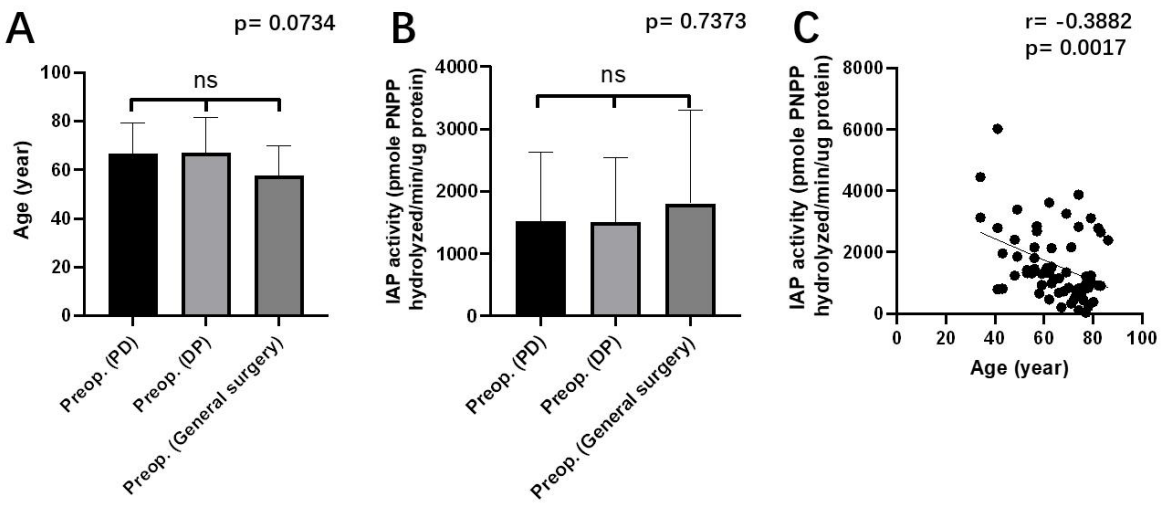


Figure 3. No significant difference in age and preoperative stool IAP activity among the 3 groups was detected (A, B). Stool IAP activity declined with age in the whole cohort (C). (Abbreviations: PD: pancreaticoduodenectomy; DP: distal pancreatectomy; Preop: preoperative; p: p value; r: r value; ns: not significant; IAP: intestinal alkaline phosphatase; PNPP: para-nitrophenyl phosphate.)

3.3. Preoperative Serum LPS Levels

Preoperative serum LPS levels were significantly and negatively correlated with stool IAP activity in the whole cohort. Blood samples were taken one day before surgery, and serum LPS levels were assayed. Figure 4A showed the difference in LPS levels among the three groups. (Preop. (PD) vs Preop. (DP) vs Preop. (General surgery): 0.7571±0.1951 vs 0.7258±0.1596 vs 0.5961±0.1598 EU/ml, p=0.0193). Subsequently, multiple comparisons indicated that the patients undergoing PD had higher serum LPS concentrations than those receiving smaller general surgery (Preop. (PD) vs Preop. (General surgery): 0.7571±0.1951 vs 0.5961±0.1598 EU/ml, p=0.0146), but had no difference with the DP patients (Preop. (PD) vs Preop. (DP): 0.7571±0.1951 vs 0.7258±0.1596 EU/ml, p=0.8629). There was no significant difference in the clinical data among the three groups of patients except for the types of disease (most patients in the PD group were diagnosed with pancreatic cancer, whereas patients in the general surgery group were not). According to previous studies, circulating LPS levels elevated in patients with liver cancer [8, 9], CRC [10], or bladder cancer compared with the healthy controls [11]. Therefore, we

hypothesized that pancreatic cancer patients also have higher LPS levels, which is further discussed in Section 3.10. In our study, serum LPS levels did not change with age in the whole cohort (Figure 4B). Preoperative data analysis showed that serum LPS levels were significantly and negatively correlated with stool IAP levels in the total study population (Figure 4C). There was no statistical significance in the relationship between LPS levels and other clinical characteristics before surgery, as shown in Table 3.

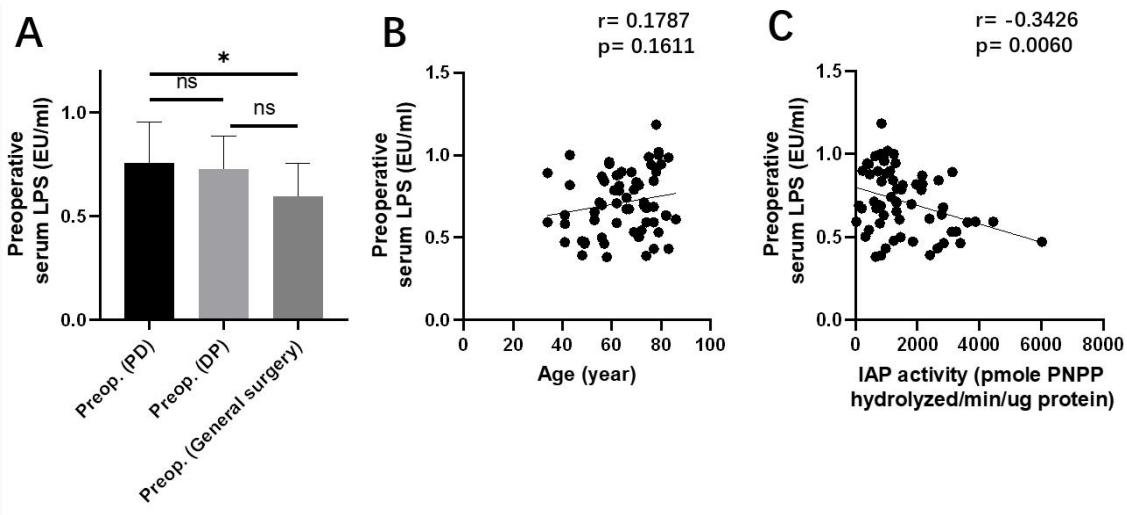


Figure 4. Patients undergoing PD had higher serum LPS levels than those in the general surgery group (A). The levels of serum LPS were not related to age (B), but negatively correlated with stool IAP activity (C). (Abbreviations: PD: pancreaticoduodenectomy; DP: distal pancreatectomy; Preop: preoperative; LPS: lipopolysaccharide; p: p value; r: r value; ns: not significant; IAP: intestinal alkaline phosphatase; PNPP: p-nitrophenyl phosphate; * p< 0.05.)

3.4. Effect of Pancreaticoduodenectomy on Stool IAP Activity

Stool IAP activity significantly decreased after PD. For the patients undergoing PD, the stool samples were collected one day before surgery and between postoperative day 10-12, but not before the patients' second postoperative bowel movement. IAP activity was analyzed as described in the materials and methods section preoperatively and postoperatively. As shown in figure 5A, the stool IAP activity levels significantly decreased after PD (Preop. (PD) vs Postop. (PD): 1522.0±1103.0 vs 721.0±915.7 pmole PNPP hydrolyzed/min/ug protein, p< 0.0001).

As mentioned before, IAP is mainly produced in the small intestine, especially the duodenum. Thus, the removal length of the proximal small intestine during PD was also recorded. Figure 5B showed that the decline in IAP activity positively correlated with the removal length of the proximal small intestine. We also studied the effects of the operative time and blood loss during surgery on the decreased level of IAP but did not find any statistically significant results in patients receiving PD (Figure 5C and Figure 5D).

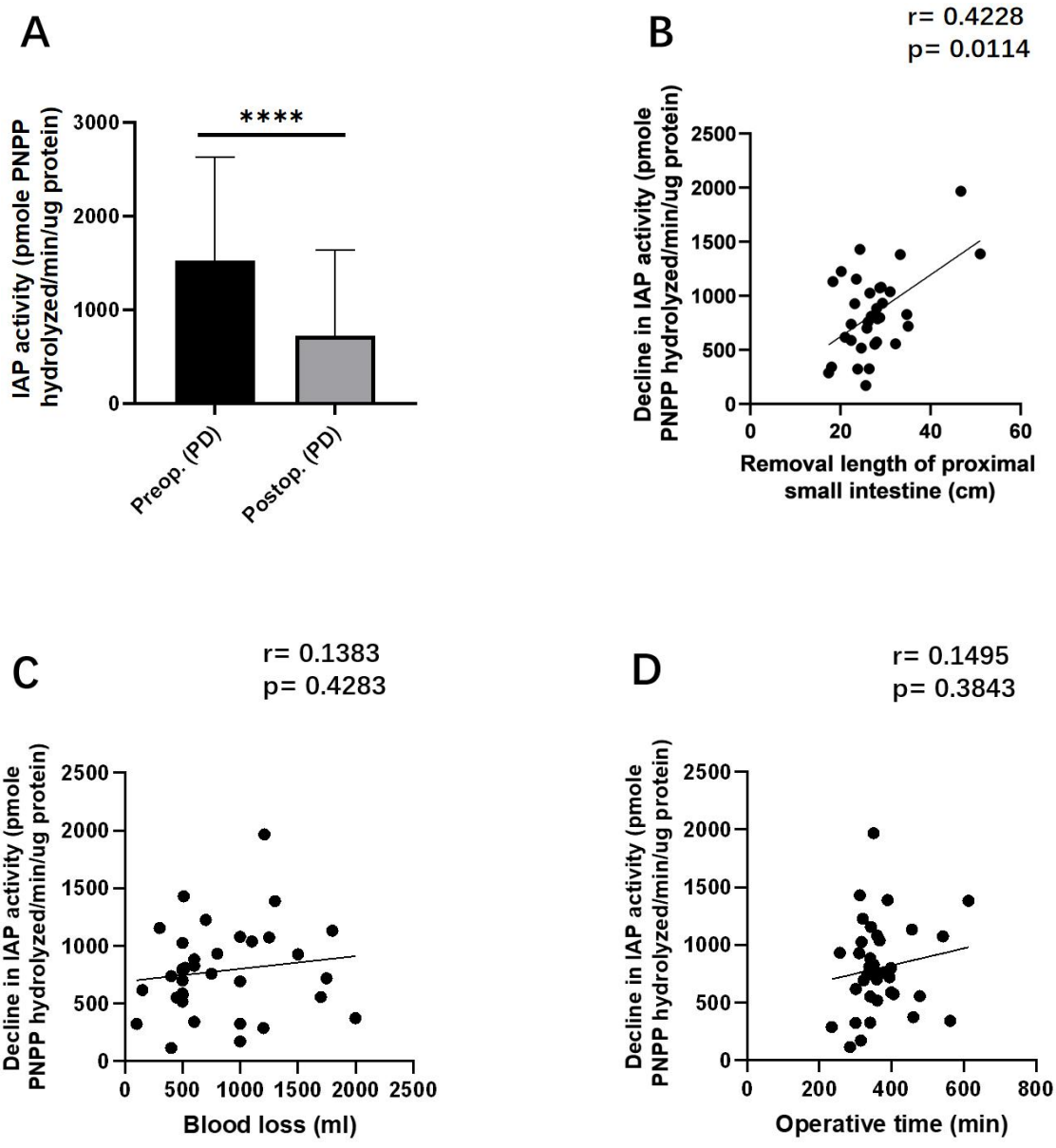


Figure 5. Stool IAP activity decreased after PD (A), which only correlated with the removal length of the proximal small intestine (B) but not with blood loss (C) or operative time (D). (Abbreviations: IAP: intestinal alkaline phosphatase; PNPP: para-nitrophenyl phosphate; AST: aspartate transaminase; Preop: preoperative; PD: pancreaticoduodenectomy; Postop: postoperative; p: p value; r: r value; **** p< 0.0001.)

3.5. Effect of Distal Pancreatectomy on Stool IAP Activity

Stool IAP activity also decreased after DP. For the patients undergoing DP, the stool samples were collected one day before surgery and between postoperative day 10-12, but not before the patients' second postoperative bowel movement. The stool IAP activity levels were also assessed before and after surgery. As shown in figure 6A, the levels of IAP also decreased after DP (Preop. (DP) vs Postop. (DP): 1512.0±1025.0 vs 1363.0±994.9 pmole PNPP hydrolyzed/min/ug protein, p= 0.0387). There was no significant difference in preoperative IAP activity between the PD and DP groups (Preop. (PD) vs Preop. (DP): 1522.0±1103.0 vs 1512.0±1025.0 pmole PNPP hydrolyzed/min/ug protein, p= 0.9786) (Figure 6C). Postoperative IAP levels were significantly higher in the latter group (Postop. (PD) vs Postop. (DP): 721.0±915.7 vs 1363.0±994.9 pmole PNPP hydrolyzed/min/ug protein, p= 0.0452) (Figure 6D). Meanwhile, the decline in IAP activity was more significant in the PD group than in the DP group (PD vs DP: 880.9±396.3 vs 149.2±220.2 pmole PNPP hydrolyzed/min/ug protein, p< 0.0001) (Figure 6B). Therefore, PD caused a more pronounced reduction in IAP levels compared to DP. Furthermore, in patients undergoing DP, there was no significant correlation between the decreased level in IAP activity and the operative time or the blood loss (Figure 6E and Figure 6F).

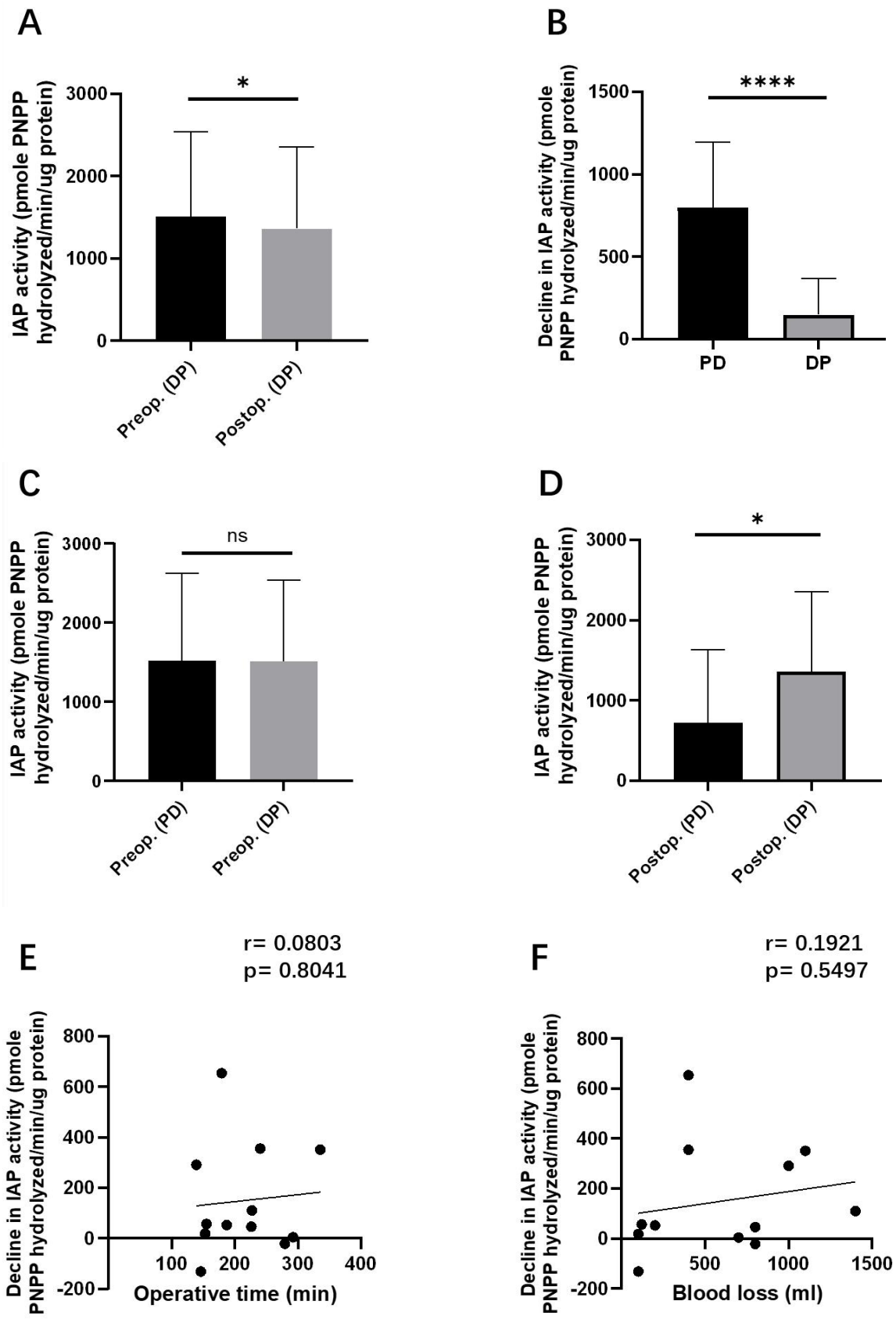


Figure 6. Stool IAP activity also decreased after DP (A). PD and DP groups were not significantly different in preoperative IAP activity (C), however, the DP group had higher postoperative IAP levels (D), therefore PD caused a more significant reduction in IAP levels compared to DP (B). Moreover, for the patients undergoing DP, the decline in IAP activity was not significantly

correlated with the operative time (E) or the amount of blood loss (F). (Abbreviations: IAP: intestinal alkaline phosphatase; PNPP: para-nitrophenyl phosphate; Preop: preoperative; DP: distal pancreatectomy; Postop: postoperative; PD: pancreaticoduodenectomy; p: p value; r: r value; ns: not significant; * p< 0.05; **** p< 0.0001.)

3.6. Effect of General Surgery on Stool IAP Activity

There was no significant decrease in stool IAP activity after general surgery. Patients undergoing cholecystectomy or hernia repair were also included as a control group. The stool samples were taken one day before surgery and between postoperative day 2 and 3 - not before the patients' second postoperative bowel movement. The stool IAP activity was assessed before and after surgery. As shown in figure 7A, the levels of IAP did not significantly decrease after the procedures (Preop. (General surgery) vs Postop. (General surgery): 1810.0±1492.0 vs 1777.0±1841.0 pmole PNPP hydrolyzed/min/ug protein, p= 0.8601). There was no significant difference in the stool IAP levels between this control group and PD group preoperatively (Preop. (General surgery) vs Preop. (PD): 1810.0±1492.0 vs 1522.0±1103.0 pmole PNPP hydrolyzed/min/ug protein, p= 0.4489) (Figure 7B). However, postoperative IAP activity levels in this control group were significantly higher than those in the PD group (Postop. (General surgery) vs Postop. (PD): 1777.0±1841.0 vs 721.0±915.7 pmole PNPP hydrolyzed/min/ug protein, p= 0.0497) (Figure 7C).

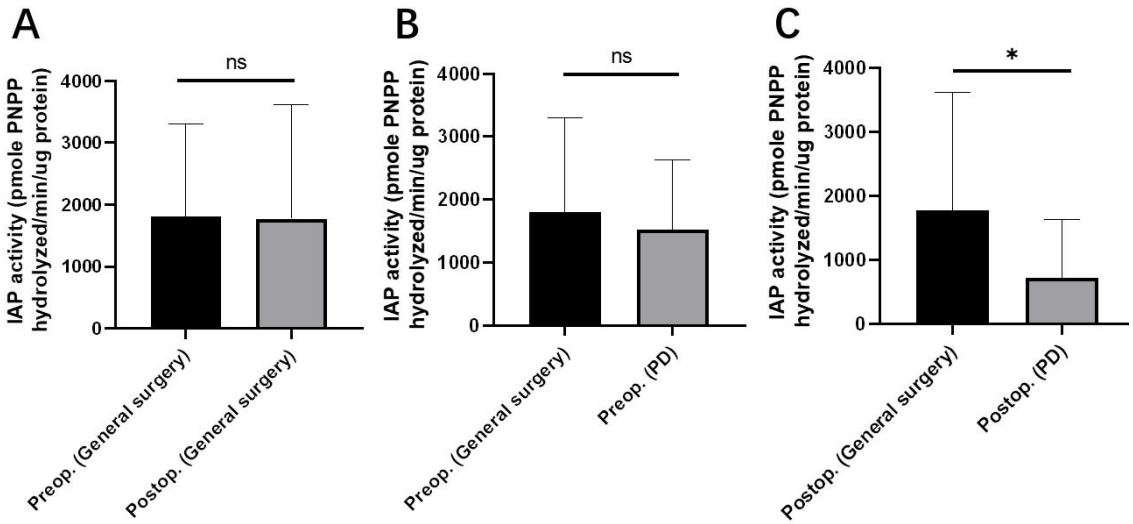
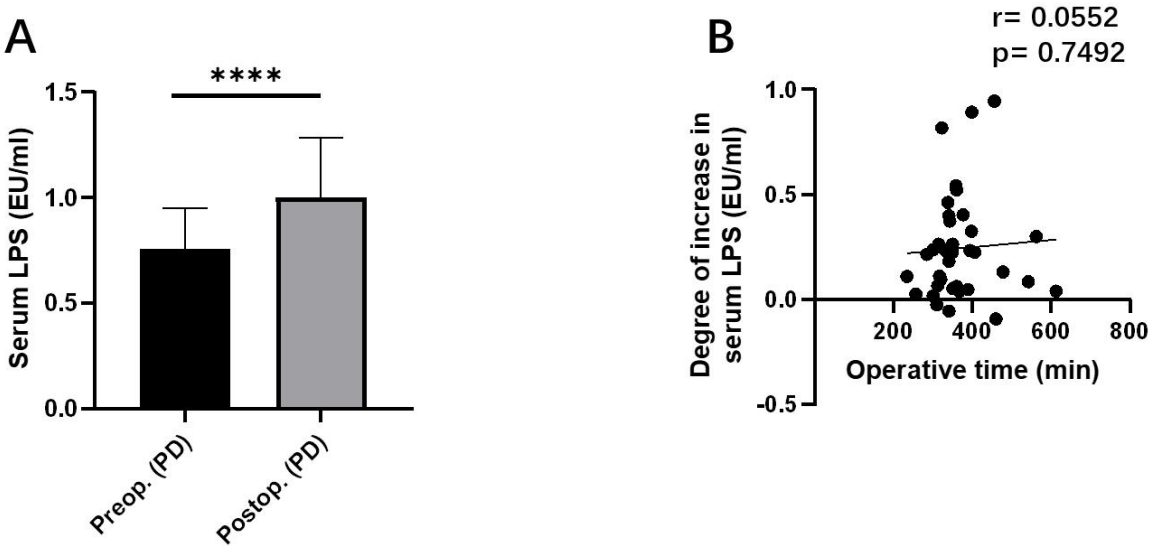


Figure 7. General surgery did not cause a significant reduction in stool IAP levels (A). There was no significant difference in stool IAP activity between the general surgery and the PD group preoperatively (B), however, in the postoperative period, the former group had significantly higher IAP levels (C). (Abbreviations: IAP: intestinal alkaline phosphatase; PNPP: para-nitrophenyl phosphate; Preop: preoperative; Postop: postoperative; PD: pancreaticoduodenectomy; p: p value; ns: not significant; * p< 0.05.)

3.7. Effect of Pancreaticoduodenectomy on Serum LPS Levels

Serum LPS levels significantly increased after PD. For the patients undergoing PD, the blood samples were taken one day before surgery and between postoperative day 10-12. The serum was extracted and LPS levels were determined by the LAL assay. As shown in figure 8A, the serum LPS levels significantly increased after PD (Preop. (PD) vs Postop. (PD): 0.7571±0.1951 vs 1.000±0.2832 EU/ml, p< 0.0001). The degree of increase in LPS levels did not significantly correlate with the operative time (Figure 8B), the amount of blood loss during surgery (Figure 8C), or age (Figure 8D).



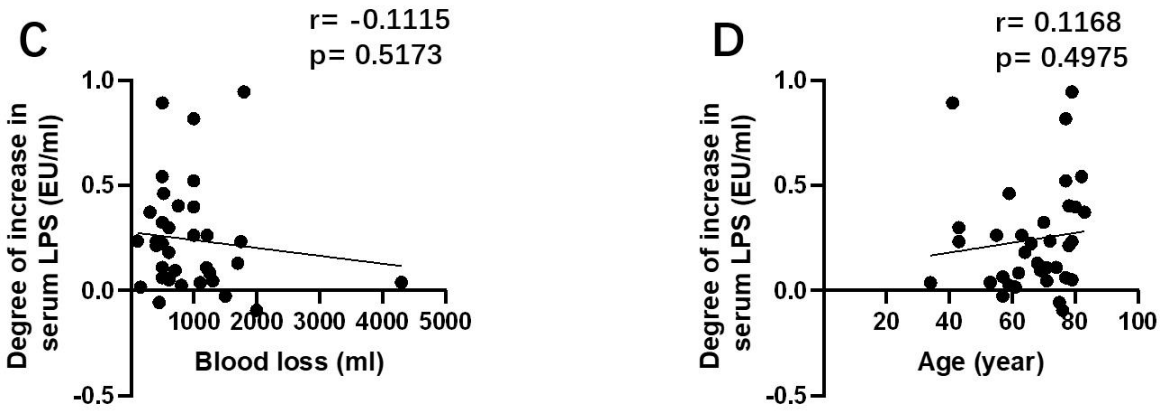


Figure 8. Serum LPS levels significantly increased after PD (A), which was not correlated with operative time (B), blood loss (C) or age (D). (Abbreviations: LPS: lipopolysaccharide; Preop: preoperative; Postop: postoperative; PD pancreaticoduodenectomy; r: r value; p: p value; **** p< 0.0001.)

3.8. Effect of Distal Pancreatectomy on Serum LPS Levels

Serum LPS levels also increased after DP. For the patients undergoing DP, the blood samples were also collected one day preoperatively and between postoperative day 10-12. The serum LPS levels were assessed before and after the procedures. As shown in figure 9A, serum LPS levels also increased after DP (Preop. (DP) vs Postop. (DP): 0.7258±0.1596 vs 0.7715±0.1912 EU/ml, p= 0.0284). The PD group and DP group were not significantly different in terms of circulating LPS levels preoperatively (Preop. (PD) vs Preop. (DP): 0.7571±0.0.1951 vs 0.7258±0.1596 EU/ml, p= 0.6186) (Figure 9C), however, the former group had higher LPS levels postoperatively (Postop. (PD) vs Postop. (DP): 1.0000±0.2832 vs 0.7715±0.1912 EU/ml, p= 0.0126) (Figure 9D). The increase in serum LPS levels was also more significant in the PD group than in the DP group (PD vs DP: 0.2431±0.2524 vs 0.0415±0.0657 EU/ml, p< 0.0001) (Figure 9B). Given all these facts, PD caused a more pronounced increase in the serum LPS levels compared to DP. The operative time and the amount of blood loss also did not significantly affect the degree of increase in LPS levels in the patients undergoing DP (Figure 9E and Figure 9F).

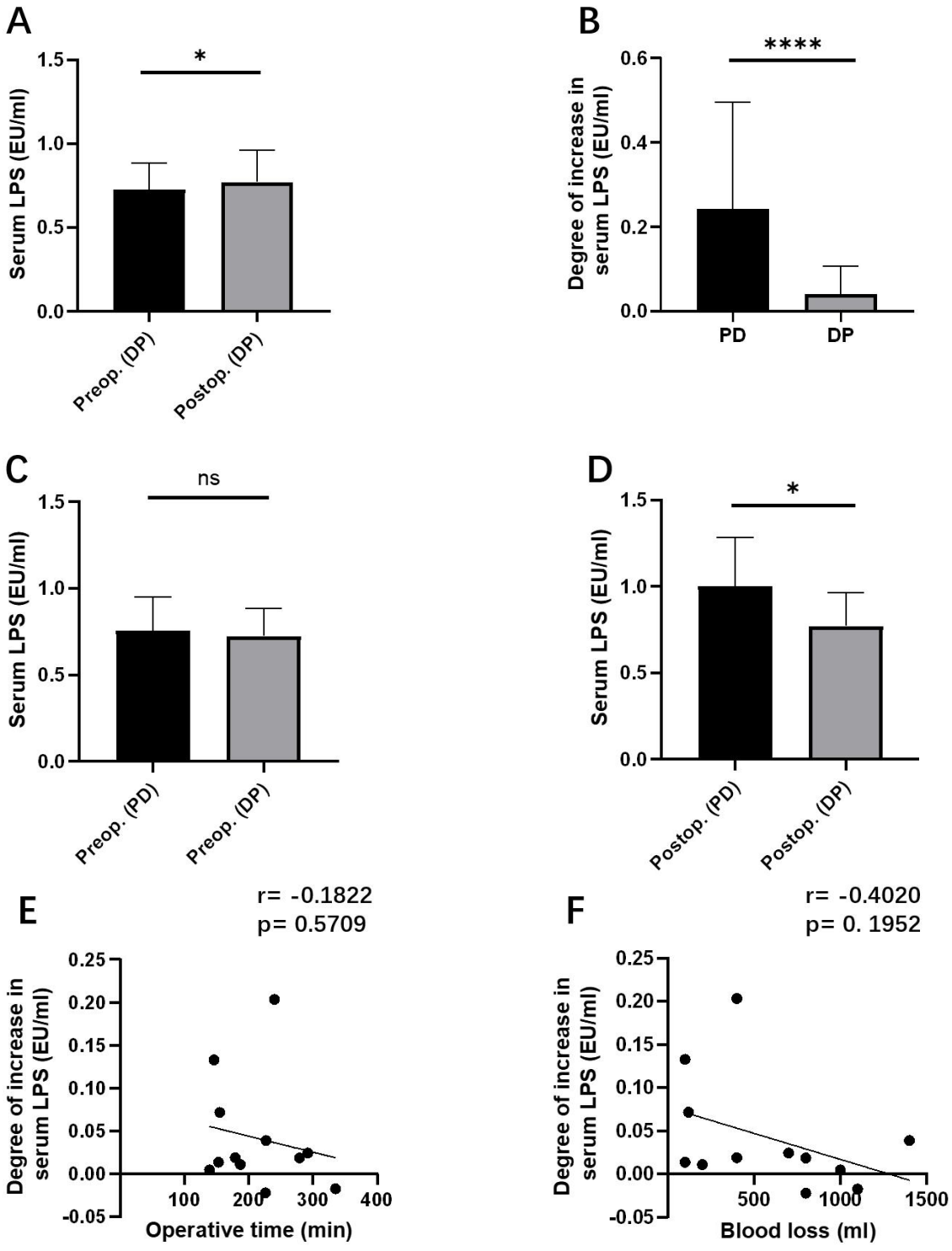


Figure 9. Serum LPS levels also increased after DP (A). There was no significant difference in the preoperative LPS levels between the PD and DP groups (C), however, the PD group had higher postoperative serum LPS levels (D), therefore PD caused a more pronounced rise in LPS levels

compared to DP (B). The degree of increase in serum LPS levels was not correlated with operative time (E) and blood loss (F). (Abbreviations: LPS: lipopolysaccharide; Preop: preoperative; Postop: postoperative; DP: distal pancreatectomy; PD: pancreaticoduodenectomy; r: r value; p: p value; ns: not significant; * p< 0.05; **** p< 0.0001.)

3.9. Effect of General Surgery on Serum LPS Levels

There was no significant increase in serum LPS levels after general surgery. While studying the effect of surgery on serum LPS levels, patients undergoing cholecystectomy or hernia repair were also included as controls. The blood samples were taken one day before surgery and between postoperative day 2-3. LPS levels were also assessed before and after the procedures. As shown in figure 10A, LPS levels didn't increase significantly after surgery (Preop. (General surgery) vs Postop. (General surgery): 0.5961±0.1598 vs 0.5450±0.2476 EU/ml, p= 0.5073). Patients in the general surgery group had lower preoperative LPS levels than the PD group (Preop. (General surgery) vs Preop. (PD): 0.5961±0.1598 vs 0.7571±0.1951 EU/ml, p= 0.0069) (Figure 10B) and this gap became more pronounced postoperatively (Postop. (General surgery) vs Postop. (PD): 0.5450±0.2476 vs 1.000±0.2832 EU/ml, p< 0.0001) (Figure 10C). This result indicated that PD caused a significant increase in serum LPS levels, whereas smaller general surgery procedures did not.

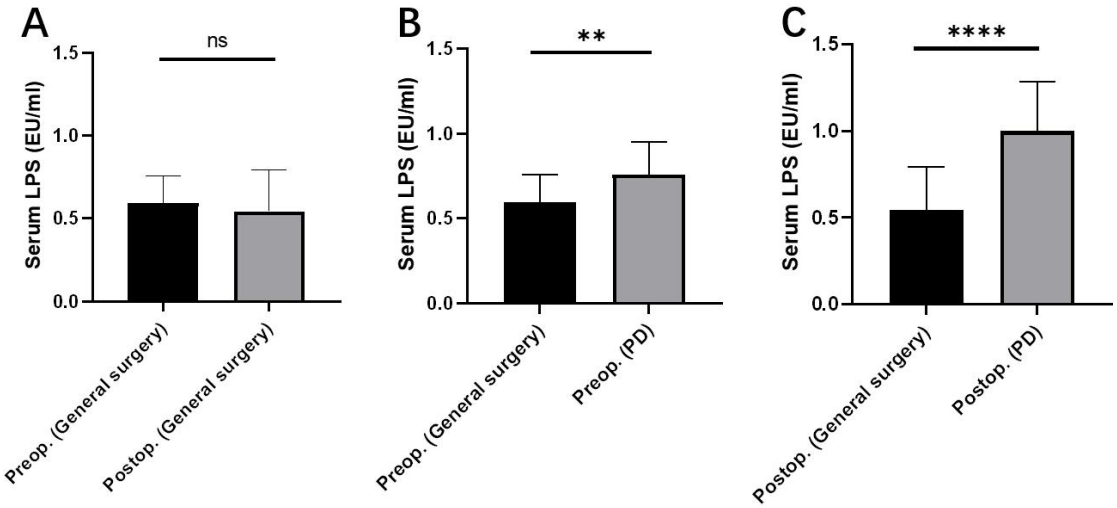


Figure 10. Smaller general surgery procedures (cholecystectomy and hernia repair) did not

cause a significant rise in serum LPS levels (A). Patients in the PD group had higher preoperative LPS levels than the general surgery group (B) and this difference became more pronounced postoperatively (C). (Abbreviations: LPS: lipopolysaccharide; Preop: preoperative; Postop: postoperative; PD: pancreaticoduodenectomy; ns: not significant; ** p< 0.01; **** p< 0.0001.)

3.10. Serum LPS Levels in Pancreatic Cancer Patients

Serum LPS levels were significantly higher in pancreatic cancer patients than in non-cancer patients before surgery. As described above in Section 3.3 (Figure 4A), the patients in the PD group had higher serum LPS levels than the general surgery group. There was no significant difference in the clinical data between the 2 groups of patients except for the types of disease (most patients in the PD group were diagnosed with pancreatic cancer, whereas patients in the general surgery group were not). According to previous studies, circulating LPS levels elevated in patients with liver cancer [8, 9], CRC [10], or bladder cancer compared with the healthy controls [11]. Therefore, we hypothesized that pancreatic cancer patients also have higher LPS levels. In the whole cohort, 26 cases were diagnosed with pancreatic cancer, 10 patients had IPMN, and 15 had cholecystitis or hernia. We divided these 51 patients into a cancer group (26 pancreatic cancer patients) and a non-cancer group (25 IPMN, cholecystitis, or hernia patients). Preoperative serum LPS levels in patients with pancreatic cancer were significantly higher than in non-cancer patients (Preop. (PC) vs Preop. (Non-cancer): 0.8089±0.1862 vs 0.6311±0.1634 EU/ml, p= 0.0007) (Figure 11A). Besides, preoperative stool IAP activity was negatively correlated with LPS concentrations in pancreatic cancer patients (r= -0.6628, p= 0.0002) (Figure 11C), and the correlation between these two markers was more pronounced in the pancreatic cancer group than in the whole study population (r= -0.3426, p= 0.0060) (Figure 4C).

There was no significant difference in the preoperative stool IAP activity levels (Preop. (PC) vs Preop. (Non-cancer): 1439.0±957.2 vs 1509.0±1302.0 pmole PNPP hydrolyzed/min/ug protein, p= 0.8270) (Figure 11B) and other basic clinical characteristics between the two groups as shown in Table 4, except for age (Figure 12A). As previously stated, serum LPS levels did not change with age in the whole

cohort. Moreover, there was no significant correlation between serum LPS levels and age in the cancer group or non-cancer group (Figure 12B and Figure 12C). Therefore, the age difference was not the main reason for the different LPS levels between the two groups.

Table 4. Demographics of Study Population.

Preoperative Variables (Mean ± SD)	**Pancreatic Cancer Group** (N=26)	**Non-Cancer Group** (N=25)	**p**
Gender (Male/Female)	18 (69.2%)/8 (30.8%)	16 (64.0%)/9 (36.0%)	0.7712
Age (Year)	70.15±11.70	60.40±12.70	**0.0063**
BMI	24.54±3.97	24.17±3.23	0.7193
AST	55.48±51.15	32.04±30.15	0.0568
Creatinine (mg/dl)	0.90±0.17	0.90±0.23	0.8869
CRP (mg/dL)	0.68±0.82	0.34±0.39	0.0670
WBC (G/L)	7.56±4.34	7.11±2.42	0.6501
RBC (T/L)	4.46±0.59	4.68±0.66	0.2134
Platelets (G/L)	279.90±87.29	261.5±76.54	0.4280
Hemoglobin (g/dl)	13.33±1.76	14.02±2.31	0.2379
GFR (ml/min)	80.35±16.75	84.12±19.72	0.4643
FBG (mg/dl)	144.0±76.66	112.4±21.42	0.0523
ALP (U/L)	300.60±551.80	98.65±90.25	0.0772
K (mmol/L)	4.45±0.59	4.33±0.65	0.4958
TBil (mg/dl)	1.30±2.01	0.49±0.18	0.0517

(Abbreviations: SD: standard deviation; BMI: body mass index; AST: aspartate transaminase; TBil: total bilirubin; FBG: fasting blood glucose; CRP: C-reactive protein; WBC: white blood cell; RBC: red blood cell; GFR: glomerular filtration rate; ALP: alkaline phosphatase; K: blood kalium. All values are expressed as mean ± SD except for gender.)

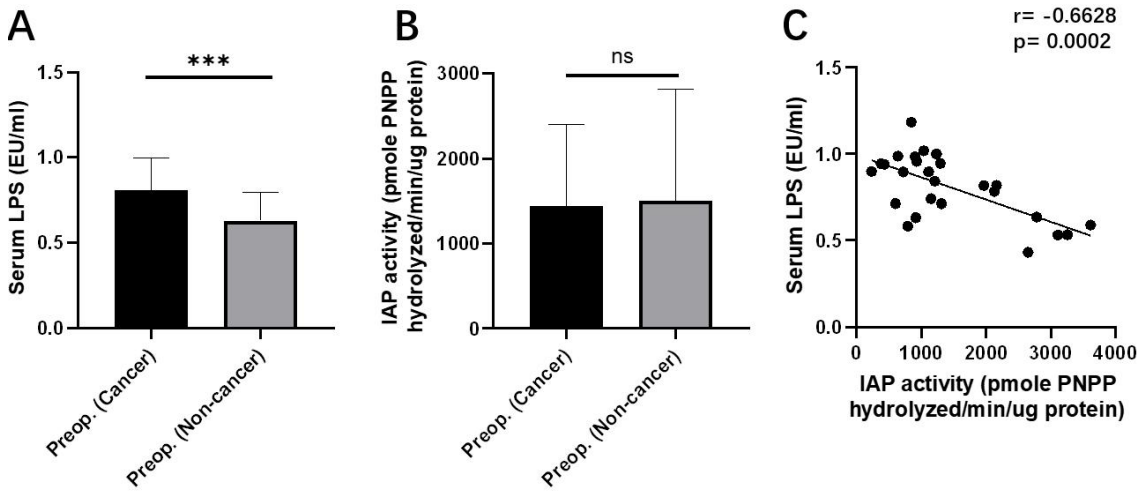


Figure 11. Pancreatic cancer patients had significantly higher preoperative serum LPS levels than non-cancer patients (A), however, there was no significant difference in stool IAP activity between the two groups (B). Stool IAP activity negatively correlated with serum LPS levels in pancreatic cancer group before surgery (C). (Abbreviations: LPS: lipopolysaccharide; Preop: preoperative; Postop: postoperative; PC: pancreatic cancer group; Non-cancer: non-cancer group; ns: not significant; *** p< 0.001.)

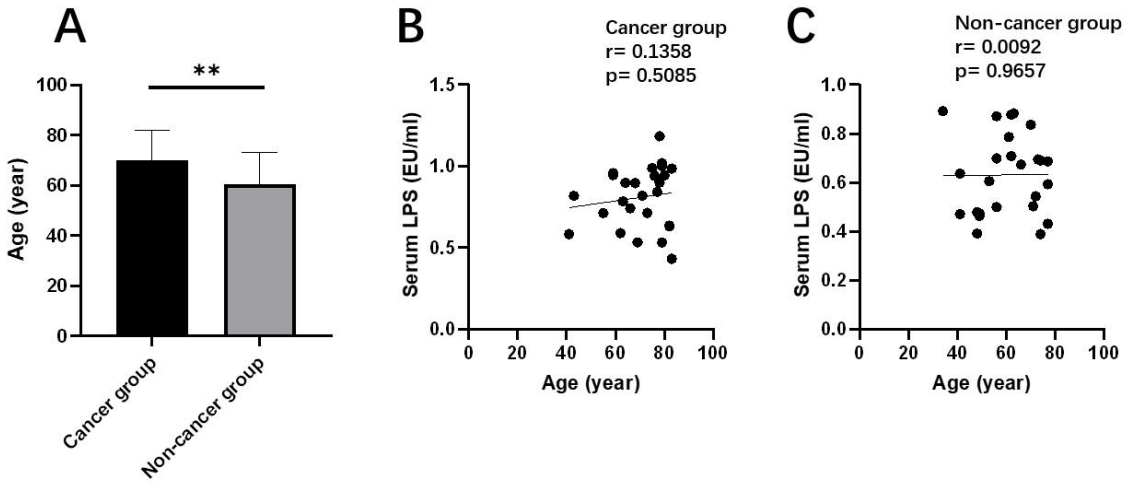


Figure 12. Patients in the cancer group were older than in the non-cancer group (A), however，LPS levels were not age-related in either group before surgery (B, C). (Abbreviations: LPS: lipopolysaccharide; r: r value; p: p value.)

3.11. Serum LPS Levels and Stool IAP Activity in Pancreatic Cancer Patients after Pancreaticoduodenectomy

Serum LPS levels were positively correlated with hospital days in pancreatic cancer patients after PD. As presented in Section 3.10 and 3.3, serum LPS levels were negatively correlated with stool IAP activity both in the pancreatic cancer patients and the whole study population before surgery. While examining the correlation between IAP, LPS and other clinical data in 23 pancreatic cancer patients after PD (Table 5), we also found that stool IAP activity had a negative correlation with serum LPS levels (Figure 14A). The average length of hospital stay of these 23 patients was 24.48±9.43 days. Furthermore, hospital days increased with higher postoperative LPS levels (Figure 13A) but were not significantly correlated with operative time and blood loss during surgery (Figure 13B and 13C). IAP activity was positively associated with the concentrations of serum FBG and K (Figure 14B and Figure 14C) in these patients after the procedures. No significant associations between IAP, LPS levels and other clinical data were detected in this group.

Table 5. Relationship and Correlation of IAP, LPS and Clinical Parameters in Pancreatic Cancer Patients after PD.

Clinical Parameters	Postoperative Stool IAP	Postoperative Serum LPS
Stool IAP	_	**r = -0.4230, p = 0.0444**
Gender	p = 0.1026	p = 0.4127
Age	r = -0.1648, p = 0.4523	r = 0.0059, p = 0.9787
BMI	r = 0.2369, p = 0.2764	r = -0.4006, p = 0.0582
AST	r = -0.0794, p = 0.7189	r = -0.2464, p = 0.2815
TBil	r = 0.3743, p = 0.0785	r = 0.0039, p = 0.9860
Creatinine	r = 0.3739, p = 0.0788	r = -0.1598, p = 0.4665
CRP	r = -0.2789, p = 0.1975	r = -0.3755, p = 0.0775
WBC	r = 0.4195, p = 0.0520	r = 0.0670, p = 0.7671
RBC	r = 0.0059, p = 0.9324	r = -0.3050, p = 0.1570
Platelets	r = 0.1070, p = 0.6270	r = -0.2935, p = 0.1740
Hemoglobin	r = -0.0572, p = 0.7953	r = -0.2644, p = 0.2227
GFR	r = -0.0492, p = 0.8235	r = 0.0406, p = 0.8540
FBG	**r = 0.5564, p = 0.0072**	r = -0.1940, p = 0.3869
ALP	r = 0.3455, p = 0.1250	r = 0.2624, p = 0.2381
K	**r = 0.5065, p = 0.0162**	r = 0.1786, p = 0.4264
Operation time	r = 0.2547, p = 0.2526	r = -0.0510, p = 0.8173
Blood loss	r = 0.1499, p = 0.4947	r = -0.0902, p = 0.6823
Hospital days	r = 0.3291, p = 0.1253	**r = 0.4188, p = 0.0467**

(Abbreviations: IAP: intestinal alkaline phosphatase; LPS: lipopolysaccharide; BMI: body mass index; AST: aspartate transaminase; TBil: total bilirubin; FBG: fasting blood glucose; CRP: C - reactive protein; WBC: white blood cell; RBC: red blood cell; GFR: glomerular filtration rate; ALP: alkaline phosphatase; K: blood kalium.)

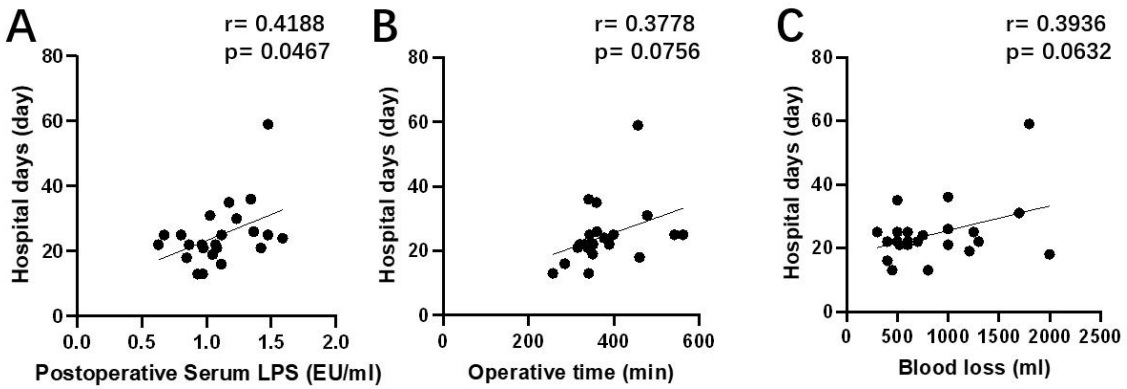


Figure 13. For patients with pancreatic cancer after the PD, hospital days increased with higher postoperative LPS levels (A) but were not significantly correlated with operative time (B) and blood loss during surgery (C). (Abbreviations: LPS: lipopolysaccharide; r: r value; p: p value.)

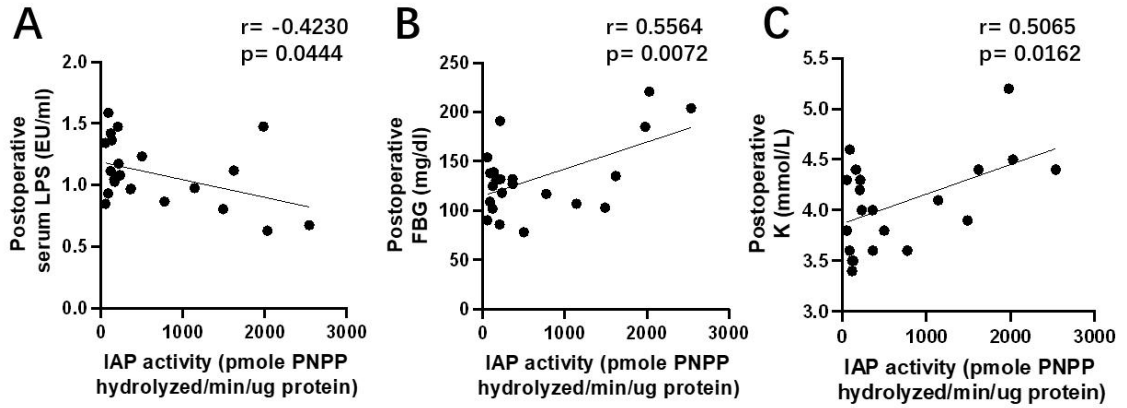


Figure 14. Serum LPS levels decreased with higher stool IAP activity (A), whereas the concentrations of serum FBG and K were positively correlated with IAP activity (B, C) in pancreatic cancer patients after PD. (Abbreviations: IAP: intestinal alkaline phosphatase; LPS: lipopolysaccharide; PNPP: para-nitrophenyl phosphate; FBG: fasting blood glucose; K: blood kalium; r: r value; p: p value.)

4. Discussion

IAP as a gut brush border enzyme is a critical regulator of intestinal bacterial homeostasis and gut barrier function [1]. In addition, IAP also functions to dephosphorylate LPS and other inflammatory mediators responsible for endotoxemia [1]. IAP deficiency has been demonstrated to be related to many diseases such as IBD, necrotizing enterocolitis (NEC), T2DM, ischemic heart disease (IHD), and aging-related liver change [3, 56, 68, 71, 73]. LPS is a principal constituent of the outer membrane of Gram-negative bacteria and a potent trigger of inflammation [5-7]. It activates TLR4-MD2 to initiate a signaling cascade that induces production of the pro-inflammatory cytokines and helps eliminate invading pathogens [86, 87]. However, overexpressed cytokines can also activate the NF-κβ pathway, which is implicated in DNA damage and cell proliferation leading to carcinogenesis [31]. LPS has been used as an inflammatory stimulus to confirm the role of TLR/MyD88/NF-κB signaling pathway in connecting inflammation and cancer progression [121]. Moreover, recent studies have reported elevated circulating LPS levels in patients with cancers (e. g., CRC, liver cancer, and bladder cancer) [8-11]. Pancreatic surgery mainly consists of two types of procedure: PD (Whipple procedure) and DP, which can remove a portion or all of the pancreas to eliminate the initial lesions. Up to now, surgical resection combined with adjuvant chemotherapy and radiotherapy remains the only curative therapeutic option for patients with early-stage pancreatic cancer [122]. Although pancreatic surgery is demonstrated to be a safe, effective, and key component of the treatment for pancreatic lesions [93], it is still associated with high morbidity and mortality rates [107-109]. Therefore, fully understanding the damage caused by pancreatic surgery to the human body and searching for the corresponding treatments are important for improving the prognosis of pancreatic lesions.

IAP is known to be predominately produced in the duodenum, which is removed during PD [15]. Therefore, theoretically speaking, PD could cause a sharp decrease in IAP levels, followed by disruption of intestinal homeostasis, leading to a series of pathologies and unfavorable prognosis. Based on these facts, it is essential to study

the changes in serum LPS levels and IAP activity in the perioperative period in patients undergoing PD.

4.1. Preoperative Stool IAP Activity and Serum LPS Levels

Stool IAP activity decreased with age in the whole cohort

In our study, we first compared the demographical characteristics and stool IAP activity between the three groups of patients (i.e., PD group, DP group, and General surgery group) and found no significant differences. When examining the correlation between IAP levels and clinical data, we found that IAP activity declined linearly with age in the whole study population. Our result was consistent with Malo's conclusion, and his group found IAP activity decreased with an average drop of 0.7 U/g stool per year [71]. Kühn et al. [3] also showed a significant decline in IAP activity with age both in mice and humans. As IAP deficiency is involved in the development of various diseases [26, 50, 69, 71], monitoring of stool IAP activity and supplementing with exogenous IAP as aging might be beneficial to prevent disease and maintain health. However, many factors such as starvation, diet, inflammation, and some special drugs modulate IAP activity [63, 123-130], leading to different research outcomes. Hamarneh et al. [17] showed that age, sex, and BMI did not influence ileal IAP activity, whereas in another study, intestinal IAP gene expression strongly correlated with BMI [131]. Hence, stool IAP data should be interpreted with caution, and more research is needed to determine the relationship between IAP activity and other clinical indicators.

Preoperative Serum LPS levels are negatively correlated with stool IAP activity

The intestinal microbiota is an important source of circulating LPS [30, 132]. Many factors such as a high-fat diet, chronic stress, and aging can cause microbial dysbiosis and disrupt gut barrier function, thus increase the translocation of bacterial products into the systemic circulation and elevate serum LPS levels [3, 12-14]. On the other hand, administration of probiotics, prebiotics, glutamine, vitamin D, and other substances could decrease blood LPS levels by maintaining intestinal homeostasis [133-136]. Overall, many factors can affect serum LPS levels, but in most cases, elevated LPS levels can be considered to be a consequence of the

alterations in intestinal homeostasis. Thus, serum LPS is not only a cause of various diseases but also a potential tool for non-invasive assessment of intestinal microbiota and gut barrier integrity in humans [16]. In our research, preoperative data analysis showed that serum LPS levels were negatively correlated with stool IAP activity in the total study population. Although IAP is well-known for its beneficial roles in intestinal homeostasis and its ability to dephosphorylate LPS, the association between stool IAP activity and serum LPS levels has not been previously explored in human beings. Our study demonstrated this relationship and suggested that IAP could influence the development and progression of many diseases by affecting the concentration of LPS in the blood.

Kaliannan et al. [19] showed that oral administration of IAP prevented the corn-oil–induced increase in LPS levels both in cecal contents and blood in mice. Besides, Kühn et al. [3] demonstrated that long-term IAP supplementation significantly reduced the levels of serum LPS and proinflammatory cytokine compared with control mice. Based on these facts, exogenous IAP supplementation may also decrease LPS levels in the human circulation and tissues, thereby reducing the incidence and improving the prognosis of various diseases. However, more clinical studies are needed to further confirm this hypothesis.

The patients in the PD group had higher preoperative LPS levels than those undergoing smaller general surgery procedures. There was no significant difference in the clinical indicators between these groups of patients except for the types of diseases (most patients in the PD group were diagnosed with pancreatic cancer, whereas patients in the general surgery group were not). Therefore, it could be argued that LPS levels may be related to cancer status, which is discussed in more detail in Section 4.3.

In contrast to our presumption, serum LPS concentrations did not change with age in our study. The imbalance of gut microbiota and decrease in barrier function mirrors age-related degeneration, indicating that there may be an age-associated increase in serum LPS level [137, 138]. Nevertheless, as previously mentioned, many factors can affect serum LPS levels. Thus, more large-scale clinical trials are needed to clarify the relationship between LPS and age.

4.2. Changes in Stool IAP Activity and Serum LPS Levels after Surgery.

Stool IAP activity significantly decreased after PD

IAP is predominantly synthesized by enterocytes and secreted in the small intestine, especially in the duodenum [32]. Many factors such as aging, inflammation, and diet can affect IAP level [3, 139, 140]. Adult and pediatric patients undergoing cardiac surgery with cardiopulmonary bypass (CPB) demonstrated a sharp decline in circulating alkaline phosphatase activity in the immediate postoperative phase [141-143]. Khailova et al. [144] tested IAP activity and IAP mRNA expression level in the intestinal tissue of piglet model undergoing CPB/deep hypothermic circulatory arrest (DHCA). They found that CPB/DHCA increased IAP mRNA expression in the ileum and colon but not in the jejunum compared to the controls [144]. Interestingly, total alkaline phosphatase activity in the ileum and colon tissue did not differ significantly between the CPB/DHCA and anesthesia control groups [144]. This finding likely reflected a combination of increased production and sustained loss or consumption of IAP [144]. During PD, the duodenum is completely removed [15], which could affect the synthesis of IAP seriously. To our knowledge, no studies have reported on IAP activity changes after PD. In our research, postoperative IAP activity decreased significantly compared to preoperative values in the PD group. Additionally, the decreased degree of IAP activity positively correlated with the removal length of the proximal small intestine, which demonstrated that duodenectomy directly affected stool IAP activity levels.

Given the important physiological roles of IAP in maintaining health, regular testing of IAP activity and supplementation with exogenous IAP after duodenal resection might be beneficial. In recent years, a human recombinant placental alkaline phosphatase (hRESCAP) has been developed as a better alternative by replacing the crown domain of human IAP with the crown domain of placental AP, which makes it more stable and efficient than the parent IAP enzyme [145]. The administration of IAP and hRESCAP has shown its efficiency in some clinical studies (Table 7) [146], and no particular safety issues have been reported until now. Oral administration of bovine IAP was shown to attenuate moderate/severe UC [61]. Bovine IAP

treatment improved renal function in patients with severe sepsis and reduced the inflammatory response in patients undergoing coronary artery bypass grafting [62, 64, 147]. The route of IAP administration appears to be an important factor as well. Previous studies have revealed that oral administration of IAP can reduce systemic inflammation and stimulate endogenous IAP production. Whereas intraperitoneal or intravenous injection was only able to decrease systemic inflammation [51, 148].

Table 7. Summary of Clinical Trials Applying IAP [146]

Study Title	Countries	Diseases	Interventions	No. Patients/ Starting Date/ Status
BIAP Modulating RA	United Kingdom	Acute RA	Drug: s.c. injections of BIAP	6/May 2011/ Completed
Safety/Efficacy Study of BIAP in Patients with Moderate to Severe UC	Czech Republic	UC	Drug: BIAP	22/May 2006/ Completed
BIAP for the Treatment of Patients with Sepsis	Belgium; Netherlands	Sepsis; MODS	Drug: BIAP Drug: Placebo	37/September 2004/Completed
Efficacy and Safety of BIAP During Heart Surgery	Netherlands	Inflammation	Drug: BIAP bolus and 8h infusion Drug: placebo bolus and 8h infusion	53/April 2010/ Completed
Microdose and FIH Study of hRESCAP	Netherlands	Healthy	Drug: hRESCAP Drug: Placebo	4/June 2013/Completed
A Study in Sepsis Patients with Renal Failure	Belgium; Netherlands	Sepsis; Bacterial Infections and	Drug: Placebo Drug: BIAP	36/May 2008/ Terminated

		Mycoses		
Preventing Systemic Inflammation After Cardiac Surgery with Alkaline Phosphatase	Australia; Austria; Belgium; Malaysia; Netherlands	Systemic Inflammation CPB	Drug: bRESCAP Drug: placebo	1250 (Estimated enrollment)/ November 2017/ Recruiting

(Abbreviations: BIAP: Bovine Intestinal Alkaline Phosphatase; s.c.: subcutaneous; RA: Rheumatoid Arthritis; UC: Ulcerative Colitis; MODS: Multiple Organ Dysfunction Syndrome; hRESCAP: Recombinant Human Placental Alkaline Phosphatase; FIH: First-In-Human; CPB: Cardiopulmonary-bypass)

In this study, the decrease of IAP activity also showed a rising trend with increasing operative time and blood loss, but these associations were not statistically significant. Until now, stool IAP data in patients undergoing surgery is scarce. Although we cannot exclude that surgery or trauma directly affects IAP activity based on our current results, the effect of the operation might have diminished significantly for most patients when their samples were taken between postoperative day 10-12.

Stool IAP activity also decreased after DP

The results of our study indicated that DP also caused a reduction in stool IAP activity, but to a much lesser extent than PD. In addition, no significant correlation between a decrease in the IAP activity and operative time or blood loss was found. Therefore, we presumed that although the operative time and blood loss have no significant impact on IAP levels between postoperative day 10-12, some factors such as inflammation and dietary changes associated with the operation can still reduce IAP activity. We also demonstrated that IAP activity was not significantly lower on the 3rd postoperative day than the preoperative baseline in patients undergoing smaller general surgery procedures, which indicated that smaller surgical procedures without upper intestinal resections did not affect IAP levels or had only minimal and short-lasting effects. Therefore, monitoring of the stool IAP

activity and supplementation with exogenous IAP might be beneficial for patients receiving major surgery, especially when they are undergoing long periods of fasting or displaying a severe inflammatory response postoperatively. However, for patients undergoing smaller general surgery, tests for IAP are not always necessary except in special cases.

The increase in serum LPS levels was more pronounced after PD than after DP

It is well established that LPS can elicit robust immune responses by stimulating TLR4-MD2 and inducing the production of pro-inflammatory cytokines. Overexpression of pro-inflammatory cytokines results in various pathophysiological consequences such as acute (sepsis) and chronic systemic inflammation, leukopenia, hypotension, and even multiorgan failure, which are detrimental to the prognosis of the operations [78, 86, 87]. Studies have reported that the levels of serum LPS or LPS components decreased at 3, 6, and 12 months after bariatric surgery [149-151]. Zhang et al. [152] showed that serum LPS concentrations were higher on postoperative day 3 than preoperative levels in patients undergoing spinal surgery. In our study, the serum LPS levels significantly elevated after PD. In the DP group, postoperative serum LPS concentrations did also increase compared with preoperative baseline values, but the increase was much smaller than the PD group. As stated above, the increase in the blood LPS levels indicates that intestinal homeostasis is altered [16]. The removal of the duodenum during PD resulted in a substantial decrease in IAP activity, which might induce intestinal dysbiosis and gut barrier dysfunction. IAP deficiency and disrupted intestinal homeostasis could at least partially explain why PD led to a more significant increase in serum LPS levels than DP. Although serum LPS levels between postoperative day 10-12 did not significantly correlate with operative time or blood loss in both PD and DP groups, other surgery-associated factors (e.g., inflammation and dietary changes) could still promote translocation of enteric-derived LPS into the bloodstream by affecting intestinal homeostasis, which would trigger a systemic inflammatory response and subsequent complications. Therefore, for patients undergoing major surgery, even without intestinal resection, it might be useful to test the postoperative serum LPS levels and take timely measures to maintain intestinal microbial homeostasis and

gut barrier function. With the function of maintaining intestinal homeostasis, IAP appears to have a low risk profile, few side effects, and might have the potential to improve the outcome of patients undergoing major surgery, especially the Whipple procedure. Numerous experiments have reported changes in blood LPS levels in different diseases [153-158], but a few experiments showed that LPS was not detectable in human plasma [159, 160]. The method used to detect LPS may be the root cause of the different results [161]. LAL test and immunoassay are currently the most commonly employed methods for LPS detection [162]. However, they are usually highly susceptible to the experimental conditions, time-consuming, and require tedious preparation and testing procedures [163-166]. Several studies have recently been devoted to the development of alternative detection methods for LPS [167-173]. For example, the biosensing technique and collaborative amplification of dual enzymes method have proven to be low cost, easy to use, and rapid in detecting LPS [162, 174].

On the 3rd postoperative day, serum content of LPS in patients receiving smaller general surgery without intestinal resection was not significantly altered compared to preoperative levels, which further confirmed that smaller general surgery procedures did not affect intestinal homeostasis or had only a minimal and short-term impact.

4.3. Stool IAP and serum LPS in pancreatic cancer patients

Serum LPS levels were higher in pancreatic cancer patients than in non-cancer patients before surgery.

According to former animal studies, intestinal dysbiosis and gut barrier dysfunction could promote translocation of gut-derived LPS into circulation and be involved in the carcinogenesis of liver cancer [175]. Several studies have also shown that oral dysbiosis can increase pancreatic cancer risk [176-178]. Others have demonstrated that circulating LPS levels were elevated in patients with liver cancer, CRC, and bladder cancer compared with the healthy controls [8-11]. Andrews et al. [92] found that exposure to LPS increased colon cancer cell adhesion to the vascular endothelium and postoperative metastatic tumor growth. LPS was also found to promote invasion of pancreatic cancer cells in vitro [179]. Given these facts,

disruption of intestinal homeostasis can lead to the increase in serum LPS levels, which is related to the development, progression, and prognosis of various forms of cancers. However, no data on serum LPS levels in pancreatic cancer patients has been reported until now.

In our study, the pancreatic cancer group had significantly higher preoperative serum LPS levels than the non-cancer group. This might be caused by a disrupted gut barrier integrity and/or intestinal microbial homeostasis, followed by elevated serum LPS levels. Furthermore, we found that preoperative serum LPS concentrations were negatively correlated with stool IAP activity in the patients with pancreatic cancer, and the correlation between these two indicators was more pronounced in cancer patients than in the whole study population. Therefore, for pancreatic cancer patients, exogenous IAP supplementation may be more effective in reducing serum LPS levels before surgery.

Postoperative LPS level correlate with the length of hospital stay

In our study, for pancreatic cancer patients undergoing PD, postoperative serum LPS levels were positively correlated with the length of hospital stay. This result suggests that postoperative serum LPS level is not only a circulating biomarker of intestinal homeostasis, but also a predictor of the prognosis after PD. Besides, there was a negative relationship between postoperative stool IAP activity and serum LPS content in these pancreatic cancer patients receiving PD. The correlations between these two markers before and after PD indicate that stool IAP can play an important role in the development, progression, and surgical prognosis of pancreatic cancer by affecting circulating LPS levels. The concentrations of serum FBG and K were positively correlated with IAP activity in pancreatic cancer patients after PD. Although previous studies haven't found the correlation between IAP and serum FBG or K, we need to notice the changes in these two indicators while supplementing the patients with exogenous IAP.

4.4. Conclusion

This study showed that PD led to a significant decrease in stool IAP levels and a significant increase in serum LPS levels. The degree of IAP reduction positively

correlated with the removal length of the proximal small intestine, which indicated that duodenectomy directly affected stool IAP activity levels. Stool IAP activity also decreased after DP, but to a much lesser extent than PD. The PD group had a more pronounced elevation of serum LPS levels compared to the control groups after surgery. Preoperative serum LPS levels were higher in the pancreatic cancer patients than in the non-cancer patients before surgery. Furthermore, stool IAP activity negatively correlated with serum LPS levels in pancreatic cancer patients before and after PD. Based on the multiple functional roles for IAP, in particular its ability to dephosphorylate bacteria-derived inflammatory mediators and its salutary role on gut barrier integrity, we propose to test the supplementation of exogenous IAP for patients undergoing PD.

References

1. Lallès J P. Recent advances in intestinal alkaline phosphatase, inflammation, and nutrition. Nutr Rev. 2019 Oct 1; 77(10): 710-724.
2. Malo M S, Alam S N, Mostafa G, Zeller S J, Johnson P V, Mohammad N, et al. Intestinal alkaline phosphatase preserves the normal homeostasis of gut microbiota. Gut. 2010 Nov; 59(11): 1476-84.
3. Kühn F, Adiliaghdam F, Cavallaro P M, Hamarneh S R, Tsurumi A, Hoda R S, et al. Intestinal alkaline phosphatase targets the gut barrier to prevent aging. JCI Insight. 2020 Mar 26; 5(6).
4. Aw W, Fukuda S. Understanding the role of the gut ecosystem in diabetes mellitus. J Diabetes Investig. 2018 Jan; 9(1): 5-12.
5. Uchiyama T, Jacobs D M. Modulation of immune response by bacterial lipopolysaccharide (LPS): multifocal effects of LPS-induced suppression of the primary antibody response to a T-dependent antigen. J Immunol. 1978 Dec; 121(6): 2340-6.
6. Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev. 2003 Dec; 67(4): 593-656.
7. Whitfield C, Trent M S. Biosynthesis and export of bacterial lipopolysaccharides. Annu Rev Biochem. 2014 83: 99-128.
8. Fedirko V, Tran H Q, Gewirtz A T, Stepien M, Trichopoulou A, Aleksandrova K, et al. Exposure to bacterial products lipopolysaccharide and flagellin and hepatocellular carcinoma: a nested case-control study. BMC Med. 2017 Apr 4; 15(1): 72.
9. Yang B, Petrick J L, Thistle J E, Pinto L A, Kemp T J, Tran H Q, et al. Bacterial Translocation and Risk of Liver Cancer in a Finnish Cohort. Cancer Epidemiol Biomarkers Prev. 2019 Apr; 28(4): 807-813.
10. de Waal G M, de Villiers W J S, Forgan T, Roberts T, Pretorius E. Colorectal cancer is associated with increased circulating lipopolysaccharide, inflammation and hypercoagulability. Sci Rep. 2020 May 29; 10(1): 8777.
11. He C, Li B, Huang L, Teng C, Bao Y, Ren M, et al. Gut microbial composition changes in bladder cancer patients: A case-control study in Harbin, China.

Asia Pac J Clin Nutr. 2020 29(2): 395-403.

12. Netto Candido T L, Bressan J, Alfenas R C G. Dysbiosis and metabolic endotoxemia induced by high-fat diet. Nutr Hosp. 2018 Dec 3; 35(6): 1432-1440.
13. Fang W, Xue H, Chen X, Chen K, Ling W. Supplementation with Sodium Butyrate Modulates the Composition of the Gut Microbiota and Ameliorates High-Fat Diet-Induced Obesity in Mice. J Nutr. 2019 May 1; 149(5): 747-754.
14. Dodiya H B, Forsyth C B, Voigt R M, Engen P A, Patel J, Shaikh M, et al. Chronic stress-induced gut dysfunction exacerbates Parkinson's disease phenotype and pathology in a rotenone-induced mouse model of Parkinson's disease. Neurobiol Dis. 2020 Feb; 135: 104352.
15. Clancy T E. Surgery for Pancreatic Cancer. Hematol Oncol Clin North Am. 2015 Aug; 29(4): 701-16.
16. Grootjans J, Thuijls G, Verdam F, Derikx J P, Lenaerts K, Buurman W A. Non-invasive assessment of barrier integrity and function of the human gut. World J Gastrointest Surg. 2010 Mar 27; 2(3): 61-9.
17. Hamarneh S R, Mohamed M M, Economopoulos K P, Morrison S A, Phupitakphol T, Tantillo T J, et al. A novel approach to maintain gut mucosal integrity using an oral enzyme supplement. Ann Surg. 2014 Oct; 260(4): 706-14; discussion 714-5.
18. Liu W, Hu D, Huo H, Zhang W, Adiliaghdam F, Morrison S, et al. Intestinal Alkaline Phosphatase Regulates Tight Junction Protein Levels. J Am Coll Surg. 2016 Jun; 222(6): 1009-17.
19. Kaliannan K, Hamarneh S R, Economopoulos K P, Nasrin Alam S, Moaven O, Patel P, et al. Intestinal alkaline phosphatase prevents metabolic syndrome in mice. Proc Natl Acad Sci U S A. 2013 Apr 23; 110(17): 7003-8.
20. Horie Y, Kato S, Ohki E, Tamai H, Ishii H. Role of endothelin in endotoxin-induced hepatic microvascular dysfunction in rats fed chronically with ethanol. J Gastroenterol Hepatol. 2001 Aug; 16(8): 916-22.
21. Vallon V, Mühlbauer B, Osswald H. Adenosine and kidney function. Physiol Rev. 2006 Jul; 86(3): 901-40.
22. Rentea R M, Liedel J L, Fredrich K, Welak S R, Pritchard K A, Jr., Oldham K T, et

al. Intestinal alkaline phosphatase administration in newborns decreases systemic inflammatory cytokine expression in a neonatal necrotizing enterocolitis rat model. J Surg Res. 2012 Oct; 177(2): 228-34.

23. Rentea R M, Liedel J L, Welak S R, Cassidy L D, Mayer A N, Pritchard K A, Jr., et al. Intestinal alkaline phosphatase administration in newborns is protective of gut barrier function in a neonatal necrotizing enterocolitis rat model. J Pediatr Surg. 2012 Jun; 47(6): 1135-42.
24. Li X, Atkinson M A. The role for gut permeability in the pathogenesis of type 1 diabetes--a solid or leaky concept? Pediatr Diabetes. 2015 Nov; 16(7): 485-92.
25. Peters E, Ergin B, Kandil A, Gurel-Gurevin E, van Elsas A, Masereeuw R, et al. Effects of a human recombinant alkaline phosphatase on renal hemodynamics, oxygenation and inflammation in two models of acute kidney injury. Toxicol Appl Pharmacol. 2016 Dec 15; 313: 88-96.
26. Bilski J, Mazur-Bialy A, Wojcik D, Zahradnik-Bilska J, Brzozowski B, Magierowski M, et al. The Role of Intestinal Alkaline Phosphatase in Inflammatory Disorders of Gastrointestinal Tract. Mediators Inflamm. 2017 2017: 9074601.
27. Schletter J, Heine H, Ulmer A J, Rietschel E T. Molecular mechanisms of endotoxin activity. Arch Microbiol. 1995 Dec; 164(6): 383-9.
28. Schauvliege R, Vanrobaeys J, Schotte P, Beyaert R. Caspase-11 gene expression in response to lipopolysaccharide and interferon-gamma requires nuclear factor-kappa B and signal transducer and activator of transcription (STAT) 1. J Biol Chem. 2002 Nov 1; 277(44): 41624-30.
29. Fujisaka S, Usui I, Nawaz A, Igarashi Y, Okabe K, Furusawa Y, et al. Bofutsushosan improves gut barrier function with a bloom of Akkermansia muciniphila and improves glucose metabolism in mice with diet-induced obesity. Sci Rep. 2020 Mar 26; 10(1): 5544.
30. Fuke N, Nagata N, Suganuma H, Ota T. Regulation of Gut Microbiota and Metabolic Endotoxemia with Dietary Factors. Nutrients. 2019 Sep 23; 11(10).
31. Kang M, Edmundson P, Araujo-Perez F, McCoy A N, Galanko J, Keku T O. Association of plasma endotoxin, inflammatory cytokines and risk of colorectal adenomas. BMC Cancer. 2013 Feb 26; 13: 91.

32. Millán J L. Alkaline Phosphatases : Structure, substrate specificity and functional relatedness to other members of a large superfamily of enzymes. Purinergic Signal. 2006 Jun; 2(2): 335-41.
33. Zimmermann H, Zebisch M, Sträter N. Cellular function and molecular structure of ecto-nucleotidases. Purinergic Signal. 2012 Sep; 8(3): 437-502.
34. Tsai L C, Hung M W, Chen Y H, Su W C, Chang G G, Chang T C. Expression and regulation of alkaline phosphatases in human breast cancer MCF-7 cells. Eur J Biochem. 2000 Mar; 267(5): 1330-9.
35. Weiss M J, Henthorn P S, Lafferty M A, Slaughter C, Raducha M, Harris H. Isolation and characterization of a cDNA encoding a human liver/bone/kidney-type alkaline phosphatase. Proc Natl Acad Sci U S A. 1986 Oct; 83(19): 7182-6.
36. Sharma U, Singh S K, Pal D, Khajuria R, Mandal A K, Prasad R. Implication of BBM lipid composition and fluidity in mitigated alkaline phosphatase activity in renal cell carcinoma. Mol Cell Biochem. 2012 Oct; 369(1-2): 287-93.
37. Calhau C, Martel F, Hipólito-Reis C, Azevedo I. Effect of P-glycoprotein modulators on alkaline phosphatase activity in cultured rat hepatocytes. Cell Physiol Biochem. 2000 10(4): 195-202.
38. Chang T C, Wang J K, Hung M W, Chiao C H, Tsai L C, Chang G G. Regulation of the expression of alkaline phosphatase in a human breast-cancer cell line. Biochem J. 1994 Oct 1; 303 (Pt 1)(Pt 1): 199-205.
39. Whyte M P, Landt M, Ryan L M, Mulivor R A, Henthorn P S, Fedde K N, et al. Alkaline phosphatase: placental and tissue-nonspecific isoenzymes hydrolyze phosphoethanolamine, inorganic pyrophosphate, and pyridoxal 5'-phosphate. Substrate accumulation in carriers of hypophosphatasia corrects during pregnancy. J Clin Invest. 1995 Apr; 95(4): 1440-5.
40. Sadeghirizi A, Yazdanparast R. Plasma membrane homing of tissue nonspecific alkaline phosphatase under the influence of 3-hydrogenkwadaphnin, an antiproliferative agent from Dendrostellera lessertii. Acta Biochim Pol. 2007 54(2): 323-9.
41. Stigbrand T. Present status and future trends of human alkaline phosphatases. Prog Clin Biol Res. 1984 166: 3-14.

42. Domar U, Nilsson B, Baranov V, Gerdes U, Stigbrand T. Expression of intestinal alkaline phosphatase in human organs. Histochemistry. 1992 Dec; 98(6): 359-64.
43. Fawley J, Gourlay D M. Intestinal alkaline phosphatase: a summary of its role in clinical disease. J Surg Res. 2016 May 1; 202(1): 225-34.
44. Estaki M, DeCoffe D, Gibson D L. Interplay between intestinal alkaline phosphatase, diet, gut microbes and immunity. World J Gastroenterol. 2014 Nov 14; 20(42): 15650-6.
45. Sussman N L, Eliakim R, Rubin D, Perlmutter D H, DeSchryver-Kecskemeti K, Alpers D H. Intestinal alkaline phosphatase is secreted bidirectionally from villous enterocytes. Am J Physiol. 1989 Jul; 257(1 Pt 1): G14-23.
46. Kühn F, Duan R, Ilmer M, Wirth U, Adiliaghdam F, Schiergens T S, et al. Targeting the Intestinal Barrier to Prevent Gut-Derived Inflammation and Disease: A Role for Intestinal Alkaline Phosphatase. Visceral Medicine. 2021 37(5): 383-393.
47. Chen K T, Malo M S, Moss A K, Zeller S, Johnson P, Ebrahimi F, et al. Identification of specific targets for the gut mucosal defense factor intestinal alkaline phosphatase. Am J Physiol Gastrointest Liver Physiol. 2010 Aug; 299(2): G467-75.
48. Cario E. Bacterial interactions with cells of the intestinal mucosa: Toll-like receptors and NOD2. Gut. 2005 Aug; 54(8): 1182-93.
49. Janssens S, Beyaert R. Role of Toll-like receptors in pathogen recognition. Clin Microbiol Rev. 2003 Oct; 16(4): 637-46.
50. Tuin A, Poelstra K, de Jager-Krikken A, Bok L, Raaben W, Velders M P, et al. Role of alkaline phosphatase in colitis in man and rats. Gut. 2009 Mar; 58(3): 379-87.
51. Riggle K M, Rentea R M, Welak S R, Pritchard K A, Jr., Oldham K T, Gourlay D M. Intestinal alkaline phosphatase prevents the systemic inflammatory response associated with necrotizing enterocolitis. J Surg Res. 2013 Mar; 180(1): 21-6.
52. Ghosh S S, Wang J, Yannie P J, Ghosh S. Intestinal Barrier Dysfunction, LPS Translocation, and Disease Development. J Endocr Soc. 2020 Feb 1; 4(2):

bvz039.

53. Park B S, Lee J O. Recognition of lipopolysaccharide pattern by TLR4 complexes. Exp Mol Med. 2013 Dec 6; 45(12): e66.
54. Moss A K, Hamarneh S R, Mohamed M M, Ramasamy S, Yammine H, Patel P, et al. Intestinal alkaline phosphatase inhibits the proinflammatory nucleotide uridine diphosphate. Am J Physiol Gastrointest Liver Physiol. 2013 Mar 15; 304(6): G597-604.
55. Zaher D M, El-Gamal M I, Omar H A, Aljareh S N, Al-Shamma S A, Ali A J, et al. Recent advances with alkaline phosphatase isoenzymes and their inhibitors. Arch Pharm (Weinheim). 2020 May; 353(5): e2000011.
56. Lallès J P. Intestinal alkaline phosphatase: multiple biological roles in maintenance of intestinal homeostasis and modulation by diet. Nutr Rev. 2010 Jun; 68(6): 323-32.
57. Moss W W, Webster W A. A numerical taxonomic study of a group of selected strongylates (Nematoda). Syst Zool. 1969 Dec; 18(4): 423-43.
58. Buchet R, Millán J L, Magne D. Multisystemic functions of alkaline phosphatases. Methods Mol Biol. 2013 1053: 27-51.
59. Brun L R, Brance M L, Lombarte M, Lupo M, Di Loreto V E, Rigalli A. Regulation of intestinal calcium absorption by luminal calcium content: role of intestinal alkaline phosphatase. Mol Nutr Food Res. 2014 Jul; 58(7): 1546-51.
60. Sasaki S, Segawa H, Hanazaki A, Kirino R, Fujii T, Ikuta K, et al. A Role of Intestinal Alkaline Phosphatase 3 (Akp3) in Inorganic Phosphate Homeostasis. Kidney Blood Press Res. 2018 43(5): 1409-1424.
61. Lukas M, Drastich P, Konecny M, Gionchetti P, Urban O, Cantoni F, et al. Exogenous alkaline phosphatase for the treatment of patients with moderate to severe ulcerative colitis. Inflamm Bowel Dis. 2010 Jul; 16(7): 1180-6.
62. Pickkers P, Heemskerk S, Schouten J, Laterre P F, Vincent J L, Beishuizen A, et al. Alkaline phosphatase for treatment of sepsis-induced acute kidney injury: a prospective randomized double-blind placebo-controlled trial. Crit Care. 2012 Jan 23; 16(1): R14.
63. Lallès J P. Intestinal alkaline phosphatase: novel functions and protective effects. Nutr Rev. 2014 Feb; 72(2): 82-94.

64. Heemskerk S, Masereeuw R, Moesker O, Bouw M P, van der Hoeven J G, Peters W H, et al. Alkaline phosphatase treatment improves renal function in severe sepsis or septic shock patients. Crit Care Med. 2009 Feb; 37(2): 417-23, e1.
65. Peters E, Stevens J, Arend J, Guan Z, Raaben W, Laverman P, et al. Biodistribution and translational pharmacokinetic modeling of a human recombinant alkaline phosphatase. Int J Pharm. 2015 Nov 10; 495(1): 122-131.
66. Alam S N, Yammine H, Moaven O, Ahmed R, Moss A K, Biswas B, et al. Intestinal alkaline phosphatase prevents antibiotic-induced susceptibility to enteric pathogens. Ann Surg. 2014 Apr; 259(4): 715-22.
67. Yang W H, Heithoff D M, Aziz P V, Sperandio M, Nizet V, Mahan M J, et al. Recurrent infection progressively disables host protection against intestinal inflammation. Science. 2017 Dec 22; 358(6370).
68. Heath M, Buckley R, Gerber Z, Davis P, Linneman L, Gong Q, et al. Association of Intestinal Alkaline Phosphatase With Necrotizing Enterocolitis Among Premature Infants. JAMA Netw Open. 2019 Nov 1; 2(11): e1914996.
69. Liu Y, Cavallaro P M, Kim B M, Liu T, Wang H, Kühn F, et al. A role for intestinal alkaline phosphatase in preventing liver fibrosis. Theranostics. 2021 11(1): 14-26.
70. Hamarneh S R, Kim B M, Kaliannan K, Morrison S A, Tantillo T J, Tao Q, et al. Intestinal Alkaline Phosphatase Attenuates Alcohol-Induced Hepatosteatosis in Mice. Dig Dis Sci. 2017 Aug; 62(8): 2021-2034.
71. Malo M S. A High Level of Intestinal Alkaline Phosphatase Is Protective Against Type 2 Diabetes Mellitus Irrespective of Obesity. EBioMedicine. 2015 Dec; 2(12): 2016-23.
72. Fiechter D, Kats S, Brands R, van Middelaar B, Pasterkamp G, de Kleijn D, et al. Bovine Intestinal Alkaline Phosphatase Reduces Inflammation After Induction of Acute Myocardial Infarction in Mice. Cardiol Res. 2011 Oct; 2(5): 236-242.
73. Malo J, Alam M J, Shahnaz M, Kaliannan K, Chandra G, Aziz T, et al. Intestinal Alkaline Phosphatase Deficiency Is Associated with Ischemic Heart Disease. Dis Markers. 2019 2019: 8473565.
74. Kuehn F, Adiliaghdam F, Hamarneh S R, Vasan R, Liu E, Liu Y, et al. Loss of

Intestinal Alkaline Phosphatase Leads to Distinct Chronic Changes in Bone Phenotype. J Surg Res. 2018 Dec; 232: 325-331.

75. Liebers V, Brüning T, Raulf M. Occupational endotoxin exposure and health effects. Arch Toxicol. 2020 Nov; 94(11): 3629-3644.
76. Kocíncová D, Lam J S. Structural diversity of the core oligosaccharide domain of Pseudomonas aeruginosa lipopolysaccharide. Biochemistry (Mosc). 2011 Jul; 76(7): 755-60.
77. Maldonado R F, Sá-Correia I, Valvano M A. Lipopolysaccharide modification in Gram-negative bacteria during chronic infection. FEMS Microbiol Rev. 2016 Jul; 40(4): 480-93.
78. Heine H, Rietschel E T, Ulmer A J. The biology of endotoxin. Mol Biotechnol. 2001 Nov; 19(3): 279-96.
79. Rietschel E T, Brade H. Bacterial endotoxins. Sci Am. 1992 Aug; 267(2): 54-61.
80. Rietschel E T, Kirikae T, Schade F U, Mamat U, Schmidt G, Loppnow H, et al. Bacterial endotoxin: molecular relationships of structure to activity and function. Faseb j. 1994 Feb; 8(2): 217-25.
81. Mazgaeen L, Gurung P. Recent Advances in Lipopolysaccharide Recognition Systems. Int J Mol Sci. 2020 Jan 7; 21(2).
82. Rietschel E T, Seydel U, Zähringer U, Schade U F, Brade L, Loppnow H, et al. Bacterial endotoxin: molecular relationships between structure and activity. Infect Dis Clin North Am. 1991 Dec; 5(4): 753-79.
83. Amarante-Mendes G P, Adjemian S, Branco L M, Zanetti L C, Weinlich R, Bortoluci K R. Pattern Recognition Receptors and the Host Cell Death Molecular Machinery. Front Immunol. 2018 9: 2379.
84. Li D, Wu M. Pattern recognition receptors in health and diseases. Signal Transduct Target Ther. 2021 Aug 4; 6(1): 291.
85. Walsh D, McCarthy J, O'Driscoll C, Melgar S. Pattern recognition receptors--molecular orchestrators of inflammation in inflammatory bowel disease. Cytokine Growth Factor Rev. 2013 Apr; 24(2): 91-104.
86. Raetz C R, Whitfield C. Lipopolysaccharide endotoxins. Annu Rev Biochem. 2002 71: 635-700.
87. Tan Y, Kagan J C. A cross-disciplinary perspective on the innate immune

responses to bacterial lipopolysaccharide. Mol Cell. 2014 Apr 24; 54(2): 212-23.

88. Meseguer V, Alpizar Y A, Luis E, Tajada S, Denlinger B, Fajardo O, et al. TRPA1 channels mediate acute neurogenic inflammation and pain produced by bacterial endotoxins. Nat Commun. 2014 5: 3125.
89. Hagar J A, Powell D A, Aachoui Y, Ernst R K, Miao E A. Cytoplasmic LPS activates caspase-11: implications in TLR4-independent endotoxic shock. Science. 2013 Sep 13; 341(6151): 1250-3.
90. Gurung P, Malireddi R K, Anand P K, Demon D, Vande Walle L, Liu Z, et al. Toll or interleukin-1 receptor (TIR) domain-containing adaptor inducing interferon-β (TRIF)-mediated caspase-11 protease production integrates Toll-like receptor 4 (TLR4) protein- and Nlrp3 inflammasome-mediated host defense against enteropathogens. J Biol Chem. 2012 Oct 5; 287(41): 34474-83.
91. Zu Y, Ping W, Deng T, Zhang N, Fu X, Sun W. Lipopolysaccharide-induced toll-like receptor 4 signaling in esophageal squamous cell carcinoma promotes tumor proliferation and regulates inflammatory cytokines expression. Dis Esophagus. 2017 Feb 1; 30(2): 1-8.
92. Andrews E J, Wang J H, Winter D C, Laug W E, Redmond H P. Tumor cell adhesion to endothelial cells is increased by endotoxin via an upregulation of beta-1 integrin expression. J Surg Res. 2001 May 1; 97(1): 14-9.
93. Torphy R J, Fujiwara Y, Schulick R D. Pancreatic cancer treatment: better, but a long way to go. Surg Today. 2020 Oct; 50(10): 1117-1125.
94. Neoptolemos J P, Kleeff J, Michl P, Costello E, Greenhalf W, Palmer D H. Therapeutic developments in pancreatic cancer: current and future perspectives. Nat Rev Gastroenterol Hepatol. 2018 Jun; 15(6): 333-348.
95. *World Health Organization. International Agency for Research on Cancer*. 2020; Available from: http://www.iarc.fr/.
96. Ducreux M, Seufferlein T, Van Laethem J L, Laurent-Puig P, Smolenschi C, Malka D, et al. Systemic treatment of pancreatic cancer revisited. Semin Oncol. 2019 Feb; 46(1): 28-38.
97. Kleeff J, Korc M, Apte M, La Vecchia C, Johnson C D, Biankin A V, et al. Pancreatic cancer. Nat Rev Dis Primers. 2016 Apr 21; 2: 16022.

98. Klein A P, Brune K A, Petersen G M, Goggins M, Tersmette A C, Offerhaus G J, et al. Prospective risk of pancreatic cancer in familial pancreatic cancer kindreds. Cancer Res. 2004 Apr 1; 64(7): 2634-8.
99. Amundadottir L, Kraft P, Stolzenberg-Solomon R Z, Fuchs C S, Petersen G M, Arslan A A, et al. Genome-wide association study identifies variants in the ABO locus associated with susceptibility to pancreatic cancer. Nat Genet. 2009 Sep; 41(9): 986-90.
100. Raimondi S, Maisonneuve P, Lowenfels A B. Epidemiology of pancreatic cancer: an overview. Nat Rev Gastroenterol Hepatol. 2009 Dec; 6(12): 699-708.
101. Wolpin B M, Chan A T, Hartge P, Chanock S J, Kraft P, Hunter D J, et al. ABO blood group and the risk of pancreatic cancer. J Natl Cancer Inst. 2009 Mar 18; 101(6): 424-31.
102. Blackford A, Parmigiani G, Kensler T W, Wolfgang C, Jones S, Zhang X, et al. Genetic mutations associated with cigarette smoking in pancreatic cancer. Cancer Res. 2009 Apr 15; 69(8): 3681-8.
103. Guo X, Cui Z. Current diagnosis and treatment of pancreatic cancer in China. Pancreas. 2005 Jul; 31(1): 13-22.
104. Pietryga J A, Morgan D E. Imaging preoperatively for pancreatic adenocarcinoma. J Gastrointest Oncol. 2015 Aug; 6(4): 343-57.
105. Zhang L, Sanagapalli S, Stoita A. Challenges in diagnosis of pancreatic cancer. World J Gastroenterol. 2018 May 21; 24(19): 2047-2060.
106. Uehara H, Iishi H, Tatsuta M. [Diagnosis of pancreatic cancer]. Nihon Rinsho. 2006 Jan; 64 Suppl 1: 101-6.
107. Bosetti C, Bertuccio P, Negri E, La Vecchia C, Zeegers M P, Boffetta P. Pancreatic cancer: overview of descriptive epidemiology. Mol Carcinog. 2012 Jan; 51(1): 3-13.
108. Oberstein P E, Olive K P. Pancreatic cancer: why is it so hard to treat? Therap Adv Gastroenterol. 2013 Jul; 6(4): 321-37.
109. Lambe M, Eloranta S, Wigertz A, Blomqvist P. Pancreatic cancer; reporting and long-term survival in Sweden. Acta Oncol. 2011 Nov; 50(8): 1220-7.
110. Adamska A, Domenichini A, Falasca M. Pancreatic Ductal Adenocarcinoma:

Current and Evolving Therapies. Int J Mol Sci. 2017 Jun 22; 18(7).

111. Grasso C, Jansen G, Giovannetti E. Drug resistance in pancreatic cancer: Impact of altered energy metabolism. Crit Rev Oncol Hematol. 2017 Jun; 114: 139-152.
112. Siegel R L, Miller K D, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018 Jan; 68(1): 7-30.
113. Burris H A, 3rd, Moore M J, Andersen J, Green M R, Rothenberg M L, Modiano M R, et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Oncol. 1997 Jun; 15(6): 2403-13.
114. Ychou M, Conroy T, Seitz J F, Gourgou S, Hua A, Mery-Mignard D, et al. An open phase I study assessing the feasibility of the triple combination: oxaliplatin plus irinotecan plus leucovorin/ 5-fluorouracil every 2 weeks in patients with advanced solid tumors. Ann Oncol. 2003 Mar; 14(3): 481-9.
115. Conroy T, Paillot B, François E, Bugat R, Jacob J H, Stein U, et al. Irinotecan plus oxaliplatin and leucovorin-modulated fluorouracil in advanced pancreatic cancer--a Groupe Tumeurs Digestives of the Federation Nationale des Centres de Lutte Contre le Cancer study. J Clin Oncol. 2005 Feb 20; 23(6): 1228-36.
116. Conroy T, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med. 2011 May 12; 364(19): 1817-25.
117. Zhu H, Li T, Du Y, Li M. Pancreatic cancer: challenges and opportunities. BMC Med. 2018 Nov 22; 16(1): 214.
118. Hammel P, Huguet F, van Laethem J L, Goldstein D, Glimelius B, Artru P, et al. Effect of Chemoradiotherapy vs Chemotherapy on Survival in Patients With Locally Advanced Pancreatic Cancer Controlled After 4 Months of Gemcitabine With or Without Erlotinib: The LAP07 Randomized Clinical Trial. Jama. 2016 May 3; 315(17): 1844-53.
119. Werner J, Combs S E, Springfeld C, Hartwig W, Hackert T, Büchler M W. Advanced-stage pancreatic cancer: therapy options. Nat Rev Clin Oncol. 2013 Jun; 10(6): 323-33.
120. Pappalardo A, Giunta E F, Tirino G, Pompella L, Federico P, Daniele B, et al.

Adjuvant Treatment in Pancreatic Cancer: Shaping the Future of the Curative Setting. Front Oncol. 2021 11: 695627.

121. Ikebe M, Kitaura Y, Nakamura M, Tanaka H, Yamasaki A, Nagai S, et al. Lipopolysaccharide (LPS) increases the invasive ability of pancreatic cancer cells through the TLR4/MyD88 signaling pathway. J Surg Oncol. 2009 Dec 15; 100(8): 725-31.
122. Jäger R, Weigel R, Forthuber B, Ganswindt U. Integrating radiation oncology into the management of pancreatic cancer. European Surgery. 2019 2019/06/01; 51(3): 139-145.
123. Hufnagel H, Bode C, Bode J C, Lehmann F G. Damage of rat small intestine induced by ethanol. Effect of ethanol on fecal excretion of intestinal alkaline phosphatase. Res Exp Med (Berl). 1980 178(1): 65-70.
124. Hodin R A, Graham J R, Meng S, Upton M P. Temporal pattern of rat small intestinal gene expression with refeeding. Am J Physiol. 1994 Jan; 266(1 Pt 1): G83-9.
125. Davis C D. Low dietary copper increases fecal free radical production, fecal water alkaline phosphatase activity and cytotoxicity in healthy men. J Nutr. 2003 Feb; 133(2): 522-7.
126. Taché S, Parnaud G, Van Beek E, Corpet D E. Polyethylene glycol, unique among laxatives, suppresses aberrant crypt foci, by elimination of cells. Scand J Gastroenterol. 2006 Jun; 41(6): 730-6.
127. Goldberg R F, Austen W G, Jr., Zhang X, Munene G, Mostafa G, Biswas S, et al. Intestinal alkaline phosphatase is a gut mucosal defense factor maintained by enteral nutrition. Proc Natl Acad Sci U S A. 2008 Mar 4; 105(9): 3551-6.
128. Wu X, Sun L, Zha W, Studer E, Gurley E, Chen L, et al. HIV protease inhibitors induce endoplasmic reticulum stress and disrupt barrier integrity in intestinal epithelial cells. Gastroenterology. 2010 Jan; 138(1): 197-209.
129. Ghosh S S, Bie J, Wang J, Ghosh S. Oral supplementation with non-absorbable antibiotics or curcumin attenuates western diet-induced atherosclerosis and glucose intolerance in LDLR-/- mice--role of intestinal permeability and macrophage activation. PLoS One. 2014 9(9): e108577.
130. Lallès J P. Intestinal Alkaline Phosphatase in Stool: A Novel Biomarker for

Metabolic Diseases. EBioMedicine. 2015 Dec; 2(12): 1866.

131. Little T J, Cvijanovic N, DiPatrizio N V, Argueta D A, Rayner C K, Feinle-Bisset C, et al. Plasma endocannabinoid levels in lean, overweight, and obese humans: relationships to intestinal permeability markers, inflammation, and incretin secretion. Am J Physiol Endocrinol Metab. 2018 Oct 1; 315(4): E489-e495.
132. Cani P D, Bibiloni R, Knauf C, Waget A, Neyrinck A M, Delzenne N M, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes. 2008 Jun; 57(6): 1470-81.
133. Ryz N R, Lochner A, Bhullar K, Ma C, Huang T, Bhinder G, et al. Dietary vitamin D3 deficiency alters intestinal mucosal defense and increases susceptibility to Citrobacter rodentium-induced colitis. Am J Physiol Gastrointest Liver Physiol. 2015 Nov 1; 309(9): G730-42.
134. Parnell J A, Klancic T, Reimer R A. Oligofructose decreases serum lipopolysaccharide and plasminogen activator inhibitor-1 in adults with overweight/obesity. Obesity (Silver Spring). 2017 Mar; 25(3): 510-513.
135. Kikuchi K, Ben Othman M, Sakamoto K. Sterilized bifidobacteria suppressed fat accumulation and blood glucose level. Biochem Biophys Res Commun. 2018 Jul 2; 501(4): 1041-1047.
136. Abboud K Y, Reis S K, Martelli M E, Zordão O P, Tannihão F, de Souza A Z Z, et al. Oral Glutamine Supplementation Reduces Obesity, Pro-Inflammatory Markers, and Improves Insulin Sensitivity in DIO Wistar Rats and Reduces Waist Circumference in Overweight and Obese Humans. Nutrients. 2019 Mar 1; 11(3).
137. Landete J M, Gaya P, Rodríguez E, Langa S, Peirotén Á, Medina M, et al. Probiotic Bacteria for Healthier Aging: Immunomodulation and Metabolism of Phytoestrogens. Biomed Res Int. 2017 2017: 5939818.
138. Sovran B, Hugenholtz F, Elderman M, Van Beek A A, Graversen K, Huijskes M, et al. Age-associated Impairment of the Mucus Barrier Function is Associated with Profound Changes in Microbiota and Immunity. Sci Rep. 2019 Feb 5; 9(1): 1437.
139. Nakaoka K, Yamada A, Noda S, Goseki-Sone M. Vitamin D-restricted high-fat

diet down-regulates expression of intestinal alkaline phosphatase isozymes in ovariectomized rats. Nutr Res. 2018 May; 53: 23-31.

140. Biesterveld B E, Koehler S M, Heinzerling N P, Rentea R M, Fredrich K, Welak S R, et al. Intestinal alkaline phosphatase to treat necrotizing enterocolitis. J Surg Res. 2015 Jun 15; 196(2): 235-40.
141. Lum G, Marquardt C, Khuri S F. Hypomagnesemia and low alkaline phosphatase activity in patients' serum after cardiac surgery. Clin Chem. 1989 Apr; 35(4): 664-7.
142. Davidson J, Tong S, Hauck A, Lawson D S, Jaggers J, Kaufman J, et al. Alkaline phosphatase activity after cardiothoracic surgery in infants and correlation with post-operative support and inflammation: a prospective cohort study. Crit Care. 2012 Aug 20; 16(4): R160.
143. Neutze J M, Drakeley M J, Barratt-Boyes B G. Serum enzymes after cardiac surgery under profound hypothermia with circulatory arrest and limited cardiopulmonary bypass. Am Heart J. 1974 Nov; 88(5): 553-6.
144. Khailova L, Robison J, Jaggers J, Ing R, Lawson S, Treece A, et al. Tissue alkaline phosphatase activity and expression in an experimental infant swine model of cardiopulmonary bypass with deep hypothermic circulatory arrest. J Inflamm (Lond). 2020 17: 27.
145. Kiffer-Moreira T, Sheen C R, Gasque K C, Bolean M, Ciancaglini P, van Elsas A, et al. Catalytic signature of a heat-stable, chimeric human alkaline phosphatase with therapeutic potential. PLoS One. 2014 9(2): e89374.
146. *Clinical Trials. gov*. 2021; Available from: https://clinicaltrials.gov/ct2/home.
147. Kats S, Brands R, Seinen W, de Jager W, Bekker M W, Hamad M A, et al. Anti-inflammatory effects of alkaline phosphatase in coronary artery bypass surgery with cardiopulmonary bypass. Recent Pat Inflamm Allergy Drug Discov. 2009 Nov; 3(3): 214-20.
148. Whitehouse J S, Riggle K M, Purpi D P, Mayer A N, Pritchard K A, Jr., Oldham K T, et al. The protective role of intestinal alkaline phosphatase in necrotizing enterocolitis. J Surg Res. 2010 Sep; 163(1): 79-85.
149. Li Y, Guan W, Ma S, Lin S, Yang N, Liu R, et al. Lipopolysaccharide and inflammatory cytokines levels decreased after sleeve gastrectomy in Chinese

adults with obesity. Endocr J. 2019 Apr 25; 66(4): 337-347.

150. Trøseid M, Nestvold T K, Rudi K, Thoresen H, Nielsen E W, Lappegård K T. Plasma lipopolysaccharide is closely associated with glycemic control and abdominal obesity: evidence from bariatric surgery. Diabetes Care. 2013 Nov; 36(11): 3627-32.
151. Cottam D R, Schaefer P A, Shaftan G W, Velcu L, Angus L D. Effect of surgically-induced weight loss on leukocyte indicators of chronic inflammation in morbid obesity. Obes Surg. 2002 Jun; 12(3): 335-42.
152. Zhang M, Zhang Y H, Fu H Q, Zhang Q M, Wang T L. Ulinastatin May Significantly Improve Postoperative Cognitive Function of Elderly Patients Undergoing Spinal Surgery by Reducing the Translocation of Lipopolysaccharide and Systemic Inflammation. Front Pharmacol. 2018 9: 1007.
153. Buford T W. (Dis)Trust your gut: the gut microbiome in age-related inflammation, health, and disease. Microbiome. 2017 Jul 14; 5(1): 80.
154. Jackson A, Forsyth C B, Shaikh M, Voigt R M, Engen P A, Ramirez V, et al. Diet in Parkinson's Disease: Critical Role for the Microbiome. Front Neurol. 2019 10: 1245.
155. Loffredo L, Ivanov V, Ciobanu N, Deseatnicova E, Gutu E, Mudrea L, et al. Is There an Association Between Atherosclerotic Burden, Oxidative Stress, and Gut-Derived Lipopolysaccharides? Antioxid Redox Signal. 2020 May 18.
156. Lubomski M, Tan A H, Lim S Y, Holmes A J, Davis R L, Sue C M. Parkinson's disease and the gastrointestinal microbiome. J Neurol. 2020 Sep; 267(9): 2507-2523.
157. Shyamali N L A, Mahapatuna S D, Gomes L, Wijewickrama A, Ogg G S, Malavige G N. Risk Factors for Elevated Serum Lipopolysaccharide in Acute Dengue and Association with Clinical Disease Severity. Trop Med Infect Dis. 2020 Nov 16; 5(4).
158. Terawaki H, Yokoyama K, Yamada Y, Maruyama Y, Iida R, Hanaoka K, et al. Low-grade endotoxemia contributes to chronic inflammation in hemodialysis patients: examination with a novel lipopolysaccharide detection method. Ther Apher Dial. 2010 Oct; 14(5): 477-82.

159. Wong J, Zhang Y, Patidar A, Vilar E, Finkelman M, Farrington K. Is Endotoxemia in Stable Hemodialysis Patients an Artefact? Limitations of the Limulus Amebocyte Lysate Assay and Role of (1→3)-β-D Glucan. PLoS One. 2016 11(10): e0164978.
160. Fulenwider J T, Sibley C, Stein S F, Evatt B, Nordlinger B M, Ivey G L. Endotoxemia of cirrhosis: an observation not substantiated. Gastroenterology. 1980 May; 78(5 Pt 1): 1001-4.
161. Fukui H. Gut-liver axis in liver cirrhosis: How to manage leaky gut and endotoxemia. World J Hepatol. 2015 Mar 27; 7(3): 425-42.
162. Huang Y, Wang L, Sha L, Wang Y, Duan X, Li G. Highly sensitive detection of lipopolysaccharide based on collaborative amplification of dual enzymes. Anal Chim Acta. 2020 Aug 22; 1126: 31-37.
163. Roslansky P F, Novitsky T J. Sensitivity of Limulus amebocyte lysate (LAL) to LAL-reactive glucans. J Clin Microbiol. 1991 Nov; 29(11): 2477-83.
164. Seki N, Muta T, Oda T, Iwaki D, Kuma K, Miyata T, et al. Horseshoe crab (1,3)-beta-D-glucan-sensitive coagulation factor G. A serine protease zymogen heterodimer with similarities to beta-glucan-binding proteins. J Biol Chem. 1994 Jan 14; 269(2): 1370-4.
165. Lim S K, Chen P, Lee F L, Moochhala S, Liedberg B. Peptide-assembled graphene oxide as a fluorescent turn-on sensor for lipopolysaccharide (endotoxin) detection. Anal Chem. 2015 Sep 15; 87(18): 9408-12.
166. Su W, Ding X. Methods of Endotoxin Detection. J Lab Autom. 2015 Aug; 20(4): 354-64.
167. Tombelli S, Minunni M, Mascini M. Analytical applications of aptamers. Biosens Bioelectron. 2005 Jun 15; 20(12): 2424-34.
168. Yoo E H, Lee S Y. Glucose biosensors: an overview of use in clinical practice. Sensors (Basel). 2010 10(5): 4558-76.
169. Yeo T Y, Choi J S, Lee B K, Kim B S, Yoon H I, Lee H Y, et al. Electrochemical endotoxin sensors based on TLR4/MD-2 complexes immobilized on gold electrodes. Biosens Bioelectron. 2011 Oct 15; 28(1): 139-45.
170. da Silva J S, Oliveira M D, de Melo C P, Andrade C A. Impedimetric sensor of bacterial toxins based on mixed (Concanavalin A)/polyaniline films. Colloids

Surf B Biointerfaces. 2014 May 1; 117: 549-54.

171. Li B R, Chen C C, Kumar U R, Chen Y T. Advances in nanowire transistors for biological analysis and cellular investigation. Analyst. 2014 Apr 7; 139(7): 1589-608.
172. Leva-Bueno J, Peyman S A, Millner P A. A review on impedimetric immunosensors for pathogen and biomarker detection. Med Microbiol Immunol. 2020 Jun; 209(3): 343-362.
173. Oliveira M D, Andrade C A, Correia M T, Coelho L C, Singh P R, Zeng X. Impedimetric biosensor based on self-assembled hybrid cystein-gold nanoparticles and CramoLL lectin for bacterial lipopolysaccharide recognition. J Colloid Interface Sci. 2011 Oct 1; 362(1): 194-201.
174. Sondhi P, Maruf M H U, Stine K J. Nanomaterials for Biosensing Lipopolysaccharide. Biosensors (Basel). 2019 Dec 21; 10(1).
175. Zhang Q, Ma C, Duan Y, Heinrich B, Rosato U, Diggs L P, et al. Gut Microbiome Directs Hepatocytes to Recruit MDSCs and Promote Cholangiocarcinoma. Cancer Discov. 2021 May; 11(5): 1248-1267.
176. Fan X, Alekseyenko A V, Wu J, Peters B A, Jacobs E J, Gapstur S M, et al. Human oral microbiome and prospective risk for pancreatic cancer: a population-based nested case-control study. Gut. 2018 Jan; 67(1): 120-127.
177. Torres P J, Fletcher E M, Gibbons S M, Bouvet M, Doran K S, Kelley S T. Characterization of the salivary microbiome in patients with pancreatic cancer. PeerJ. 2015 3: e1373.
178. Michaud D S, Izard J, Wilhelm-Benartzi C S, You D H, Grote V A, Tjønneland A, et al. Plasma antibodies to oral bacteria and risk of pancreatic cancer in a large European prospective cohort study. Gut. 2013 Dec; 62(12): 1764-70.
179. Tao M, Liu L, Shen M, Zhi Q, Gong F R, Zhou B P, et al. Inflammatory stimuli promote growth and invasion of pancreatic cancer cells through NF-κB pathway dependent repression of PP2Ac. Cell Cycle. 2016 15(3): 381-93.

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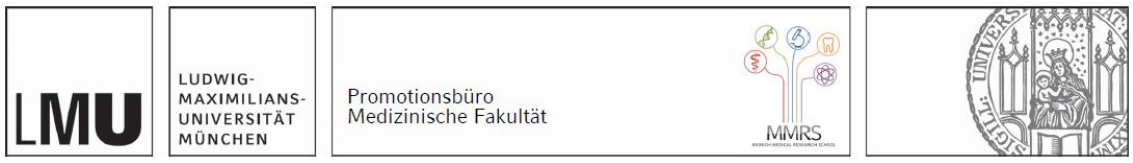
Affidavit



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I hereby declare, that the submitted thesis entitled:

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Signature doctoral candidate

List of publications

Kühn F, Duan R, Ilmer M, Wirth U, Adiliaghdam F, Schiergens T, S, Andrassy J, Bazhin A, V, Werner J: Targeting the Intestinal Barrier to Prevent Gut-Derived Inflammation and Disease: A Role for Intestinal Alkaline Phosphatase. Visc Med 2021. doi: 10.1159/000515910