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Interplay Between Gut Homeostasis-Regulating Enzyme Intestinal Alkaline Phosphatase and Bacterial Lipopolysaccharides in Patients undergoing Abdominal Surgery for Cancer

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Qiang Li
aus
Henan, Volksrepublik China

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Ludwig-Maximilians-Universität zu München

Erstes Gutachten:	Prof. Dr. med. Florian Kühn
Zweites Gutachten:	Priv. Doz. Dr. med. Christian Schulz
Drittes Gutachten:	Priv. Doz. Dr. Dr. Patrick Scheiermann

Mitbetreuung durch den Dekan:	Prof. Dr. med. Thomas Gudermann

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Zusammenfassung

Hintergrund: Die Intestinale alkalische Phosphatase (IAP) ist entscheidend für die Aufrechterhaltung der intestinalen Homöostase, indem sie bakterielle Populationen reguliert, die Darmbarriere stärkt und proinflammatorische Moleküle wie Lipopolysaccharide (LPS) dephosphoryliert. LPS, ein Virulenzfaktor gramnegativer Bakterien, ist mit verschiedenen Tumorentitäten (Pankreas, Dickdarm, Leber) und allgemein schlechteren chirurgischen Ergebnissen im postoperativen Verlauf assoziiert. Diese Studie untersucht das Zusammenspiel von IAP und LPS sowie deren therapeutisches und prädiktives Potenzial nach abdominalen Tumorresektionen.

Studiendesign: Prä- und postoperative Blut-, Stuhl- und Darmproben wurden von 118 Patienten am LMU Klinikum München entnommen, die sich einer abdominellen Operation unterzogen. Davon hatten 91 Patienten eine Tumorerkrankung. Zusätzlich dienten 8 Blutproben von gesunden Personen als Kontrollen. Die IAP-Aktivität wurde im Stuhl und im Serum mit der para-Nitrophenylphosphat (pNPP)-Methode oder mit dem humanen IAP-ELISA-Test analysiert. Mit dem Limulus-Amebozyten-Lysat-Assay (LAL) wurde der LPS-Gehalt im Serum gemessen.

Ergebnisse: Unsere Studie zeigte eine signifikante negative Korrelation zwischen präoperativer Stuhl-IAP und präoperativem Serum-LPS ($r=-0,514$, $p=0,029$). Es zeigte sich eine positive Korrelation zwischen präoperativer Stuhl- und Serum-IAP ($r=0,524$, $p=0,040$), während die präoperative Serum-IAP eine negative Korrelation mit Serum-LPS aufwies ($r=-0,397$, $p<0,001$). Präoperative Serum-IAP und LPS in Verbindung mit verschiedenen klinischen Parametern ergab, dass höhere präoperative Serum-IAP-Werte negativ mit IL-6 ($r=-0,334$, $p=0,020$) und CRP ($r=-0,348$, $p=0,001$) und positiv mit Albumin ($r=0,425$, $p<0,001$) korrelierten. Patienten mit Pankreas- ($p=0,0144$) und Kolorektaltumor (CRC) ($p=0,0349$) zeigten niedrigere präoperative Serum-IAP-Werte, während sich bei Lebertumoren im Vergleich zu gesunden Kontrollen kein signifikanter Unterschied ergab ($p=0,0984$). Bei allen abdominalchirurgischen Patienten ($p=0,0071$) und insbesondere bei allen Tumorresektionen ($p=0,0128$) konnte eine signifikante postoperative Reduktion der Serum-LPS-Werte im Vergleich zu den präoperativen Werten nachgewiesen werden, was einen potenziellen Einfluss der Operationen auf die LPS-Werte zeigt.

Höhere präoperative Serum-IAP-Werte waren mit einem günstigeren postoperativen Verlauf (Clavien-Dindo ≤ II) nach abdominalen Eingriffen assoziiert (p=0,0016). Eine ROC-Analyse identifizierte einen Schwellenwert der präoperativen Serum-IAP, der chirurgische Ergebnisse effektiv vorhersagen könnte (AUC=0,749; Youden-Index=0,4620). Höhere präoperative Serum-IAP-Werte korrelierten mit besseren chirurgischen Ergebnissen und kürzeren Aufenthalten auf der Intensivstation bei Tumorpatienten (r=-0,375, p=0,012).

Schlussfolgerung: Serum-IAP könnte als zusätzlicher Marker zur Vorhersage chirurgischer Ergebnisse und zur Steuerung des perioperativen Managements dienen. Weitere Studien sind erforderlich, um die Rolle der IAP als möglicher Prädiktor chirurgischer Ergebnisse zu untersuchen.

Abstract

Background: Intestinal alkaline phosphatase (IAP) is a key player in preserving gut health, regulating bacterial populations, promoting gut barrier integrity, and dephosphorylating proinflammatory molecules like lipopolysaccharides (LPS). LPS, a virulence factor in gram-negative bacteria, triggers inflammatory responses. It is associated with various cancers (such as pancreatic, colorectal, and liver cancer) and negatively contributes to surgical outcomes (sepsis, postoperative cognitive function). IAP's potential in pancreatic, colorectal, and liver health is a reassuring aspect of our research, with current studies highlighting its critical interplay and potential therapeutic implications across diverse health conditions.

Study Design: The study encompassed 118 patients from the Department of General, Visceral and Transplant Surgery at Ludwig-Maximilians-University between June 22nd, 2022, and September 15th, 2023. Among them, 91 had cancer (25 pancreatic, 21 colorectal, 14 liver, 31 other adenocarcinomas), and 27 had non-cancer cases. Additionally, 8 blood samples from healthy individuals served as controls. We conducted the Para-Nitrophenyl Phosphate (PNPP) method to assess the stool IAP activity of 23 preoperative samples. Additionally, we utilized the Limulus Amebocyte Lysate (LAL) assay to measure serum LPS levels of 113 preoperative and 35 postoperative samples and the Human IAP ELISA assay to quantify serum IAP concentrations of 83 preoperative samples.

Results: Our study revealed a significant negative correlation between preoperative stool IAP and preoperative serum LPS ($r=-0.514$, $p=0.029$), reinforcing previous findings in pancreatic cancer patients and the entire study population. Moreover, a positive correlation between preoperative stool and serum IAP emerged ($r=0.524$, $p=0.040$), while preoperative serum IAP displayed a negative correlation with serum LPS ($r=-0.397$, $p<0.001$). The analysis of preoperative serum IAP and LPS alongside various clinical parameters showed that preoperative serum IAP values displayed a negative association with preoperative IL-6 levels ($r=-0.334$, $p=0.020$) and CRP ($r=-0.348$, $p=0.001$), as well as a positive relationship with albumin ($r=0.425$, $p<0.001$).

Preoperative serum IAP levels were notably lower in the cancer group compared to healthy controls ($p=0.0012$). Preoperative serum IAP levels were lower in patients diagnosed with pancreatic ($p=0.0144$) and colorectal cancer (CRC) ($p = 0.0349$). However, no significant differences in preoperative serum IAP were detected when comparing liver cancer and healthy controls ($p=0.0984$). In all abdominal surgery patients ($p=0.0071$) and

all cancer resected patients (p=0.0128), respectively, a significant reduction in postoperative serum LPS levels compared to preoperative levels was observed, indicating a potential impact of surgeries on LPS levels.

We showed that higher preoperative serum IAP levels were associated with a favorable outcome (Clavien-Dindo ≤ II) following abdominal surgery ($p = 0.0016$). A ROC analysis identified a cutoff value for preoperative serum IAP that could predict surgical outcomes effectively (AUC = 0.749; Youden-Index = 0.4620). Higher preoperative serum IAP levels correlated with better abdominal surgical outcomes, also indicated by shorter intensive care unit (ICU) stays ($r = -0.375$, $p = 0.012$).

Among patients undergoing surgery for all cancer resection, preoperative serum IAP values were lower in the Clavien-Dindo > II, in which III or higher is relevant to complications where intervention is required, compared to the Clavien-Dindo II or lower group ($p = 0.0096$). Notably, a negative correlation existed between preoperatively IAP and hospital stay ($r = -0.437$, $p < 0.001$) in cancer resection patients. There was a negative correlation between preoperative serum IAP and the length of ICU stay among cancer patients ($r = -0.413$, $p = 0.008$). Among liver cancer patients undergoing surgery, our study identified a significant negative correlation between preoperative serum IAP levels and hospital stay ($r = -0.603$, $p = 0.038$). Additionally, when comparing patients based on ICU admission following liver cancer surgeries, those admitted ($p = 0.0485$).

Conclusion: Our study emphasizes the potential of preoperative serum IAP as a valuable biomarker for predicting surgical outcomes, particularly in abdominal and cancer resection surgeries. Higher preoperative serum IAP levels were associated with more favorable outcomes, lower Clavien-Dindo grade, and shorter ICU and hospital stays, while lower IAP levels were linked to higher serum LPS and elevated inflammatory markers, such as IL-6 and CRP. This suggests that serum IAP could serve as a critical prognostic marker, particularly in predicting recovery and complications after abdominal and cancer surgeries. Additionally, while LPS levels did not predict surgical outcomes as strongly as IAP, their significant postoperative reduction highlights their relevance in post-surgical inflammation and gut integrity. Further studies are needed to refine the clinical application of both biomarkers in managing postoperative complications.

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List of Abbreviations

Abbreviation	Full Name
AEG	adenocarcinoma of the gastroesophageal junction
AJs	adherens junctions
Alb	albumin
ALP	alkaline phosphatase
AMI	acute myocardial infarction
ASA	American Society of Anesthesiologists
AST	aspartate transaminase
Barthel cog	Barthel cognitive score
Barthel mot	Barthel motility score
BCA	bicinchoninic acid
BMI	body mass index
BSA	bovine serum albumin
Ca	calcium
CA19-9	carbohydrate antigen 19-9
CD	Crohn's disease
CEA	carcinoembryonic antigen
CRC	colorectal cancer
Crea	creatinine
CRP	C-reactive protein
DPBS	Dulbecco's phosphate- buffered saline
DSV	desulfovibrio vulgaris
ECM	extracellular matrix
EU	endotoxin unit
FBG	fasting blood glucose
GCAP	germ cell alkaline phosphatase
GFR	glomerular filtration rate
Hb	hemoglobin
HCC	hepatocellular carcinoma
HCl	hydrochloride
IAP	intestinal alkaline phosphatase
IBD	inflammatory bowel disease
ICU	Intensive Care Unit
IECs	intestinal epithelial cells
IHD	ischemic heart disease
IL	interleukin
IL-6	interleukin 6
IPMN	intraductal papillary mucinous neoplasm
IQR	interquartile range
LAL	Limulus amebocyte lysate
LBP	LPS-binding protein
LPS	lipopolysaccharides
LSECs	liver sinusoidal endothelial cells
MDSC	myeloid-derived suppressive cells
MgCl2	magnesium chloride
min	minutes
ml	*milliliter*
mM	millimole per liter
NEC	necrotizing enterocolitis

NF-κB	nuclear factor kappa B
ng	nanogram
nm	nanometer
OD	optical density
p	p-value
PD	pancreaticoduodenectomy
PD-1	programmed cell death-1
PD-L1	programmed cell death ligand-1
PH	potential of hydrogen
Pi	phosphate
PLAP	placental alkaline phosphatase
PNPP	Para-Nitrophenyl Phosphate
Preop	preoperative
ROC	receiver operated characteristics
SD	standard deviation
Tbil	total bilirubin
T1D	type 1 diabetes
TEER	transepithelial electric resistance
TJs	tight junctions
TLR2	toll-like receptor 2
TLR4	Toll-like receptor 4
TNAP	tissue nonspecific alkaline phosphatase
TNF	tumor necrosis factor
TNF-α	tumor necrosis factor-α
TRAIL	TNF-related apoptosis inducing ligand
U	unit
UC	ulcerative colitis
UICC	Union for International Cancer Control
vs	versus
WBC	white blood cell
ZnCl2	zinc chloride
ZO	zonula occludens
µg	microgram
µl	microliter
µM	micromole per liter
%	percentage
°C	degree celsius

1. Introduction

1.1 Intestinal Alkaline Phosphatase (IAP)

1.1.1 Alkaline Phosphatase

Alkaline phosphatases (ALP) are membrane-bound glycoproteins that catalyze the hydrolysis of phosphate monoesters at basic pH values [1]. They trigger cellular responses, leading to significant physiological and immunological changes [1, 2]. ALP includes tissue nonspecific alkaline phosphatase (TNAP) and three tissue-specific forms: placental alkaline phosphatase (PLAP), germ cell alkaline phosphatase (GCAP), and intestinal alkaline phosphatase (IAP) [3-6]. TNAP is mainly found in the liver, bone, and kidney and is associated with hypophosphatasia [7], a rare genetic disorder characterized by the abnormal development of bones and teeth [8]. PLAP is abundant in the placenta, while GCAP shares similarities with PLAP and can be re-expressed in cancer cells [5, 6]. IAP is primarily found in the gastrointestinal tract, particularly in the duodenum, and its significance in inflammatory conditions is notable [9]. In the context of Alzheimer's disease, IAP has been found to In Alzheimer's disease, intestinal alkaline phosphatase (IAP) has been shown to play a regulatory role in the metabolism of amyloid-beta, a protein that forms plaques in the brains of Alzheimer's patients [10, 11] . Oral IAP supplementation could present a novel and safe approach to preventing human inflammatory and age-related diseases [9].

1.1.2 IAP and Its Function

IAP, a product of enterocytes in the duodenum, plays a crucial role in our body's natural defense mechanisms [12]. It regulates calcium, phosphate, and lipid absorption and dephosphorylates proinflammatory molecules [9, 13, 14]. Moreover, IAP acts as a guardian of gut barrier integrity, protecting against chronic inflammation and helping maintain gut homeostasis [15, 16].

Working Mechanisms of IAP

Support the preservation of gut barrier integrity.

The structural components of tight junctions (TJs) and adherens junctions (AJs) within the mucosal layer are pivotal for maintaining the integrity of the gut barrier. [15, 17]. TJs comprise proteins such as claudin, occludin, and zonula occludens (ZO)-1, whereas AJs

contain cadherins, α-catenin, β-catenin, and afadin [18]. Dysfunction in TJs can lead to a "leaky gut" associated with inflammatory diseases [19]. Barrier integrity assessment involves measurements such as transepithelial electric resistance (TEER) and paracellular permeability determined by 4kDa FITC-Dextran flux. Studies suggest that Desulfovibrio vulgaris (DSV) has the potential to disrupt TJs by decreasing TEER, elevating FITC-flux, up-regulating snail protein expression, inducing nuclear translocation of snail, and disrupting occludin staining at the junctions.

Nevertheless, pretreatment with IAP hinders DSV-induced paracellular permeability, snail expression, and occludin staining disruption [16]. Research indicates that the lack of IAP markedly decreases the expression levels of tight junction proteins in intestinal tissues [9]In IAP knockout mice, there are reduced levels of ZO-1, ZO-2, and occludin, while the overexpression of the IAP gene results in elevated mRNA levels of ZO-1 and ZO-2 in Caco-2 and T84 cells. Additionally, the introduction of 'exogenous IAP,' which refers to IAP that is produced outside the body and then administered, stimulates the expression of claudin1, ZO-1, and ZO-3 in fed mice and significantly increases the levels of claudin1, occludin, ZO-1, ZO-2, and ZO-3 in starved mice. [20, 21].

Dephosphorylation

IAP regulates intestinal bacterial populations and inflammatory status through its dephosphorylation activity. IAP operates with other dephosphorylated nucleotides, including adenosine diphosphate and adenosine monophosphate [22]Besides, lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteri, initiates a type of inflammation known as 'systemic inflammation' by activating the Toll-like receptor 4 (TLR4) pathway. Systemic inflammation is a widespread chronic inflammation affecting multiple organ systems. It is often associated with various diseases, including sepsis, rheumatoid arthritis, and inflammatory bowel disease. [23]. The lipid A component of LPS is crucial for its binding to TLR4, leading to toxicity. The lipid-A segment includes crucial phosphate groups. ALP, including IAP, is vital in removing these phosphate groups. As a result, this activity impedes the binding of LPS to TLRs, ultimately reducing the activity of LPS. [24].

Regulation of intestinal PH

IAPs actively contribute to regulating the alkaline pH in the duodenum through the ecto-purinergic pH regulatory system, which includes the interplay between IAP and P2Y receptors. ATP activates P2Y receptors, stimulating the secretion of bicarbonate. Inhibition of IAP increases ATP concentration, further activating P2Y receptors and enhancing bicarbonate production. Therefore, bicarbonate neutralizes the acidic intestinal environment by reacting with H+ ions, producing carbon dioxide. Conversely, an increased duodenal surface pH can potentially boost IAP activity. The increased IAP activity facilitates the degradation of ATP, reducing ATP levels. Consequently, this decrease in ATP levels decreases the stimulation of P2Y receptors, resulting in reduced bicarbonate release. This mechanism forms a negative feedback loop that helps regulate and maintain duodenal pH. [25, 26]

1.1.3 IAP against Various Diseases from Different Organs

The administration of exogenous IAP protects against inflammation in the intestines and the systemic environment across different diseases, suggesting its potential as a therapeutic agent. [24] Significantly, IAP has been safely administered to humans, and the progress in developing human recombinant forms of IAP represents a notable advancement. The human recombinant IAP is currently in phase 2 clinical trials, emphasizing the crucial role of this enzyme in therapeutic applications [24].

IAP and the Connection to Pancreatic Disease

Recent research has underscored the contribution of IAP in preventing metabolic syndrome induced by a high-fat diet in mice. Both endogenous and orally supplemented IAP hinder the absorption of LPS linked to dietary fat. Oral IAP supplementation prevents and reverses metabolic syndrome in mice, showcasing promising therapeutic potential. Furthermore, supplementation with IAP improves the lipid profile in mice following a standard, low-fat chow diet. These results indicate a distinctive therapeutic approach for individuals at risk of metabolic syndrome [27].

Additionally, increased IAP levels exhibit protective effects against diabetes, regardless of obesity. The concept of a 'temporal IAP profile' emerges as a valuable predictive tool for detecting the initial stages of metabolic syndrome, including early diabetes [28]. Furthermore, studies suggest that heightened intestinal permeability is an inherent characteristic of type 1 diabetes (T1D) in both human subjects and animal models. IAP’s ability to

alleviate intestinal permeability represents a potential therapeutic intervention targeting the gut-pancreatic axis in T1D [29].

IAP contributes to mitigating metabolic syndrome, holds potential as a predictive tool for early diabetes, and offers therapeutic implications for addressing intestinal permeability in type 1 diabetes.

IAP and the connection to Colorectal Disease

Given the crucial function of IAP in safeguarding the gut barrier, it represents a promising therapeutic candidate for enhancing outcomes in inflammatory diseases associated with gut barrier dysfunction. This includes inflammatory bowel disease (IBD), which comprises Crohn's disease (CD) and ulcerative colitis (UC) [24].

Inflammation in the intestine, linked to TLR4 mechanisms and characterized by IAP desialylation resulting in the buildup of LPS-phosphate, was mitigated by the administration of IAP or the antiviral neuraminidase inhibitor, zanamivir. This therapeutic strategy maintained the abundance and functionality of IAP [30]. Mice receiving oral supplementation of calf IAP during antibiotic treatment demonstrated effective and adequate resistance to infections caused by S. Typhimurium and C. difficile. The supplemented animals preserved their weight, exhibited diminished clinical severity and gut inflammation, were shielded from antibiotic-associated diarrhea and C. difficile-associated disease, and displayed enhanced survival rates [31]. In studies involving rats with intestinal necrotizing enterocolitis (NEC) injury, increased doses of supplemental enteral IAP reduced the expression of proinflammatory cytokines such as interleukin (IL)-1β, IL-6, and TNF-α. Early enteral IAP supplementation may decrease NEC-related injury, counteract the effects of a proinflammatory cascade, and preserve intestinal epithelial barrier function [32, 33].

IAP and the Connection to Liver Disease

IAP is crucial in detoxifying LPS, maintaining intestinal tight junction proteins, and preserving gut barrier integrity. Liver fibrosis often correlates with gut barrier dysfunction, with the LPS-TLR4 pathway implicated in both conditions. During liver fibrosis, decreased endogenous IAP levels might contribute to gut barrier dysfunction, exacerbating fibrosis. Supplementing orally with IAP safeguards the gut barrier and alleviates the progression of liver fibrosis through a mechanism mediated by TLR4 [34].

Bacterially derived components from the gut significantly activate inflammatory pathways in the liver, contributing to the pathogenesis of alcoholic liver disease. IAP effectively detoxifies various bacterial pro-inflammatory factors. Administering IAP protects mice from alcohol-induced hepatotoxicity and steatosis. As a result, the oral supplementation of IAP offers a novel therapeutic strategy to prevent alcoholic-related liver disease in humans [35].

IAP has protective effects on the gut barrier function, which can influence the development of liver fibrosis, and it has potential as a therapeutic intervention in alcoholic liver disease.

Other IAP Related Diseases

IAP plays various potential therapeutic roles, spanning its influence on renal inflammation, acute myocardial infarction (AMI)- related inflammatory responses, and ischemic heart disease (IHD) to its crucial involvement in connecting gut health with bone metabolism [35, 36].

Bovine IAP reduces pro-inflammatory responses during the acute phase following AMI in a mouse model involving permanent left anterior descending coronary artery ligation. This underscores the potential of bovine IAP as an innovative therapeutic agent for reducing the pro-inflammatory response post-AMI, potentially decreasing the occurrence of complications related to cardiogenic shock [35]. Notably, IAP deficiency is associated with IHD in mice and humans, suggesting that elevated IAP levels might offer protection against IHD [36].

IAP plays a significant role in calcium absorption, inhibiting inflammatory mediators like lipopolysaccharides and maintaining a healthy gut microbiota. Given the impact of the microbiome on bone metabolism and health, probiotic administration has demonstrated positive effects on bone health [35]. The diverse functions of IAP indicate its crucial role in bridging gut and bone health. A lack of IAP results in persistent alterations in bone formation, possibly attributed to dysbiosis and the dissemination of proinflammatory mediators throughout the body [37]. Additionally, exogenous supplementation of IAP effectively reduces renal inflammation and damage caused by ischemia-reperfusion-induced acute kidney injury [28, 38].

1.2 LPS

1.2.1 Definition of LPS

LPS serves as a crucial virulence factor in gram-negative bacteria. Positioned within the bacterial outer membrane, its hydrophobic features, comprised of fatty acid chains, firmly attach the molecule to the bacterial membrane [35]. Constituting 10% to 15% of the molecules in the outer membrane, LPS accounts for a substantial 75% of the overall surface area of gram-negative bacteria [39]. The bacterial cellular envelope is vital for preserving cellular morphology and structural stability. As the initial barrier, the cellular wall is crucial in safeguarding bacteria from the host environment and serves as the initial line of defense. The bacterial cell surface's structure and makeup significantly influence the interaction between microbes and the host's immune [40]. LPS is a potent stimulator of both inborn and acquired immune responses, and it triggers cascades that lead to tissue destruction [39].

1.2.2 The Relationship between LPS and IAP

LPS, present in the Gram-negative bacterial outer membrane, forms an LBP-LPS complex upon interacting with LPS-binding protein (LBP). This complex initiates inflammation by binding to TLRs on the surfaces of host cells [41]. Upon TLR activation, the secretion of inflammatory mediators, including IL-1, IL-6, and TNF, is heightened [23, 42, 43]. However, IAP can neutralize bacterial LPS by removing phosphate groups [13]. Lipid A is accountable for the harmful properties of LPS, featuring a pair of phosphate groups linked to glucosamines. This structure enables LPS binding to TLR4, consequently initiating inflammation [13]. Nevertheless, removing one phosphate group from lipid A by IAP results in the formation of monophosphoryl lipid A, which is significantly less toxic and is 100-fold less potent than unmodified lipid A [44-46]. Hence, IAP effectively averts inflammatory processes that would ensue without LPS dephosphorylation. This preventive action against inflammation may play a role in preserving the stability of the intestinal barrier. Additionally, IAP-induced autophagy hinders the transcription of IL-1β mRNA in the presence of LPS by influencing the activation of nuclear factor kappa B (NF-κB) [47]. IAP mitigated the LPS toxicity in intestinal epithelial cells (IECs), suppressed the NF-κB activation, and impeded the infiltration and displacement of microbial pathogens into the IECs, thereby aiding in maintaining intestinal barrier integrity [48].

The association between IAP and LPS may also influence the weight changes observed in mice. Given IAP's essential role in regulating lipid absorption, it is plausible that IAP-deficient mice experienced more significant weight gain due to increased dietary fat absorption. However, the impact of circulating LPS levels cannot be dismissed. Indeed, the absence of IAP led to heightened intestinal permeability, potentially contributing to elevated LPS levels and subsequently increased weight gain [49].

IAP proves advantageous in mitigating intestinal permeability and inflammation induced by LPS [50]. Through the neutralization of LPS, IAP has the potential to halt a series of pro-inflammatory signals in the intestine, thereby promoting the advantageous development of the microbiota [51]. Furthermore, endogenous and exogenous IAP have demonstrated the ability to inhibit LPS absorption in mice [27].

1.2.3 LPS and the connection to Cancer

LPS and Pancreatic Cancer

A systemic increase in LPS frequently accompanies pancreatic cancer [52]. LPS exposure caused distinct changes in the expression of 3083 genes in AsPC-1 cells and 2584 genes in PANC-1 cells. Notably, the PI3K/Akt/mTOR pathway, identified as a top canonical pathway, was impacted by LPS in various pancreatic cancer cell lines [52]. Among the genes stimulated with Porphyromonas gingivalis LPS in the pancreas, Reg3G ranked among the top 10 in terms of expression levels. A thorough analysis revealed a noticeable rise in the expression of Reg3G in the Porphyromonas gingivalis-LPS group compared to the control group. Additionally, the expression of Reg3A increased by 11-fold in the Porphyromonas gingivalis-LPS group. Moreover, image analysis revealed that the proportion of Reg3A/G-positive cells was higher in the Porphyromonas gingivalis-LPS group than in the control [53]. Detecting intra-tumoral LPS as an indicative marker for gram-negative bacterial colonization may be an unfavorable predictor for the effectiveness of gemcitabine in late-stage pancreatic cancer [54].

LPS and its connection to Colorectal Cancer (CRC)

Circulating levels of LPS can exacerbate body-wide inflammation, contributing to the formation of an abnormal blood clotting system. This persistent inflammation and triggered coagulation system are involved in the genesis of tumors [55]. Colitis-associated colorectal tumorigenesis is linked to LPS production by intestinal flora [56].

Metastatic dissemination is orchestrated through three key stages: initial cell adhesion to the extracellular matrix (ECM) and subsequent detachment through ECM degradation, resulting in invasion. LPS, particularly at a concentration of 1 μg/ml, is recognized as a potent stimulator of these steps in human colon cancer cells [57].

LPS levels were elevated in colon tumor tissue compared to adjacent normal mucosa. In addition, LPS was proven to promote the progression of CRC. For instance, LPS was proven to stimulate the expression of LINC00152 by positively regulating the histone acetylation of its promoter, which reduced the efficiency of YY1 binding, a repressor factor. Then, increased LINC00152 encouraged the migration and invasion of CRC [58].

LPS is also implicated in CRC therapy. It was reported that abundant LPS in CRC tissue implanted in its original anatomical site is associated with unfavorable responses to the therapy strategy of anti-programmed cell death ligand-1 (PD-L1) monoclonal antibody therapy. Removing gram-negative bacteria in the gastrointestinal tract with polymyxin B, removing gram-negative bacteria in the gastrointestinal, or blocking TLR4 alleviates the suppressive immune environment and enhances the infiltration of T cells into CRC tumors. Moreover, an engineered fusion protein was designed to target LPS, which coding sequence incorporated into a nanoparticle system. This system selectively produces the LPS-trapping protein, effectively preventing LPS access to the tumor site. Consequently, this nano-trapping strategy markedly enhances the immune microenvironment and improves anti-PD-L1 mAb therapy for CRC tumors [59].

LPS and its connection to Liver Cancer

Chronic liver congestion promotes the development of hepatocellular carcinoma (HCC) and liver fibrosis via Sphingosine 1-phosphate production from LPS-induced capillarized liver sinusoidal endothelial cells. It has been proven that chronic liver congestion enhances HCC and metastatic liver tumor growth in a murine model of chronic liver congestion through partial inferior vena cava ligation. Moreover, LPS originating from the gut seems to induce capillarization of liver sinusoidal endothelial cells, initiating HCC, the most common form of liver cancer [60].

LPS in other Cancers

LPS promotes lung tumorigenesis through chronic inflammation, leading to T-cell exhaustion and the upregulation of the programmed cell death-1 (PD-1)/programmed cell

death ligand-1 (PD-L1) axis. This, in turn, enhances lung tumorigenesis induced by tobacco carcinogens, fostering an immunosuppressive microenvironment characterized by the accumulation of myeloid-derived suppressive cells (MDSC) and regulatory T cells [61]. Additionally, LPS induces the expansion of primary human lung cancer through the TLR4/ROS/miR-21 pathway [62].

Gram-negative bacteria, the primary origin of LPS, are enriched in breast tumor microbiota. Treating breast cancer cells with LPS increases S100A7 expression in vitro. Overexpression of S100A7 downregulates TLR4 and upregulates RAGE expression in breast cancer cells. Overall, these results imply that LPS from the commensal breast tissue microbiota might contribute to increased breast tumor burden through a unique signaling axis involving S100A7/TLR4/RAGE [63].

Besides, LPS influences the proliferation and glucose metabolism in cervical cancer cells via modulation of the FRA1/MDM2/p53 signaling pathway [64]. Intratumoral LPS-induced enhancement of the development of prostate cancer by activating the NF-κB-IL6-STAT3 signaling pathway in mice [65]. Bacterial LPS-related genes can also serve as biomarkers for predicting progression-free survival in patients with gastric cancer [66].

1.2.4 LPS and Surgery

LPS preconditioning induces endotoxin tolerance, which may protect against surgery-induced cognitive impairment in aging mice. The effectiveness depends on the timing of the application. Preconditioning with low-dose LPS could significantly alleviate neuroinflammation induced by surgery and cognitive decline in aging mice. This approach may offer a novel way to prevent postoperative cognitive dysfunction and potentially address other forms of memory impairment [67].

Fecal LPS concentrations were markedly elevated in individuals experiencing prolonged postoperative ileus. LPS exacerbated inflammation and dysfunction in mice, while polymyxin B reduced these effects. Additionally, LPS induced increased phosphorylation of p38 in mice. Administration of a p38 inhibitor effectively mitigated intestinal inflammation and dysmotility. LPS exacerbates inflammation in the intestinal muscularis by activating p38 signaling, thereby worsening postoperative ileus. Prophylactic treatment targeting bacterial sources of LPS during the perioperative period shows promise in alleviating prolonged postoperative ileus [68].

Bariatric surgeries has gained popularity as a treatment option, leading to long-term weight reduction and amelioration of obesity-associated conditions. Levels of LPS and LBP were observed to decrease following various weight loss surgeries. CD14, the receptor for LPS and toll-like receptor 2 (TLR2) and TLR4, also exhibited reduced levels post-surgery. The changes in LPS and its constituents following bariatric surgeries appear to be associated with the specific surgical technique employed and the restriction of caloric intake [69]. A temporary decrease in LPS levels post-bariatric surgeries is contingent on the surgical intervention employed and the patient's prior glycemic status, with sleeve gastrectomy demonstrating the most significant short-term impact on LPS [70].

1.3 Aim of the Study

Our research project sought to explore the relationship between preoperative serum LPS levels and stool IAP activity patients undergoing abdominal surgery. We also aimed to investigate the impact of preoperative serum IAP and serum LPS levels on surgical outcomes, such as hospital stay, ICU stay, and complication rate. Understanding the significance of these markers could lead to interventions to enhance gut health before surgeries, potentially improving patient outcomes and recovery. Furthermore, we delved into the roles of preoperative serum IAP and serum LPS in individuals with various types of cancer, including pancreatic-, colorectal-, liver-, and lung cancer. With the known association of LPS with these cancers as well as the beneficial functions of IAP, such as anti-inflammation and gut barrier preservation, our work aimed to further shed light on the interplay between these markers in cancer patients.

2. Materials and Methods

2.1 Materials

2.1.1 Devices

Devices	Manufacturer
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 Concumables

Concumables	Manufacturer
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

2.1.3 Chemicals

Chemicals	Manufacturer
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 (MgCl2.6H2O)	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 (Zncl2)	208086, Merck, Germany
80% Ethanol	1004051526001, CLN GmbH Chemikalien Laborbedarf, Germany

2.1.4 Buffers and Solutions

Solutions	Composition
1M MgCl2 solution	20.3 g MgCl2.6H2O
	100 ml Distilled water
10mM ZnCl2 solution	0.1364g ZnCl2
	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 MgCl2 100µl 10mM ZnCl2 99ml Distilled water pH 8.0

2.1.5 Software and Tools

Software and Tools	Manufacturer
Microsoft Excel	Microsoft, USA
Xiantao academic tools	Xiantao, China

2.1.6 Commercial Kits

Commercial Kits	Manufacturer
Pierce™ Chromogenic Endotoxin Quant Kit	A39553, Thermo Scientific, USA
Human Alkaline Phosphatase, Intestinal (ALPI) ELISA Kit	MBS167167 MyBioSource, USA

2.2 Methods

2.2.1 Study Design, Patients and Clinical Data

This study was designed as a prospective observational study conducted at the Department of General, Visceral, and Transplant Surgery, Ludwig-Maximilians-University Hospital Munich. Between June 2022 and September 2023, preoperative blood and stool samples were prospectively collected from patients undergoing abdominal surgery. The study consisted of two main components: a cross-sectional analysis comparing preoperative serum IAP and LPS levels among cancer patients, non-cancer patients, and healthy controls, and a prospective cohort analysis investigating the association between preoperative IAP and LPS levels and postoperative surgical outcomes. Ethical approval (project

number: 21-0285) for the study was obtained before patient enrollment, and written informed consent was obtained from all participants before inclusion. Preoperative and postoperative samples were obtained from various groups: 25 pancreatic cancer patients, 21 CRC patients, 14 liver cancer patients, 31 patients with other cancer types (including gastric cancer, liposarcoma, angiosarcoma, adenocarcinoma of the gastroesophageal junction (AEG), gallbladder carcinoma, peritoneal metastases of endometrial cancer or leiomyosarcoma), 27 non-cancer patients (diagnosed with sigmoid diverticulitis, chronic pancreatitis, ileostomy, incisional hernia, short bowel syndrome, blind-loop syndrome, chronic cholecystitis, cholecystolithiasis, Hartmann procedure, ovarian + peritoneal cysts, intraductal papillary mucinous neoplasm (IPMN), or liver cyst, etc.), and 8 healthy controls, among these participants. This data was collected between June 2022 and September 2023 at our institution. Clinical characteristics, such as gender, age, Barthel motility score, Barthel cognitive score, American Society of Anesthesiologists (ASA) score, body mass index (BMI), blood group, Union for International Cancer Control (UICC) stage, blood loss, operation time, Intensive Care Unit (ICU) stay, the total length of hospital stay, and Clavien-Dindo grade were recorded. Additionally, routine laboratory values, including preoperative interleukin 6 (IL-6), preoperative C-reactive protein (CRP), preoperative white blood cell count (WBC), preoperative hemoglobin (Hb), preoperative glomerular filtration rate (GFR), preoperative creatinine (Crea), preoperative fasting blood glucose (FBG), preoperative HbA1c, preoperative alkaline phosphatase (ALP), preoperative total bilirubin (Tbil), preoperative albumin (Alb), and preoperative aspartate transaminase (AST), preop carcinoembryonic antigen (CEA), preop carbohydrate antigen 19-9 (CA19-9), were assessed.

2.2.2 Collection and Preservation of Samples

Patients provided blood samples both before abdominal surgeries and on postoperative days 10. The samples were centrifuged at 2000x g for 10 minutes at 15°C. The serum layer was then extracted and stored at -80°C. Stool samples were collected one day before surgeries, preserved also on the 10^{th} postoperative day. Storage took place in a -80°C refrigerator until testing.

2.2.3 Human Alkaline Phosphatase, Intestinal ELISA Assay

It was ensured that all reagents, standard solutions, and samples were at ambient temperature before utilization. The assay was conducted at room temperature. For serum samples,

they were allowed to clot for 10-20 minutes at ambient temperature. Subsequently, they were centrifuged at 2000-3000 rpm for 20 minutes. The supernatant was retrieved, excluding any sediment. The standard was reconstituted by combining 120 µl of the standard solution (320 ng/ml) with 120 µl of standard diluent, resulting in a 160 ng/ml standardized stock solution. The standard solution was let to stand undisturbed for 15 minutes with mild stirring before dilution. Duplicate data points for standards were created by progressively diluting the standardized stock solution (160 ng/ml) at a 1:2 ratio using a standard diluent to produce 80 ng/ml, 40 ng/ml, 20 ng/ml, and 10 ng/ml solutions. The standard diluent functioned as the zero standard (0 ng/ml).

Recommended dilutions for standard solutions are as follows:

160ng/ml	Standard No.5	120ul original standard + 120ul standard diluent
80ng/ml	Standard No.4	120ul No.5 + 120ul standard diluent
40ng/ml	Standard No.3	120ul No.4 + 120ul standard diluent
20ng/ml	Standard No.2	120ul No.3 + 120ul standard diluent
10ng/ml	Standard No.1	120ul No.2 + 120ul standard diluent

50 µl of different standard solutions were added to the corresponding wells. 40 µl of the sample was added to the respective wells, and 10 µl of anti-IAP antibody was added to these sample wells. Subsequently, 50 µl of streptavidin-horseradish peroxidase (HRP) was added to both sample and standard wells (excluding the blank control wells). After thorough mixing, the plate was sealed and incubated for 60 minutes at 37°C. After incubation, the seal was removed, and the plate was rinsed with a wash buffer five times.Subsequently, 50 µl of substrate solution A was added to each well, followed by the addition of 50 µl of substrate solution B. The plate was then covered with a new sealer and incubated for 10 minutes at 37°C in darkness. After the incubation, 50 µl of stop solution was added to each well to stop the reaction. The blue color immediately changed to yellow. The optical density (OD) values of each well were promptly assessed using a microplate reader configured to 450nm within 10 minutes of introducing the stop solution.

2.2.4 Bicinchoninic Acid (BCA) Assay

1) Calculation of BCA Working Reagent Volume:
   The experiment commenced with determining the necessary volume of the BCA working reagent.
2) Solution A and Solution B Combination:
   Following the reagent calculation, solution A and solution B were combined at a ratio of 50:1.
3) Preparation of BSA Standard Solutions:
   Standard bovine serum albumin (BSA) solutions were prepared at 31.25 to 2000 µg/ml concentrations.
4) Sample Pipetting and Plate Shaking:
   25 µl of each unidentified or standard specimen was pipetted in triplicate into specified microplate compartments, using distilled water as a blank. Subsequently, 200 µl of the operational reagent was added to each well, and the plate was shaken thoroughly on a plate shaker for 30 seconds.
5) Incubation and Measurement:
   The plate was covered and incubated at 37°C for 30 minutes. After reaching room temperature, each well's absorbance at 562 nm was measured using a spectrophotometer. Data analysis included subtracting the average absorbance of the blank-corrected triplicates from the average absorbance of all the standards and unknown samples. A standard curve was then constructed for protein concentration determination.

2.2.5 Para-Nitrophenyl Phosphate (PNPP) Method

1）Stool Sample Processing:
   Thawed stool samples were diluted 1:30 in stool dilution buffer (10 mM Tris HCl, pH 8.0; 1 mM MgCl2; 10 µM ZnCl2) and then incubated on ice for 30 minutes. The stool mixture was homogenized on a shaker for 10 minutes, then centrifuged at 10,000g for another 10 minutes to obtain the supernatant for analysis.
2）Preparation of L-phenylalanine Inhibitor and Standard IAP Solutions:
   L-phenylalanine, a selective inhibitor of IAP, 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 various concentrations: 10, 5, 2.5, 1.25, 0.625, 0.3125, and 0.15625 U/ml.

3）Microplate Setup for Standards and Unknown Samples:

Each standard solution (25 μL) was added in triplicate to designated microplate wells, with distilled water as the blank. An equal volume (25 μL) of each unknown sample was added to designated microplate wells, with six wells assigned per sample.

4）PNPP Solution Dispensing and Incubation:

175 μL of PNPP solution was dispensed into the first three wells, while L-phenylalanine plus PNPP solution was added to the other three wells simultaneously. Standard and control wells received an identical volume of PNPP solution. The plate was incubated for 10 minutes at 37°C.

5）Measurement and Calculation of IAP Activity:

After cooling to room temperature, the absorbance of each well was measured at 405 nm with a spectrophotometer. The average absorbance of the blank-corrected triplicates at 405 nm was subtracted from all individual standard and unknown sample triplicate measurements. A standard curve was created by plotting the average blank-corrected OD values of each IAP standard against its activity (U/ml). The standard curve enabled calculation of each unknown sample's activity. The average difference between the groups with and without L-phenylalanine represented the specific activity of IAP.

2.2.6 Limulus Amebocyte Lysate (LAL) Assay

1) Reagent Preparation and Sample Retrieval:

Reagents were brought to room temperature before use. Frozen serum samples were taken from the -80°C freezer, thawed on ice, vortexed, and centrifuged at 5000 rpm for 10 minutes at 4°C. The resulting serum was carefully transferred to another tube to eliminate platelets and other sediments. A second centrifugation step at 10,000 rpm for 10 minutes at 4°C was performed to ensure complete sediment removal.

2) Sample Dilution and Heat-Shocking:

In a separate tube, the supernatant was diluted 50 times with endotoxin-free water. The diluted samples were then heat-treated at 70°C for 15 minutes and subsequently cooled on ice until they were ready for testing.

3) Preparation of Endotoxin Standard Solutions:

Endotoxin standard solutions were prepared using lyophilized E. coli endotoxin and endotoxin-free water in the Pierce™ Chromogenic Endotoxin Quant Kit, resulting in final standard endotoxin concentrations of 0.1, 0.05, 0.025, and 0.01 EU/ml. After

reconstitution or before each use, standard solutions were thoroughly vortexed for 15 minutes to ensure uniform distribution.

4) Amebocyte Lysate and Chromogenic Substrate Solution Preparation:
   Amebocyte lysate was reconstituted with endotoxin-free water (1.7 ml per vial) immediately before use and gently swirled for dissolution. Chromogenic Substrate solution was prepared by adding 3.4 ml of endotoxin-free water to each vial, gently mixed to dissolve the powder, and pre-warmed to 37°C for no longer than 10 minutes before use.
5) Microplate Setup and Incubation:
   A 96-well plate was pre-warmed in a heating block at 37±1°C for 10 minutes. While maintaining the plate at 37±1°C, 50 µl of blank, standard, and unknown samples were added in triplicate to the designated wells. Endotoxin-free water was used as the blank control. Subsequently, 50 µl of LAL reagent was pipetted into each well, marking the start time. The plate was gently tapped to facilitate mixing after adding the LAL solution to all wells. Covered with a lid, the plate was returned to the heating block to incubate at 37±1°C for the specified duration.
6) Chromogenic Substrate Reaction and Measurement:
   Following incubation, 100 µl of prewarmed Chromogenic Substrate solution was added to each well. The plate was tapped again to mix and then incubated at 37±1°C for 6 minutes. Subsequently, 50 µl of stop reagent (25% acetic acid) was sequentially added to each well. The plate was removed from the heating block and gently tapped for mixing post-stop reagent addition, and the absorbance at 405 nm was measured using a spectrophotometer. The mean 405 nm absorbance of the blank-corrected triplicates was subtracted from the mean absorbance of each individual standard and unknown sample triplicate. A standard curve was generated by plotting the average blank-corrected OD values of each LPS standard against its endotoxin concentration in EU/ml, allowing for the determination of the endotoxin concentration in each unknown sample.

2.2.7 Statistical Analysis

The Shapiro-Wilk Normality Test was used to check normality, while Levene's test (based on Mean) was used to examine the homogeneity of variance. Continuous variables conforming to a normal distribution were presented as mean ± standard deviation (SD) or

median with appropriate interquartile range (IQR). Otherwise, if the continuous variables did not meet standard distribution criteria, they were presented as median (IQR).

When continuous variables satisfied normal distribution and homogeneity of variance, the T-test was the recommended test for comparing the two groups. Statistical significance was established at $r > 0.3000$ and $p < 0.05$. Conversely, if continuous variables did not conform to normal distribution, the Wilcoxon test was recommended for comparison between the two groups, and statistical significance was set at $r > 0.3000$ and $p < 0.05$.

Specific tests were applied for categorical variables based on theoretical frequency and total sample size, with a significance level of $p < 0.05$ considered statistically significant.

Correlations between two continuous data sets were evaluated using Pearson's test if both data sets were close to a normal distribution, presenting p and r values. The significance threshold was set at $r > 0.3000$ and $p < 0.05$. If either of the continuous data sets deviated from a normal distribution, Spearman's test was used for correlation assessment, presenting p and r values with the same significance criteria.

Different statistical tests were utilized for comparing continuous datasets based on normality and homogeneity of variance criteria, with a significance level of $p < 0.05$ indicating statistical significance.

For comparisons involving three or more datasets, the Kruskal-Wallis test is applied to determine whether there are statistically significant differences among the medians of three or more independent groups. Subsequently, Dunn's test is conducted for pairwise comparisons between each independent group to accurately identify which groups differ, with a significance level of $p < 0.05$ indicating statistical significance.

Receiver-operated characteristics (ROC) curves were constructed to determine cutoff values of preoperative serum IAP values that yield the joint maximum sensitivity and specificity regarding the clavier as a predictive result. The Xiantao Academic Online Website (https://www.xiantaozi.com/products) was utilized for online analysis.

3. Results

3.1 Patients‘ Characteristics

Blood and stool samples were collected from 118 patients at the Department of General, Visceral, and Transplant Surgery, Ludwig-Maximilians-University, between June 22, 2022, and September 15, 2023. Among these patients, 91 were diagnosed with cancer, while 27 were non-cancer patients. Within the cancer group, there were 25 pancreatic cancer patients, 21 with CRC, and 14 diagnosed with liver cancer. Table 1 provides the baseline characteristics of these patients. Additionally, 8 blood samples were obtained from healthy individuals to serve as controls.

Table 1. Patient baseline characteristics

Characteristics	Non-Cancer	Cancer	P Value
N	27	91	
Gender, N (%)			0.088
Female	16 (13.6)	37 (31.4)	
Male	11 (9.3)	54 (45.8)	
Age (Year), Median (IQR)	62 (47.5, 69)	68 (59, 76)	**0.013**
Barthel mot, N (%)			0.069
100	20 (16.9)	82 (69.5)	
<100	7 (5.9)	9 (7.6)	
Barthel cog, N (%)			1.000
90	26 (22)	87 (73.7)	
<90	1 (0.8)	4 (3.4)	
ASA physical status classification, N (%)			**< 0.001**
3	15 (12.8)	82 (70.1)	
2	12 (10.3)	8 (6.8)	
BMI, Median (IQR)	23 (21.5, 28)	25 (22, 28)	0.628

Blood Group, N (%)			0.897
B+	3 (3.5)	10 (11.8)	
A+	6 (7.1)	25 (29.4)	
O+	7 (8.2)	18 (21.2)	
Others	4 (4.7)	12 (14.1)	
Preop Il-6 (pg/ml), Median (IQR)	4.3 (1.825, 13.8)	5.4 (2.8, 9.65)	0.762
Preop CRP (mg/dl), Median (IQR)	0.3 (0.1, 0.6)	0.3 (0.1, 0.8)	0.630
Preop WBC (g/L), Median (IQR)	6.79 (5.84, 8.15)	6.72 (5.725, 8.535)	0.855
Preop Hb (g/dl), Mean ± SD	12.793 ± 1.7802	12.87 ± 1.9917	0.856
Preop GFR (ml/min), Mean ± SD	77.963 ± 25.86	79.066 ± 20.029	0.815
Preop Crea (mg/dl), Median (IQR)	0.9 (0.8, 1.1)	0.9 (0.8, 1)	0.726
Preop FBG (mg/dl), Median (IQR)	97 (89.5, 104.5)	103 (93.5, 125.5)	**0.038**
Preop HbA1c (%), Mean ± SD	5.6333 ± 0.30551	6.1667 ± 1.3377	0.506
Preop ALP (U/L), Median (IQR)	80 (65, 100)	92 (74.25, 124.25)	**0.027**
Preop Tbil (mg/dl), Median (IQR)	0.4 (0.3, 0.7)	0.5 (0.3, 0.6)	0.647
Preop Alb (g/dl), Median (IQR)	4.3 (3.7, 4.5)	4.3 (4, 4.5)	0.704
Preop AST (U/L), Median (IQR)	24 (18, 34)	28 (22, 39)	0.113
Preop CEA (ng/ml), Median (IQR)	1.8 (1.8, 2.2)	3.1 (1.8, 5.8)	**0.041**
Preop CA19-9 (U/ml), Median (IQR)	8.9 (7.2, 29.2)	24.1 (9.5, 129)	0.072

(Abbreviations: Preop: preoperative; Barthel mot: Barthel motility score; Barthel cog: Barthel cognitive score; ASA: American Society of Anesthesiologists; BMI: body mass

index; IL-6: interleukin 6; CRP: C-reactive protein; WBC: white blood cell; Hb: hemoglobin; GFR: glomerular filtration rate; Crea: creatinine; FBG: fasting blood glucose; ALP: alkaline phosphatase; Tbil: total bilirubin; Alb: albumin; AST: aspartate transaminase; CEA: carcinoembryonic antigen; CA19-9: carbohydrate antigen 19-9. Statistical notation: SD: standard deviation; IQR: interquartile range. Presentation of data: Continuous variables adhering to normal distribution are expressed as mean Mean ± SD; Continuous variables not adhering to normal distribution are expressed as Median (IQR); Categorized variants are described as N%.)

3.2 Correlation of IAP, LPS, and Clinical Parameters Across the Entire Cohort

We collected a total of 112 preoperative serum samples and 23 stool samples from our participants. Of these, all 112 serum samples underwent testing for serum LPS values, and 83 serum samples were tested for serum IAP values. Additionally, the 23 preoperative stool samples were examined for preoperative stool IAP values, with 18 of these samples having paired preoperative serum LPS values.

Upon inclusion of the 18 paired samples in this study, we observed a continued negative correlation between preoperative stool IAP and preoperative serum LPS in our participants ($r = -0.514$, $p = 0.029$) (Figure 1A). Furthermore, our findings indicated a positive correlation between preoperative stool IAP and preoperative serum IAP values ($r=0.524$, $p=0.040$) (Figure 1B). Additionally, there is a negative correlation between preoperative serum IAP and preoperative serum LPS ($r=-0.397$, $p<0.001$) (Figure 1C).

Our study primarily focused on serum LPS and serum IAP as indicators in patients undergoing general surgeries. Table 2 illustrates the relationship between these two indicators and various clinical parameters within the entire study. Notably, we observed a negative association between preoperative serum IAP values and preoperative IL-6 ($r=-0.334$, $p=0.020$) (Table 2). Preoperative serum IAP also displayed a positive correlation with preoperative albumin ($r = 0.425$, $p<0.001$) (Table 2). Additionally, Table 2 highlights the negative relationship between preoperative serum IAP and preoperative CRP ($r=-0.348$, $p=0.001$).

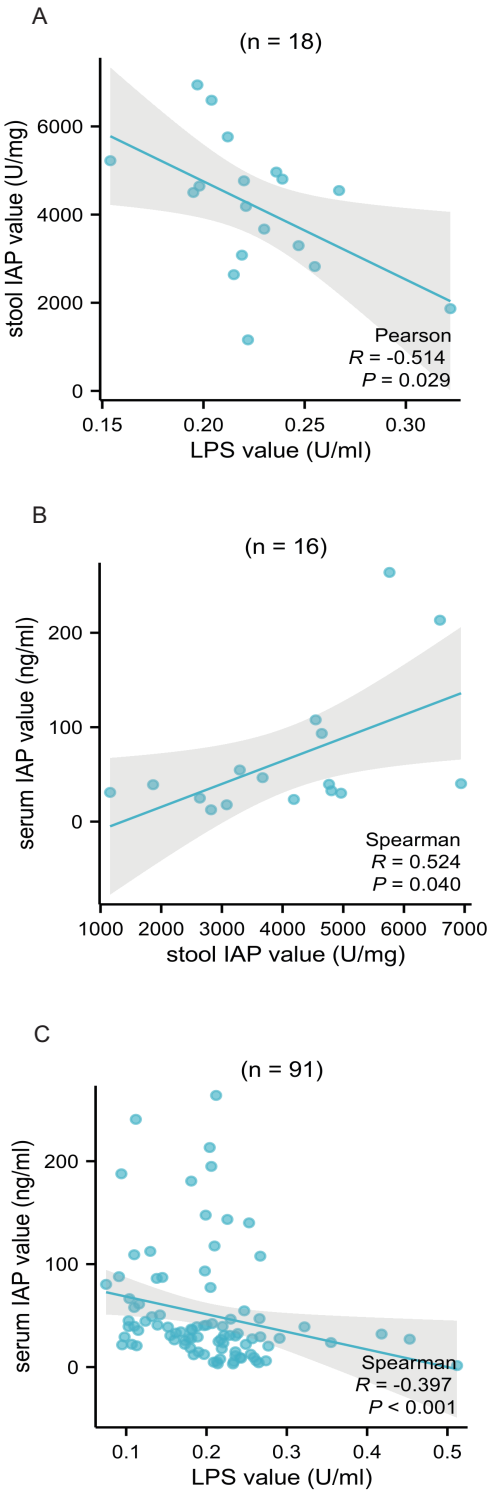


Figure 1: Correlation of IAP, LPS, and clinical parameters across the entire cohort.

A) The preoperative stool IAP value was negatively correlated to the preoperative serum LPS value (n = 18, r = -0.514, p = 0.029, analyzed by Pearson's test).

B) The preoperative serum IAP value was positively correlated with the preoperative stool IAP value (n = 16, r = 0.524, p = 0.040, analyzed by Spearman's test).

C) preoperative serum IAP value negatively correlated with preoperative serum LPS value (n = 91, r = -0.397, p<0.001, analyzed by Spearman's test).

(Abbreviations: IAP: Intestinal Alkaline Phosphatase; LPS: Lipopolysaccharides)

Table 2. Correlation of preoperative serum IAP/LPS values with clinical parameters in the entire cohort.

Clinical Parameters	serum IAP value (ng/ml)	LPS value (U/ml)
Gender	P = 0.8501	P = 0.1835
Age	R = -0.030 p = 0.790	R = -0.066, p = 0.485
Barthel mot.	P = 0.1020	P=0.5765
Barthel cog.	P = 0.2795	P = 0.9722
ASA	P = 0.8413	P = 0.6121
BMI	R = 0.001 p = 0.991	R = 0.081, p = 0.395
Blood Group	P = 0.5748	P = 0.4645
Preop IL-6	**R = -0.334, p = 0.020**	R = 0.241, p = 0.044
Preop CRP	**R = -0.348, p = 0.001**	R = 0.105, p = 0.268
Preop WBC	R = -0.080, p = 0.471	R = 0.112, p = 0.238
Preop Hb	R = 0.234, p = 0.033	R = -0.042, p = 0.660
Preop GFR	R = -0.039, p = 0.724	R = 0.067, p = 0.483
Preop Crea	R = 0.033, p = 0.765	R = 0.029, p = 0.763
Preop FBG	R = 0.049, p = 0.659	R = 0.015, p = 0.878
Preop HbA1c	R = 0.185, p = 0.447	R = -0.125, p = 0.570
Preop ALP	R = -0.088, p = 0.434	R = -0.084, p = 0.385
Preop Tbil	R = 0.110, p = 0.334	R = -0.124, p = 0.195
Preop Alb	**R = 0.425, p < 0.001**	R = -0.253, p = 0.014
Preop AST	R = 0.060, p = 0.601	R = -0.008, p =0 .933
Preop CEA	R = -0.201, p = 0.203	R = 0.101, p = 0.417
Preop CA19-9	R = -0.101, p = 0.531	R = -0.048 p = 0.699

(Abbreviations: Preop: preoperative; Barthel mot: Barthel motility score; Barthel cog: Barthel cognitive score; ASA: American Society of Anesthesiologists; BMI: body mass

index; IL-6: interleukin 6; CRP: C-reactive protein; WBC: white blood cell; Hb: hemoglobin; GFR: glomerular filtration rate; Crea: creatinine; FBG: fasting blood glucose; ALP: alkaline phosphatase; Tbil: total bilirubin; Alb: albumin; AST: aspartate transaminase; CEA: carcinoembryonic antigen; CA19-9: carbohydrate antigen 19-9.)

3.3 Preoperative Serum IAP and Cancer

We enlisted eight healthy participants as controls for comparison. In contrast to the healthy control group, the cancer group exhibited a significantly lower preoperative serum IAP level (cancer vs. healthy control: 30.341 (22.081, 40.470) vs 110.22 (70.243, 144.480) ng/ml, $p = 0.0012$) (Figure 2A). There was no significant difference in preoperative serum IAP levels when comparing the cancer group to the non-cancer group (cancer vs. non-cancer: 30.341 (22.081, 40.470) vs. 28.173 (12.524, 86.145) ng/ml, $p = 1$) (Figure 2A).

We also explored preoperative serum IAP values in different cancer types compared to healthy control and non-cancer groups (Figure 2B). Preoperative serum IAP levels were significantly lower in pancreatic cancer patients (pancreatic cancer vs. healthy control: 30.864 (26.850, 42.801) vs. 110.22 (70.243, 144.480) ng/ml, $p = 0.0144$) and CRC patients (CRC vs healthy control: 32.82 (22.5, 40.326) vs 110.22 (70.243, 144.480) ng/ml, $p = 0.0349$) in comparison to those in healthy controls. (Figure 2B). No significant difference was observed in the liver cancer group when compared to healthy controls (liver cancer vs. healthy control: 38.109 (28.397, 39.483) vs. 110.22 (70.243, 144.480) ng/ml, $p = 0.0984$) (Figure 2B). Furthermore, no significant differences were found in the preoperative serum IAP values for pancreatic cancer (pancreatic cancer vs. non-cancer: 30.864 (26.850, 42.801) vs. 28.173 (12.524, 86.145) ng/ml, $p = 1$), CRC (CRC vs. non-cancer: 32.82 (22.5, 40.326) vs. 28.173 (12.524, 86.145) ng/ml, $p = 1$), and liver cancer groups (liver cancer vs. non-cancer: 38.109 (28.397, 39.483) vs. 28.173 (12.524, 86.145) ng/ml, $p = 1$) compared to the non-cancer group (Figure 2B).

Furthermore, participants were categorized into two groups based on the UICC stage for pancreatic cancer. No significant difference was observed in preoperative serum IAP levels between the UICC stage I/II and III/IV groups in pancreatic cancer (stage I/II vs stage III/IV: 33.419 ± 6.9406 vs 42.736 ± 32.013 ng/ml, $p = 0.5566$) (Figure 2C).

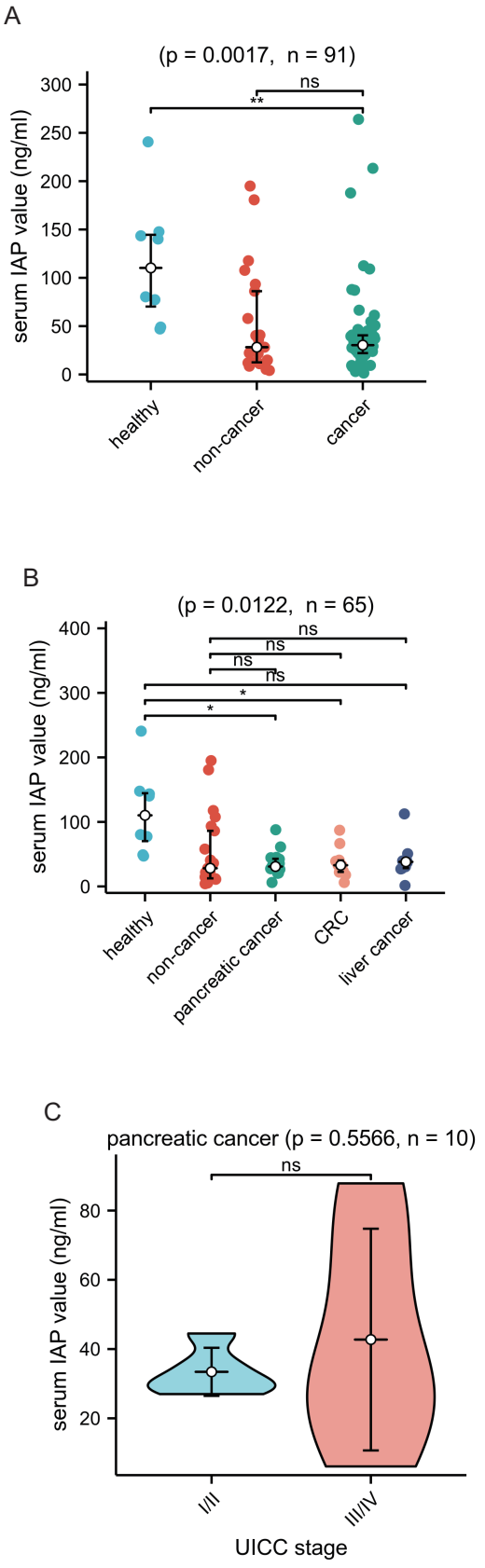

A
(p = 0.0017, n = 91)
ns
**
serum IAP value (ng/ml)
300
250
200
150
100
50
0
healthy
non-cancer
cancer
B
(p = 0.0122, n = 65)
ns
ns
ns
ns
*
*
serum IAP value (ng/ml)
400
300
200
100
0
healthy
non-cancer
pancreatic cancer
CRC
liver cancer
C
pancreatic cancer (p = 0.5566, n = 10)
ns
serum IAP value (ng/ml)
80
60
40
20
I/II
III/IV
UICC stage

Figure 2: IAP and cancer.

A) Preoperative serum IAP values were lower in the cancer group compared to the healthy group (p = 0.0012); No significant difference was observed in preoperative serum IAP values between the cancer group and the non-cancer group (p = 1). The p-value for within-group comparisons is 0.0017. The analysis was conducted on a sample size of 91.
B) Preoperative serum IAP values were lower in the pancreatic cancer group (p = 0.0144) and CRC group (p = 0.0349) compared to the healthy group; No significant difference was observed in preoperative serum IAP values between the liver cancer group and the healthy group (p = 0.0984); No significant difference was observed in preoperative serum IAP values between the pancreatic cancer group and non-cancer group (p = 1); No significant difference was observed in preoperative serum IAP values between the CRC group and non-cancer group (p = 1); No significant difference was observed in preoperative serum IAP values between the liver cancer group and non-cancer group (p = 1); The p-value for within-group comparisons is 0.0122. The analysis was conducted on a sample size of 65.
C) We divided the pancreatic cancer patients into UICC I/II stage and III/IV stage; there is no significant difference in preoperative serum IAP between UICC I/II stage and III/IV stage (p = 0.5566). The analysis was conducted on a sample size of 65.

(Abbreviations: IAP: intestinal alkaline phosphatase; ns: insignificant; * p< 0.05; ** p< 0.01.)

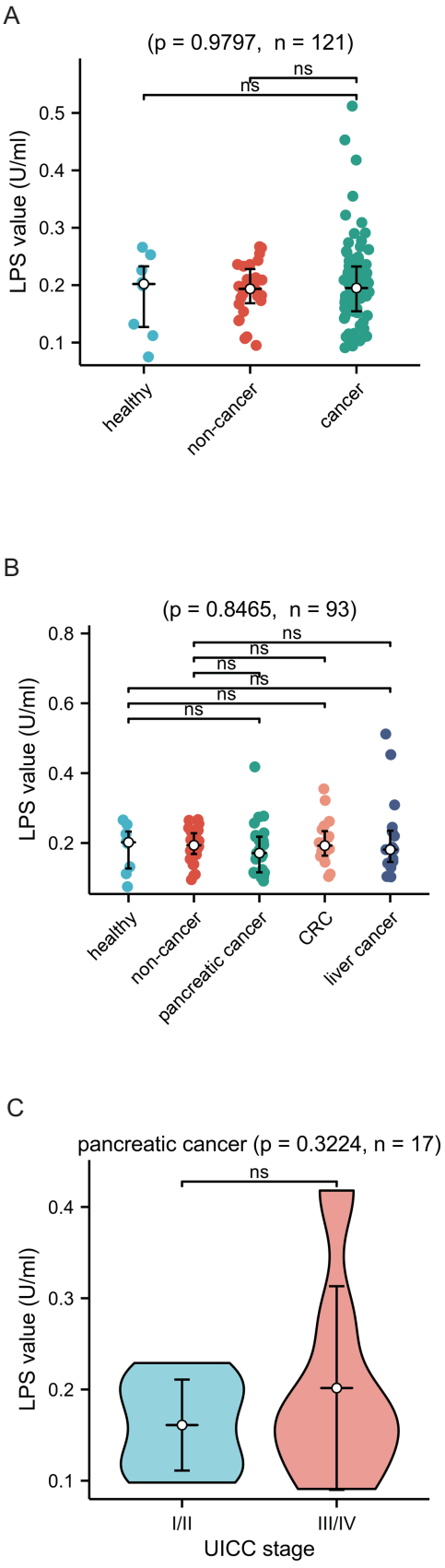
3.4 Preoperative Serum LPS and Cancer

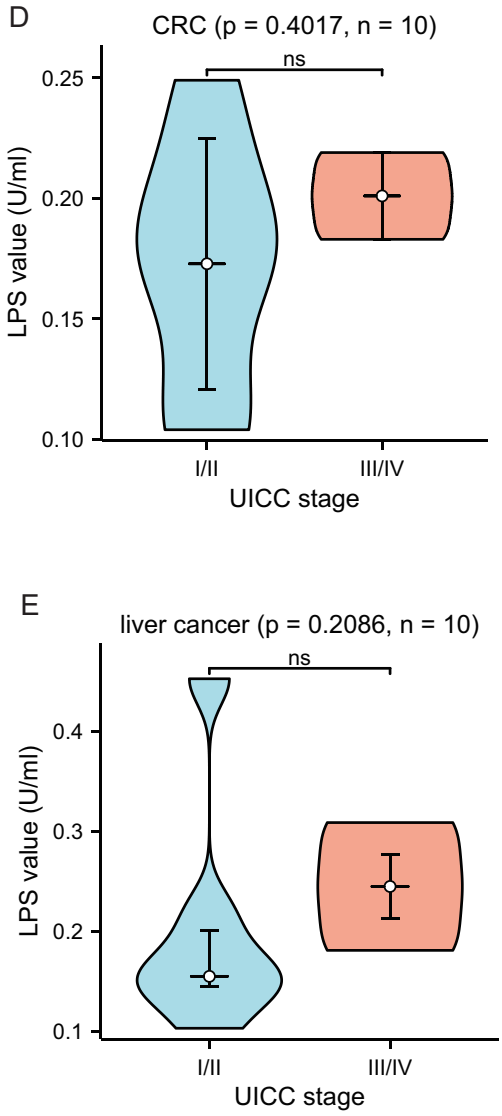
There was no significant difference in preoperative serum LPS levels in the cancer group when compared to the healthy control group (cancer vs. healthy control: 0.195 (0.1545, 0.2325) vs 0.202 (0.127, 0.2328) U/ml, p = 1) or compared to the non-cancer group (cancer vs non-cancer: 0.195 (0.1545, 0.2325) vs 0.1935 (0.1685, 0.228) U/ml, p = 1) individually (Figure 3A).

Additionally, we analyzed preoperative serum LPS values in different specific cancer types compared to healthy control and non-cancer groups (Figure 3B). The findings revealed no significant difference between pancreatic cancer and the healthy control group

(pancreatic cancer vs. healthy control: 0.171 (0.116, 0.218) vs. 0.202 (0.127, 0.2328) U/ml, p = 1), nor between pancreatic cancer and the non-cancer group (pancreatic cancer vs. non-cancer: 0.171 (0.116, 0.218) vs 0.1935 (0.1685, 0.228) U/ml, p = 1) (Figure 3B). Similarly, there was no significant difference observed between CRC and healthy control groups (CRC vs. healthy control: 0.1925 (0.1638, 0.234) vs 0.202 (0.127, 0.2328) U/ml, p = 1) or between CRC and non-cancer groups (CRC vs non-control: 0.1925 (0.1638, 0.234) vs 0.1935 (0.1685, 0.228) U/ml, p = 1) (Figure 3B). Likewise, there was no significant difference between the liver cancer group and the healthy control group (liver cancer vs healthy control: 0.181 (0.1458, 0.2353) vs 0.202 (0.127, 0.2328) U/ml, p = 1), nor between liver cancer and the non-cancer group (0.1935 (0.1685, 0.228) U/ml, p = 1) (refer to Figure 3B).

Furthermore, upon stratifying participants based on UICC stages for pancreatic cancer, CRC, and liver cancer, no significant differences were found in preoperative serum LPS levels between UICC stage I/II and III/IV groups in pancreatic cancer (stage I/II vs. stage III/IV: 0.161 ± 0.0499 vs. 0.2016 ± 0.1116 U/ml, p = 0.3224), CRC (stage I/II vs. stage III/IV: 0.1729 ± 0.0522 vs 0.201 ± 0.018 U/ml, p = 0.4017), and liver cancer (stage I/II vs stage III/IV: 0.155 (0.1445, 0.2005) vs 0.245 (0.213, 0.277) U/ml, p = 0.2086) (Figures 3C-E).


A
(p = 0.9797, n = 121)
ns
ns
LPS value (U/ml)
0.5
0.4
0.3
0.2
0.1
healthy
non-cancer
cancer
B
(p = 0.8465, n = 93)
ns
ns
ns
ns
ns
ns
LPS value (U/ml)
0.8
0.6
0.4
0.2
healthy
non-cancer
pancreatic cancer
CRC
liver cancer
C
pancreatic cancer (p = 0.3224, n = 17)
ns
LPS value (U/ml)
0.4
0.3
0.2
0.1
I/II
III/IV
UICC stage



-

Figure 3: Preoperative serum LPS and cancer.

A) There was no significant difference in preoperative serum LPS between the cancer group and the healthy group (p = 1); there was no significant difference in preoperative serum LPS between the cancer group and the non-cancer group (p = 1); The p-value for within-group comparisons is 0.9797. The analysis was conducted on a sample size of 121.

B) There was no significant difference in preoperative serum LPS between pancreatic cancer group and healthy group (p = 1); there was no significant difference in preoperative serum LPS between the CRC group and the healthy group (p = 1); there was no significant difference in preoperative serum LPS between liver cancer group and healthy group (p = 1); there was no significant difference in preoperative serum LPS between pancreatic cancer group and non-cancer group (p = 1); there was no significant difference in preoperative serum LPS between CRC group and non-cancer group (p = 1); there was no significant difference in preoperative serum LPS between liver cancer group and non-cancer group (p = 1). The p-value for within-group comparisons is 0.8465. The analysis was conducted on a sample size of 93.

C) We divided the pancreatic cancer patients into UICC I/II stage and III/IV stage. There is no significant difference in preoperative serum LPS between the two stages (p = 0.3224). The analysis was conducted on a sample size of 17.

D) We divided the CRC patients into UICC I/II stage and III/IV stage. There is no significant difference in preoperative serum LPS between the two (p = 0.4017). The analysis was conducted on a sample size of 10.

E) We divided the liver cancer patients into UICC I/II stage and III/IV stage. There is no significant difference in preoperative serum LPS between the two (p = 0.2086). The analysis was conducted on a sample size of 10.

(Abbreviations: LPS: lipopolysaccharides; ns: not significant.)

3.5 Preoperative Serum IAP in Patients undergoing Abdominal surgery

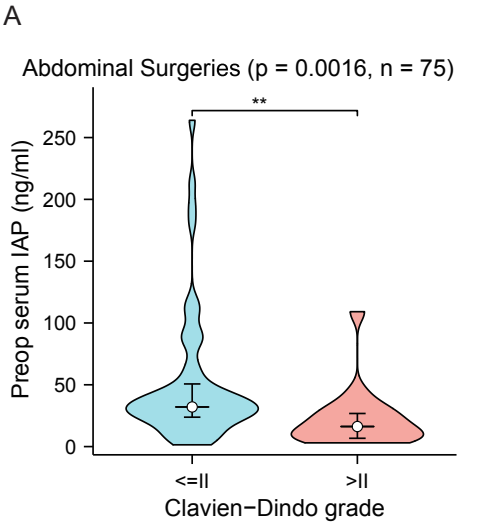
We examined preoperative serum IAP values in two distinct groups: Clavien-Dindo grade <= II and Clavien-Dindo grade > II. We observed a significant difference in preoperative serum IAP levels between these groups (Clavien-Dindo grade > II vs. Clavien-Dindo grade <= II: 16.233 (6.716, 26.782) vs. 32.074 (23.81, 50.682) ng/ml, p = 0.0016) (refer to Figure 4A).

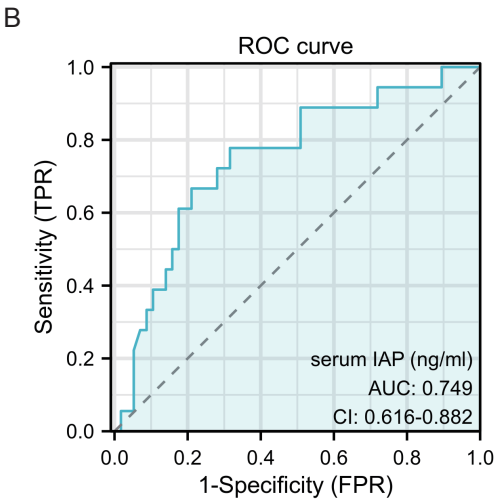
Considering Clavien-Dindo grade <= II as indicative of a favorable abdominal surgical outcome with fewer complications, we constructed ROC plots to assess the relationship between surgical outcomes and preoperative serum IAP (area under the curve (AUC) = 0.749, Youden-Index = 0.4620) (Figure 4B). The ROC analysis identified a cutoff value of 27.289 ng/ml for preoperative serum IAP that optimally balanced sensitivity and specificity in predicting surgical outcomes (Table 3). Patients with preoperative serum IAP

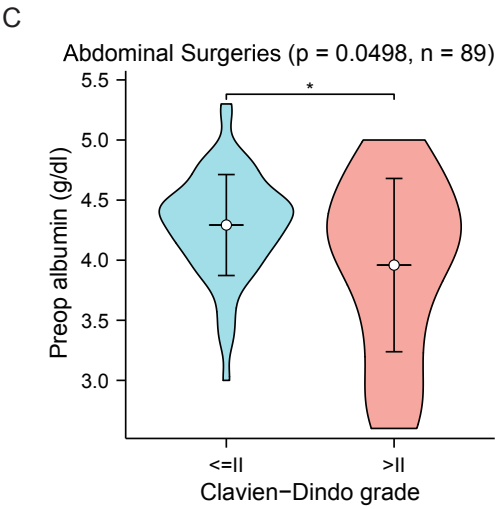
levels higher than 27.289 ng/ml were more likely to be Clavien-Dindo grade <= II after surgeries, while those with levels lower than this cutoff were prone to severe postoperative complications of Clavien-Dindo grade above II.

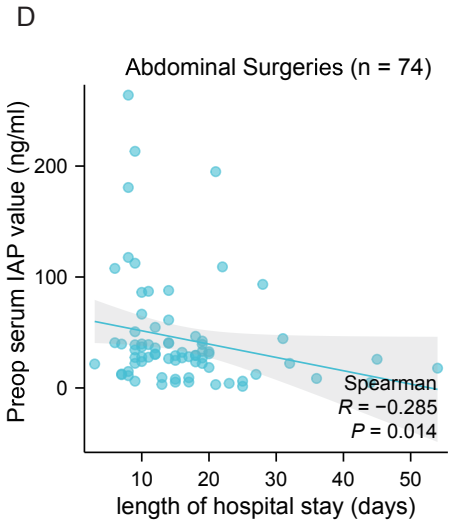
Moreover, as previously mentioned, there was a positive correlation between preoperative serum IAP and preoperative albumin levels ($r = 0.443$, $p < 0.001$) (Table 2). Additionally, we observed that preoperative albumin levels were significantly higher in the Clavien-Dindo grade <= II group compared to the higher-grade group (Clavien-Dindo grade > II vs Clavien-Dindo grade <= II: 3.9591 ± 0.7209 vs 4.2925 ± 0.4201 g/dl, $p = 0.0498$) (Figure 4C).

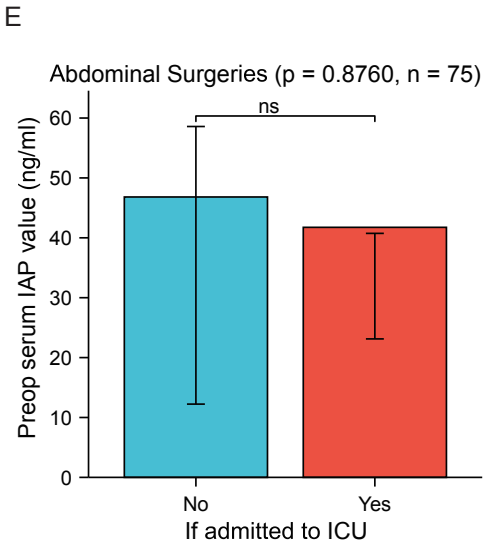
Furthermore, we investigated the role of preoperative serum IAP in relation to the length of hospital stay and ICU stay following abdominal surgeries. We found no significant correlation between hospital stay and preoperative serum IAP ($r = -0.285$, $p = 0.014$) (Figure 4D). When categorizing patients based on ICU admission, we noted no significant difference in preoperative serum IAP levels between the two groups (Yes vs No: 29.328 (23.123, 40.743) vs 28.173 (12.237, 58.584) ng/ml, $p = 0.8760$) (Figure 4E). A negative correlation was observed between the length of ICU stay and preoperative serum IAP among patients who had been in the ICU ($r = -0.375$, $p = 0.012$) (Figure 4F).


A
Abdominal Surgeries (p = 0.0016, n = 75)
**
Preop serum IAP (ng/ml)
250
200
150
100
50
0
<=II
>II
Clavien−Dindo grade


B
ROC curve
Sensitivity (TPR)
1.0
0.8
0.6
0.4
0.2
0.0
serum IAP (ng/ml)
AUC: 0.749
CI: 0.616-0.882
0.0
0.2
0.4
0.6
0.8
1.0
1-Specificity (FPR)


C
Abdominal Surgeries (p = 0.0498, n = 89)
*
Preop albumin (g/dl)
5.5
5.0
4.5
4.0
3.5
3.0
<=II
>II
Clavien−Dindo grade


D
Abdominal Surgeries (n = 74)
Preop serum IAP value (ng/ml)
200
100
0
Spearman
R = −0.285
P = 0.014
10
20
30
40
50
length of hospital stay (days)


E
Abdominal Surgeries (p = 0.8760, n = 75)
ns
Preop serum IAP value (ng/ml)
60
50
40
30
20
10
0
No
Yes
If admitted to ICU

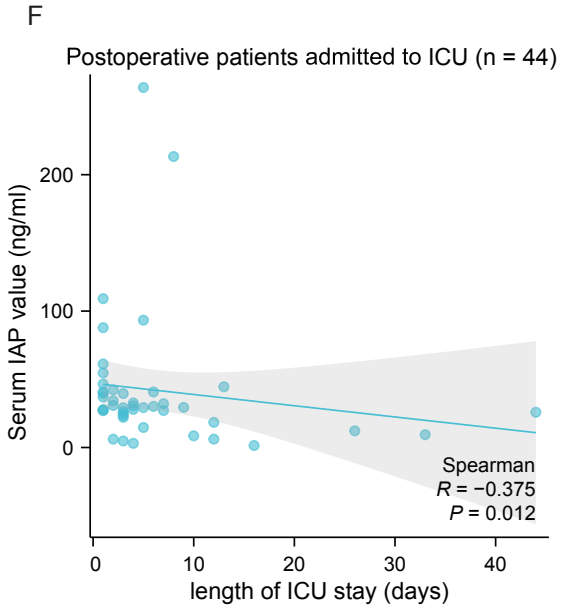

F
Postoperative patients admitted to ICU (n = 44)
Serum IAP value (ng/ml)
200
100
0
Spearman
R = −0.375
P = 0.012
0
10
20
30
40
length of ICU stay (days)

Figure 4: IAP in patients undergoing abdominal surgery.

A) We divided the abdominal postoperative patients into Clavien-Dindo grade <= II and > II groups. The preop serum IAP value was lower in the Clavien-Dindo > II group compared to the Clavien-Dindo <= II group (p = 0.0016). The analysis was conducted on a sample size of 75.

B) ROC plots to assess the relationship between surgical outcomes (Clavien-Dindo grade <= II) and preoperative serum IAP.

C) We divided the abdominal postoperative patients into Clavien-Dindo grade <= II and > II groups. The preop albumin value was lower in the Clavien-Dindo > II group compared to the Clavien-Dindo <= II group (p = 0.0498). The analysis was conducted on a sample size of 89.

D) There was no relationship between preoperative serum IAP and length of hospital stay (r = -0.285; p = 0.014) when all abdominal surgery procedures were included. The analysis was conducted on a sample size of 74.

E) We divided the abdominal postoperative patients into two groups according to whether they had been in the ICU. The two groups had no significant difference in preoperative serum IAP value (p = 0.8760). The analysis was conducted on a sample size of 75.

F) The length of stay in the ICU for postoperative patients had a negative correlation with preoperative serum IAP (p = 0.012, r = -0.375). The sample size for the analysis was 44.

(Abbreviations: IAP: intestinal alkaline phosphatase; preop: preoperative; ROC: receiver operator characteristic curve; AUC: area under the curve; * p< 0.05; ** p< 0.01; ns: insignificant.)

Table 3. Distribution of cases based on ROC cutoffs

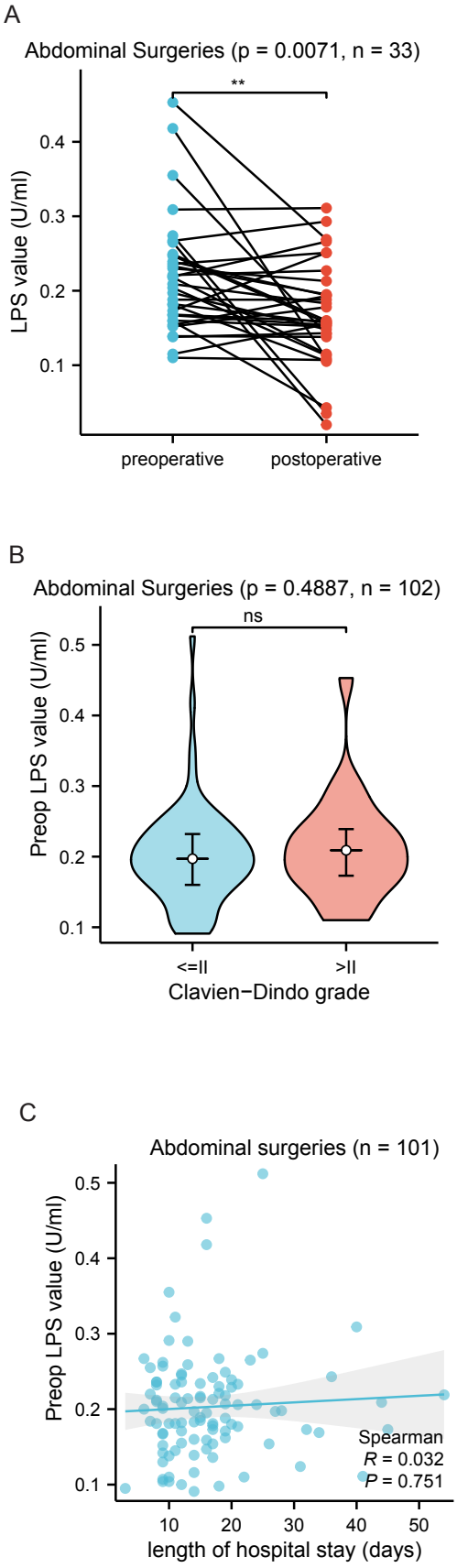
	Preop IAP < 27.289ng/ml	Preop IAP > 27.289ng/ml
Clavien-Dindo grade <= II	18	39
Clavien-Dindo grade > II	14	4

(Abbreviations: IAP: intestinal alkaline phosphatase; preop: preoperative; ROC: receiver operator characteristic curve)

3.6 Preoperative Serum LPS in Patients undergoing Abdominal Surgery

During our study involving participants undergoing abdominal surgery, we observed a significant decrease in postoperative serum LPS levels compared to the preoperative serum LPS levels (preoperative LPS value vs. postoperative LPS value: 0.209 (0.167, 0.247) vs 0.157 (0.138, 0.195) U/ml, p = 0.0071) (Figure 5A).

Additionally, we investigated preoperative serum LPS levels in both Clavien-Dindo grade <=II and Clavien-Dindo grade >II groups. Our findings indicated no significant difference between the Clavien-Dindo grade >II group and the Clavien-Dindo grade <=II group (Clavien-Dindo grade >II vs Clavien-Dindo grade <=II: 0.209 (0.173, 0.239) vs 0.197 (0.16, 0.232) U/ml, p=0.4887) (Figure 5B). Furthermore, we looked into the role of preoperative serum LPS in abdominal surgery, particularly in defining the length of hospital stay and ICU admission/stay as surgical outcomes. Our analysis revealed no significant correlation between hospital stay and preoperative serum LPS (r = 0.032, p = 0.751) (Figure 5C). Categorizing participants based on their ICU admission post abdominal surgeries, we found no substantial difference in preoperative serum LPS levels between the two groups (Yes vs No: 0.2 (0.168, 0.236) vs 0.188 (0.154, 0.233) U/ml, p = 0.5174) (Figure 5D). Moreover, no significant linear correlation was observed between the length of ICU stay and preoperative serum LPS levels among patients who had been in the ICU (r = 0.264, p = 0.034) (Figure 5E).


A
Abdominal Surgeries (p = 0.0071, n = 33)
**
LPS value (U/ml)
0.4
0.3
0.2
0.1
preoperative
postoperative
B
Abdominal Surgeries (p = 0.4887, n = 102)
ns
Preop LPS value (U/ml)
0.5
0.4
0.3
0.2
0.1
<=II
>II
Clavien−Dindo grade
C
Abdominal surgeries (n = 101)
Preop LPS value (U/ml)
0.5
0.4
0.3
0.2
0.1
Spearman
R = 0.032
P = 0.751
10
20
30
40
50
length of hospital stay (days)

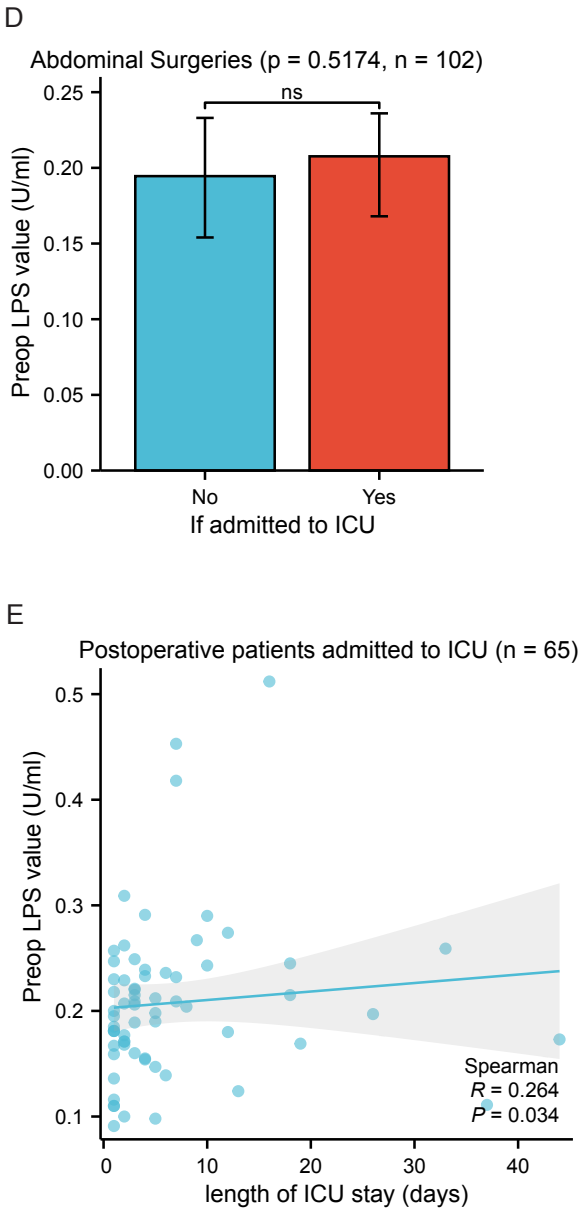


Figure 5: Preoperative serum LPS in patients undergoing abdominal surgery.

A) The Preoperative serum LPS value decreased after abdominal surgery (p = 0.0071). The analysis was conducted on a 33-sample size.

B) We divided all the postoperative abdominal patients into two groups according to their Clavien-Dindo grade： those with Clavien-Dindo grade <= II and those with Clavien-Dindo grade > II. There was no significant difference in preoperative serum LPS between Clavien-Dindo grade <= II and Clavien-Dindo grade > II groups (p = 0.4887). The analysis was conducted on a sample size of 102.

C) In all general surgeries, there was no significant correlation between preoperative serum LPS value and the length of hospital stay (p = 0.751, r = 0.032). The analysis was conducted on a sample size of 101.

D) We divided the abdominal postoperative patients into two groups according to whether they had been in the ICU. The two groups had no significant difference in preoperative serum LPS value ($p = 0.5174$). The analysis was conducted on a sample size of 102.

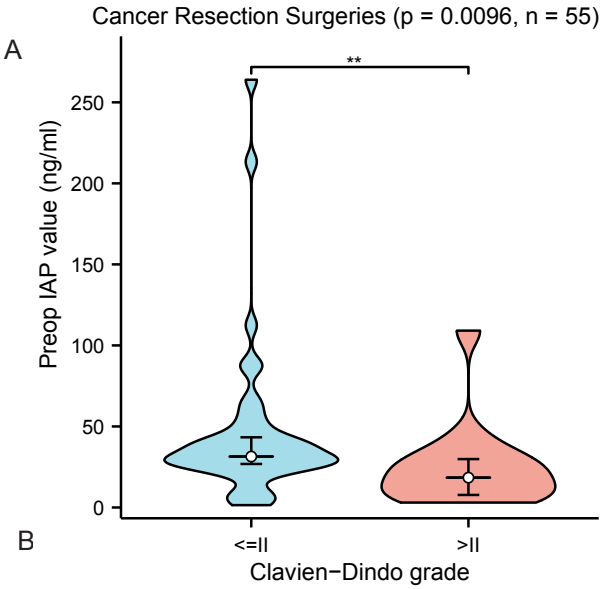
E) For postoperative patients in the ICU, there was no significant correlation between preoperative serum LPS value and the length of ICU stay ($p = 0.034$, $r = 0.264$). The analysis was conducted on a sample size of 65.

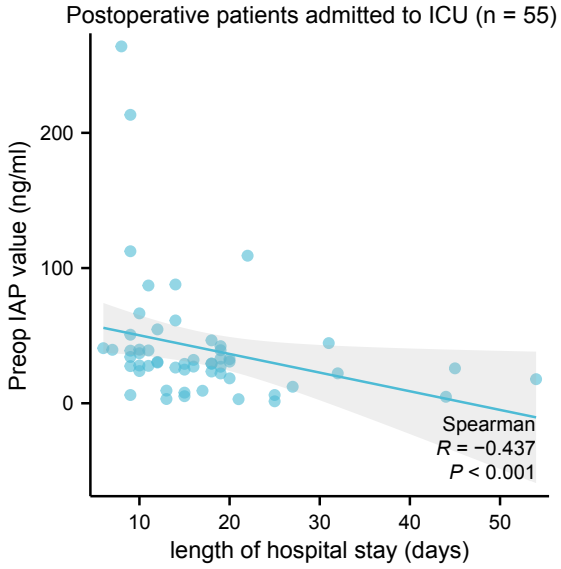
(Abbreviations: preop: preoperative; LPS: lipopolysaccharides; ns: insignificant; ** $p< 0.01$.)

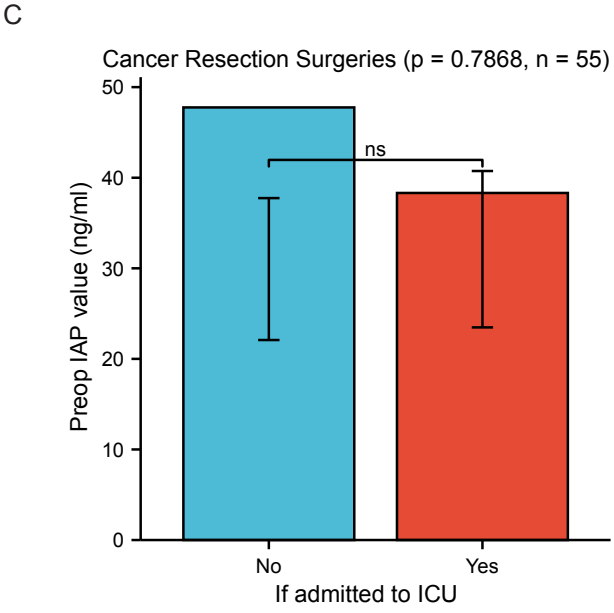
3.7 Preoperative Serum IAP in Patients undergoing Cancer Resection

Among patients undergoing cancer resection surgeries, patients in Clavien-Dindo grade >II group had lower preoperative serum IAP value compared to the patients in Clavien-Dindo grade <=II group (Clavien-Dindo grade >II vs Clavien-Dindo grade <=II: 31.33 (26.85, 43.31) vs 18.415 (7.738, 29.873) U/ml, $p = 0.0096$) (Figure 6A).

Furthermore, we investigated the impact of preoperative serum IAP on surgical outcomes, specifically hospital stay and ICU admission/stay, in cancer resection surgeries. We found a significant negative correlation between hospital stay and preoperative serum IAP ($r = -0.437$, $p < 0.001$) (Figure 6B). When categorizing participants based on ICU admission after abdominal surgeries, there was no significant difference in preoperative serum IAP levels between the two groups (Yes vs No: 30.578 (23.477, 40.741) vs 28.541 (22.081, 33.749) ng/ml, $p = 0.7868$) (Figure 6C). Among cancer patients admitted to the ICU, a negative linear correlation was observed between the length of ICU stay and preoperative IAP ($r = -0.413$, $p = 0.008$) (Figure 6D).


A
Cancer Resection Surgeries (p = 0.0096, n = 55)
**
Preop IAP value (ng/ml)
250
200
150
100
50
0
B
<=II
>II
Clavien−Dindo grade


Postoperative patients admitted to ICU (n = 55)
Preop IAP value (ng/ml)
200
100
0
Spearman
R = −0.437
P < 0.001
10
20
30
40
50
length of hospital stay (days)


C
Cancer Resection Surgeries (p = 0.7868, n = 55)
ns
Preop IAP value (ng/ml)
50
40
30
20
10
0
No
Yes
If admitted to ICU

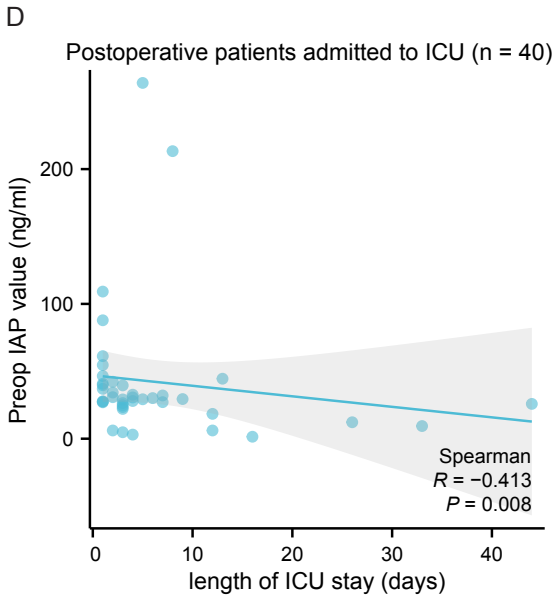


Figure 6: Preoperative serum IAP in patients undergoing cancer resection.

A) We divided all the postoperative cancer resection patients into two groups according to their Clavien-Dindo grade： those with Clavien-Dindo grade <= II and those with Clavien-Dindo grade > II. Those with Clavien-Dindo grade > II had lower preoperative serum IAP than those with Clavien-Dindo grade <= II (p = 0.0096). The analysis was conducted on a sample size of 55.

B) In all cancer resection surgeries, preoperative serum IAP negatively correlated with the length of hospital stay (p = 0.751, r = 0.032). T(p < 0.001, r = -0.437). The analysis was conducted on a sample size of 55.

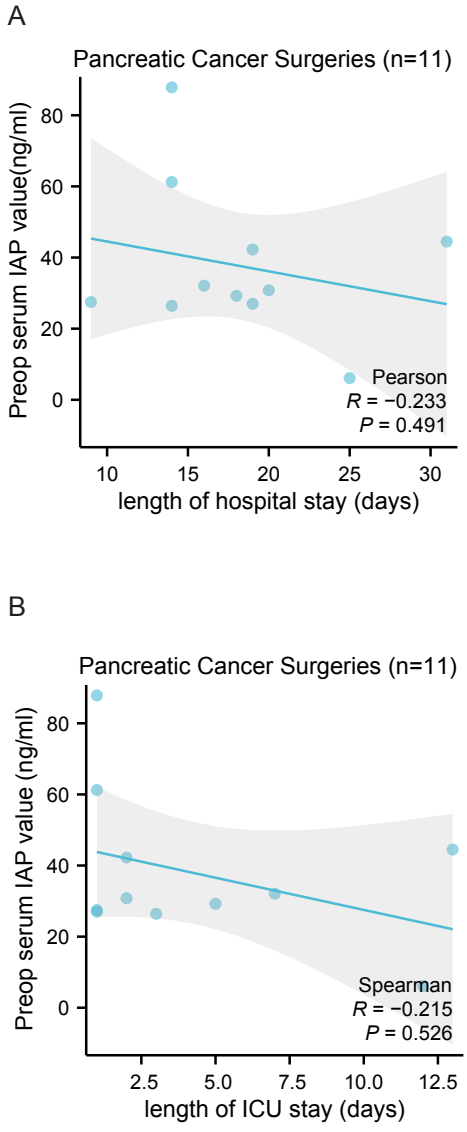
C) We divided the postoperative cancer resection patients into two groups according to whether they had been in the ICU. The two groups had no significant difference in preoperative serum IAP value (p = 0.7868). The analysis was conducted on a sample size of 55.

D) For postoperative cancer resection patients in the ICU, the preoperative serum IAP value negatively correlated with the length of ICU stay (p = 0.008, r = -0.413). The analysis was conducted on a sample size of 40.

(Abbreviations: IAP: intestinal alkaline phosphatase; ICU: intensive care unit; ns: insignificant; ** p< 0.01.)

3.7.1 Preoperative Serum IAP in Patients undergoing Pancreatic Cancer Resection

Among pancreatic cancer patients undergoing surgery, we found no significant linear correlation between the length of hospital stay and preoperative serum IAP (r = -0.233, p = 0.491) (Figure 7A). There is not enough data for us to group the participants regarding whether they had been in the ICU after pancreatic cancer resection surgeries. Among the postoperative pancreatic cancer patients in the ICU, we found no significant linear correlation between preoperative IAP value and ICU stay (r = -0.215, p = 0.526) (Figure 7B).



-

Figure 7: Preoperative serum IAP in patients undergoing pancreatic cancer resection surgeries.

A) In pancreatic cancer resection surgeries, there was no significant relationship between preoperative serum IAP and the length of hospital stay (p = 0.491, r = -0.233). The analysis was conducted on a sample size of 11.
B) For postoperative pancreatic cancer resection patients in the ICU, there was no significant relationship between preoperative serum IAP and the length of ICU stay (p = 0.526, r = -0.215). The analysis was conducted on a sample size of 11.

(Abbreviations: IAP: intestinal alkaline phosphatase; preop: preoperative.)

3.7.2 Preoperative Serum IAP in Patients undergoing CRC Resection Surgeries

Among CRC patients undergoing surgery, we observed no significant difference in preoperative IAP values between the Clavien-Dindo grade >II group and the Clavien-Dindo grade <=II group (Clavien-Dindo grade >II vs Clavien-Dindo grade <=II: 32.646 (25.229, 32.82) vs 45.148 (23.373, 71.638) ng/ml, p = 0.6286) (Figure 8 A). Additionally, we investigated the role of preoperative serum IAP in CRC resection, specifically regarding its association with hospital stay. Our analysis indicated no significant correlation between hospital stay and preoperative serum IAP (r = -0.546, p = 0.205) (Figure 8B).

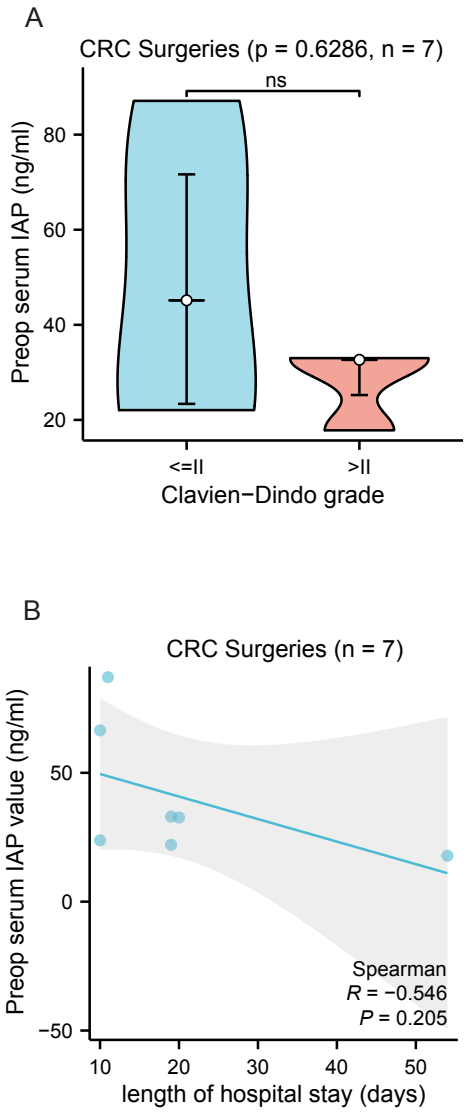


Figure 8: Preoperative serum IAP in patients undergoing CRC resection

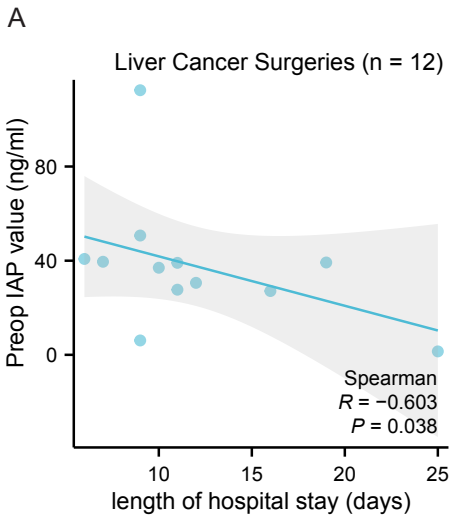
A) We divided all the postoperative CRC resection patients into two groups according to their Clavien-Dindo grade： those with Clavien-Dindo grade <= II and those with Clavien-Dindo grade > II. There was no significant difference in preoperative serum IAP between the Clavien-Dindo grade > II group and the Clavien-Dindo grade <= II group (p = 0.6286). The analysis was conducted on a sample size of 7.

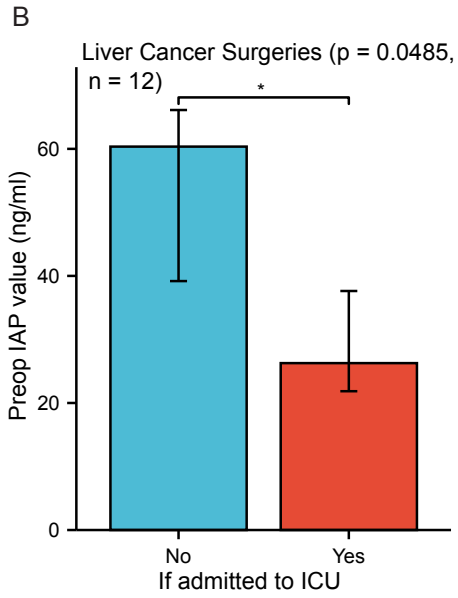
B) In all CRC resection surgeries, There was no significant correlation between preoperative serum IAP and the length of hospital stay (p = 0.205, r = -0.546). The analysis was conducted on a sample size of 7.

(Abbreviations: IAP: intestinal alkaline phosphatase; preop: preoperative; CRC: colorectal cancer)

3.7.3 Preoperative Serum IAP in Patients undergoing Liver Cancer Resection

Among liver cancer patients undergoing surgery, we discovered a negative correlation between hospital stay and preoperative serum IAP ($r = -0.603$, $p = 0.038$) (refer to Figure 9A). Further, when categorizing participants based on their admission to the ICU following liver cancer surgeries, we noted a difference in preoperative serum IAP levels between the two groups (Yes vs No: 29.124 (21.85, 37.627) vs 44.954 (39.19, 66.117) ng/ml, $p = 0.0485$) (see Figure 9B). However, no significant linear correlation was observed between the length of ICU stay and preoperative serum IAP ($r = -0.586$, $p = 0.127$) (Figure 9C).


A
Liver Cancer Surgeries (n = 12)
Preop IAP value (ng/ml)
80
40
0
Spearman
R = −0.603
P = 0.038
10
15
20
25
length of hospital stay (days)


B
Liver Cancer Surgeries (p = 0.0485,
n = 12)
*
Preop IAP value (ng/ml)
60
40
20
0
No
Yes
If admitted to ICU

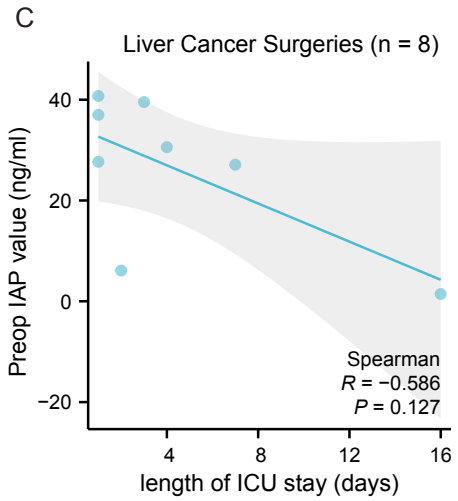

C
Liver Cancer Surgeries (n = 8)
Preop IAP value (ng/ml)
40
20
0
−20
Spearman
R = −0.586
P = 0.127
4
8
12
16
length of ICU stay (days)

Figure 9: Preoperative serum IAP in patients undergoing liver cancer resection

A）In all liver cancer resection surgeries, preoperative serum IAP negatively correlated with the length of hospital stay (p = 0.038, r = -0.603). The analysis was conducted on a sample size of 12.

B）We divided the postoperative liver cancer resection patients into two groups according to whether they have been in ICU. The postoperative liver cancer resection patients who have been in the ICU had lower preoperative serum IAP values compared to those who have not been in the ICU (p = 0.0485). The analysis was conducted on a sample size of 12.

C）In the postoperative liver cancer resection patients in the ICU, there was no significant correlation between preoperative serum IAP value and the length of ICU stay (p = 0.127, r = -0.586). The analysis was conducted on a sample size of 8.

(Abbreviations: IAP: intestinal alkaline phosphatase; ns: not significant; preop: preoperative; * p< 0.05)

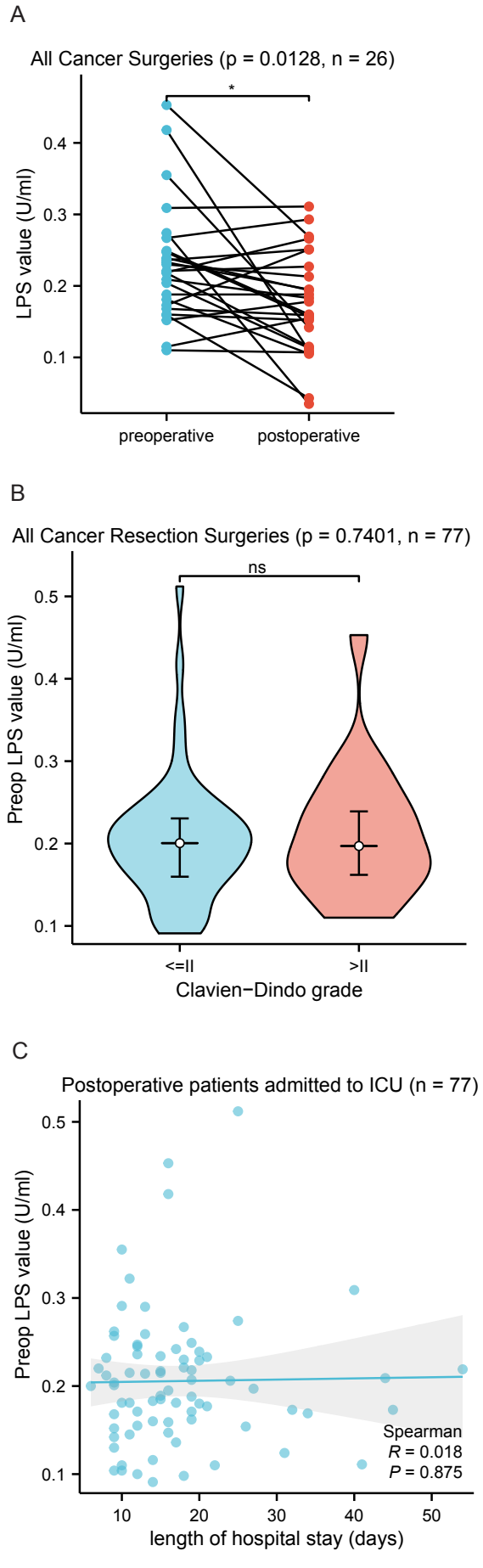
3.8 Preoperative Serum LPS in Patients undergoing Cancer Resection Surgeries

Within the cohort undergoing cancer resection surgeries in our study, we observed a decrease in LPS values post-surgeries (pre-operative LPS value vs postoperative LPS value: 0.22 (0.175, 0.2485) vs 0.169 (0.1218, 0.2235) U/ml, p = 0.0128) (Figure 10A).

Additionally, we investigated preoperative serum LPS values in both the Clavien-Dindo grade <=II and Clavien-Dindo grade >II groups, finding no significant difference between the two (Clavien-Dindo grade >II vs Clavien-Dindo grade <=II: 0.197 (0.162, 0.239) vs 0.2005 (0.1598, 0.2305) U/ml, p = 0.7401) (Figure 10B).

Moreover, in assessing the role of preoperative serum LPS in cancer resection surgeries regarding hospital stay and ICU admission/stay as surgical outcomes, we noted no significant correlation between hospital stay and preoperative serum LPS (r = 0.018, p = 0.875) (Figure 10C). When categorizing participants based on ICU admission post-cancer resection surgeries, there was no significant difference in preoperative serum LPS levels between the two groups (Yes vs No: 0.204 (0.1685, 0.2375) vs 0.1855 (0.1468, 0.2185) U/ml, p = 0.3928) (Figure 10D). Moreover, no linear correlation was observed between

the length of ICU stay and preoperative serum LPS among cancer resection patients ($r = 0.257$, $p = 0.050$) (Figure 10E).


A
All Cancer Surgeries (p = 0.0128, n = 26)
*
LPS value (U/ml)
0.4
0.3
0.2
0.1
preoperative
postoperative
B
All Cancer Resection Surgeries (p = 0.7401, n = 77)
ns
Preop LPS value (U/ml)
0.5
0.4
0.3
0.2
0.1
<=II
>II
Clavien−Dindo grade
C
Postoperative patients admitted to ICU (n = 77)
Preop LPS value (U/ml)
0.5
0.4
0.3
0.2
0.1
Spearman
R = 0.018
P = 0.875
10
20
30
40
50
length of hospital stay (days)

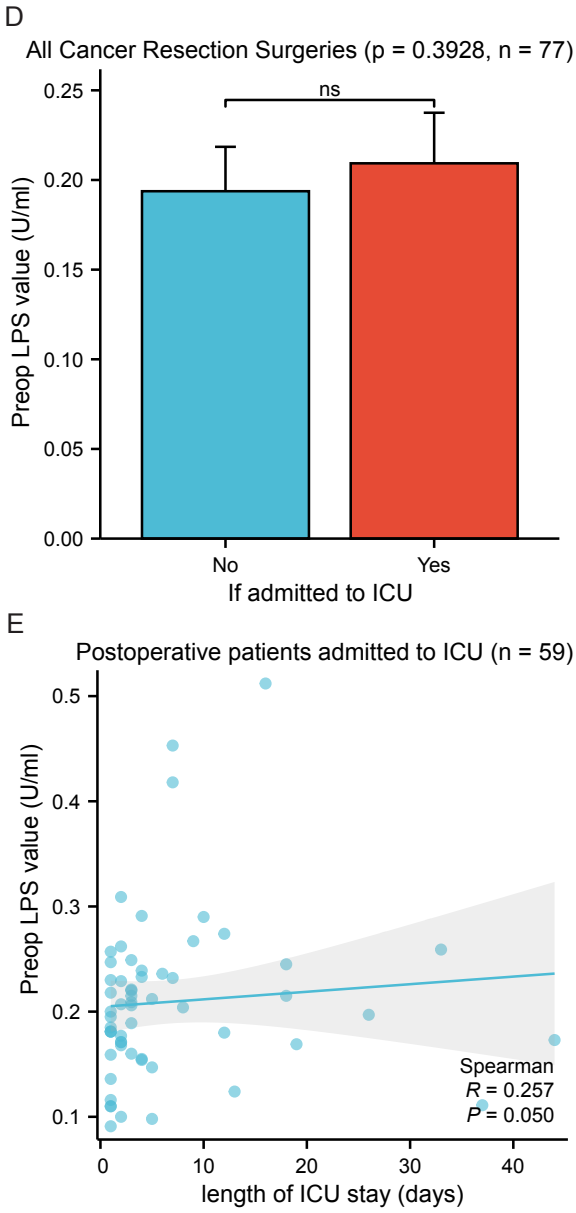


Figure 10: Preoperative serum LPS in patients undergoing cancer resection surgeries.

A) Preoperative serum LPS decreased after cancer resection surgeries (p = 0.0128). The analysis was conducted on a sample size of 55.

B) We divided all the postoperative cancer resection patients into two groups according to their Clavien-Dindo grade： those with Clavien-Dindo grade <= II and those with Clavien-Dindo grade > II. There was no significant difference in preoperative serum LPS between the Clavien-Dindo grade > II group and the Clavien-Dindo grade <= II group. (p = 0.7401). The analysis was conducted on a sample size of 77.

C) In all cancer resection surgeries, there was no significant difference between preoperative serum LPS and the length of hospital stay ($p = 0.875$, $r = 0.018$). The analysis was conducted on a sample size of 77.

D) We divided the postoperative cancer resection patients into two groups according to whether they had been in the ICU. The two groups had no significant difference in preoperative serum LPS value ($p = 0.3928$). The analysis was conducted on a sample size of 77.

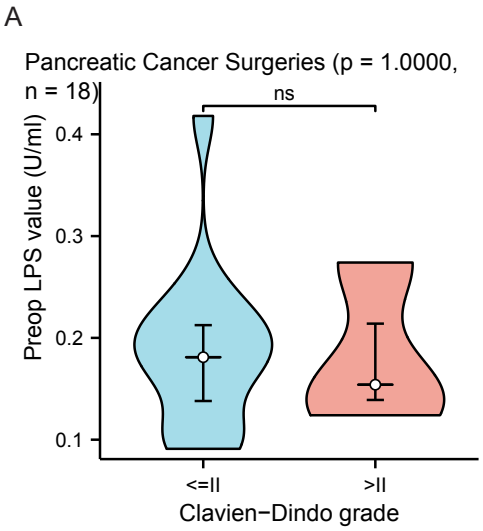
E) In the postoperative cancer resection patients in the ICU, there was no significant relationship between preoperative serum LPS and the length of ICU stay ($p = 0.050$, $r = 0.257$). The analysis was conducted on a sample size of 59.

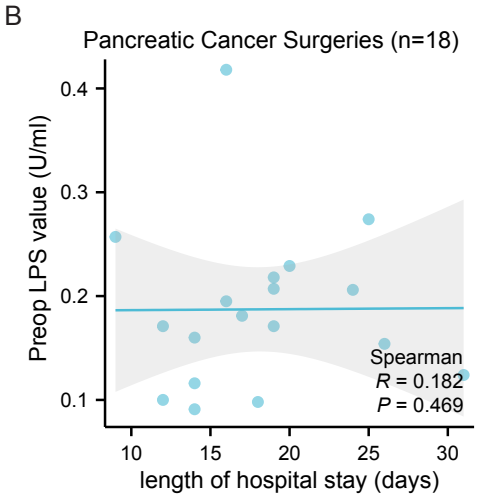
(Abbreviations: preop: preoperative; LPS: lipopolysaccharides; ns: insignificant.)

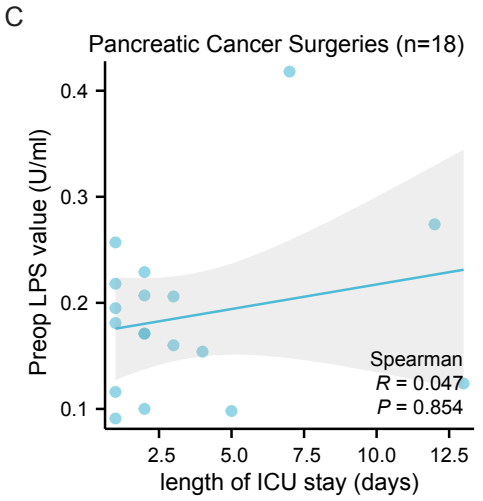
3.8.1 Preoperative Serum LPS in Patients undergoing Pancreatic Cancer Resection

We observed no difference in preoperative serum LPS values between the Clavien-Dindo grade >II group and the Clavien-Dindo grade <=II group (Clavien-Dindo grade >II vs Clavien-Dindo grade <=II: 0.154 (0.139, 0.214) vs 0.181 (0.138, 0.2125) U/ml, $p = 1$) (Figure 11A).

Additionally, we examined the role of preoperative serum LPS in pancreatic surgeries concerning its association with hospital stay and ICU admission/stay as surgical outcomes. Our analysis revealed no significant correlation between hospital stay and preoperative serum LPS ($r = 0.182$, $p = 0.469$) (Figure 11B). Unfortunately, there wasn't enough available data to categorize participants based on ICU admission following pancreatic cancer resection surgeries. Furthermore, no significant linear correlation was found between the length of ICU stay and preoperative serum LPS ($r = 0.047$, $p = 0.854$) (Figure 11C).


A
Pancreatic Cancer Surgeries (p = 1.0000, n = 18)
ns
Preop LPS value (U/ml)
0.4
0.3
0.2
0.1
<=II
>II
Clavien−Dindo grade


B
Pancreatic Cancer Surgeries (n=18)
Preop LPS value (U/ml)
0.4
0.3
0.2
0.1
Spearman
R = 0.182
P = 0.469
10
15
20
25
30
length of hospital stay (days)


C
Pancreatic Cancer Surgeries (n=18)
Preop LPS value (U/ml)
0.4
0.3
0.2
0.1
Spearman
R = 0.047
P = 0.854
2.5
5.0
7.5
10.0
12.5
length of ICU stay (days)

-

Figure 11: Preoperative serum LPS in patients undergoing pancreatic cancer resection surgeries.

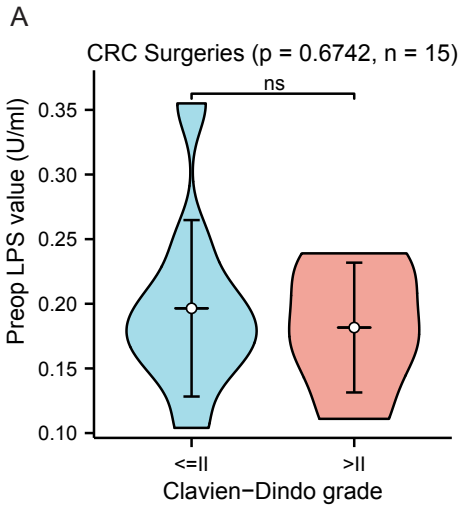
A) We divided all the postoperative pancreatic cancer resection patients into two groups according to their Clavien-Dindo grade： those with Clavien-Dindo grade <= II and those with Clavien-Dindo grade > II. There was no significant difference in preoperative serum LPS between the Clavien-Dindo grade > II group and the Clavien-Dindo grade <= II group. (p = 1). The analysis was conducted on a sample size of 18.
B) In all pancreatic cancer resection surgeries, there was no significant difference between preoperative serum LPS and the length of hospital stay (p = 0.469, r = 0.182). The analysis was conducted on a sample size of 18.
C) In the postoperative pancreatic cancer resection patients in the ICU, there was no significant relationship between preoperative serum LPS and the length of ICU stay (p = 0.854, r = 0.047). The analysis was conducted on a sample size of 18.

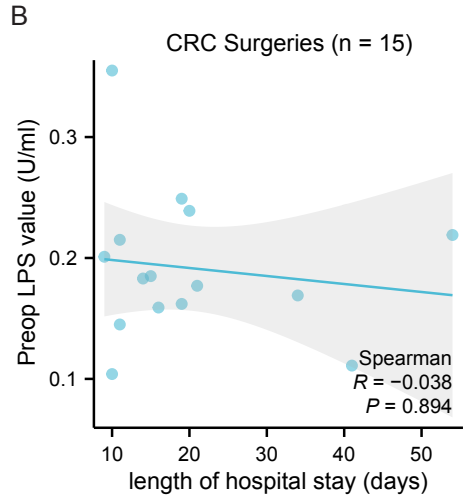
(Abbreviations: preop: preoperative; LPS: lipopolysaccharides; ns: insignificant.)

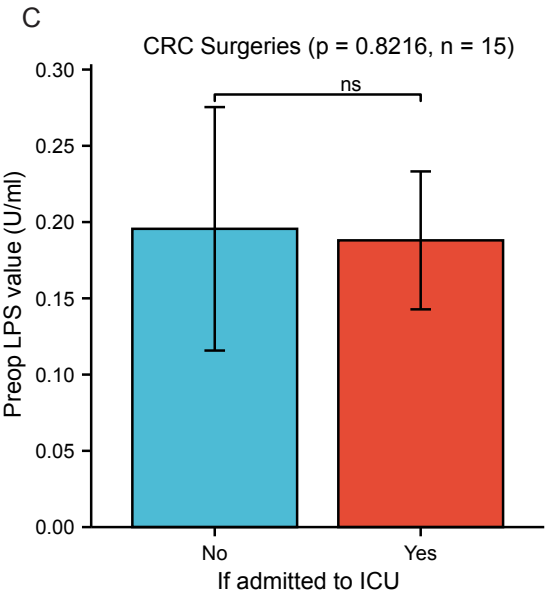
3.8.2 Preoperative Serum LPS in Patients undergoing CRC Resection

Among CRC patients undergoing surgery, we observed no significant difference in preoperative LPS levels between the Clavien-Dindo grade >II group and the Clavien-Dindo grade <=II group (Clavien-Dindo grade >II vs Clavien-Dindo grade <=II: 0.1816 ± 0.0502 vs 0.1965 ± 0.0683 U/ml, p = 0.6742) (Figure 12A).

Additionally, we investigated the role of preoperative serum LPS in CRC resection surgeries concerning its correlation with hospital stay and ICU admission/stay as surgical outcomes. Our analysis indicated no correlation between hospital stay and preoperative serum LPS (r = -0.038, p = 0.894) (Figure 12B). Categorizing CRC surgery participants based on their ICU admission post-surgeries, we found no significant difference in preoperative serum LPS levels between the two groups (Yes vs No: 0.188 ± 0.0452 vs 0.1956 ± 0.0798 U/ml, p = 0.8216) (refer to Figure 12C). Moreover, no significant linear correlation was observed between the length of ICU stay and preoperative serum LPS (r = -0.180, p = 0.670) (Figure 12D).


A
CRC Surgeries (p = 0.6742, n = 15)
ns
Preop LPS value (U/ml)
0.35
0.30
0.25
0.20
0.15
0.10
<=II
>II
Clavien−Dindo grade


B
CRC Surgeries (n = 15)
Preop LPS value (U/ml)
0.3
0.2
0.1
Spearman
R = −0.038
P = 0.894
10
20
30
40
50
length of hospital stay (days)


C
CRC Surgeries (p = 0.8216, n = 15)
ns
Preop LPS value (U/ml)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
No
Yes
If admitted to ICU

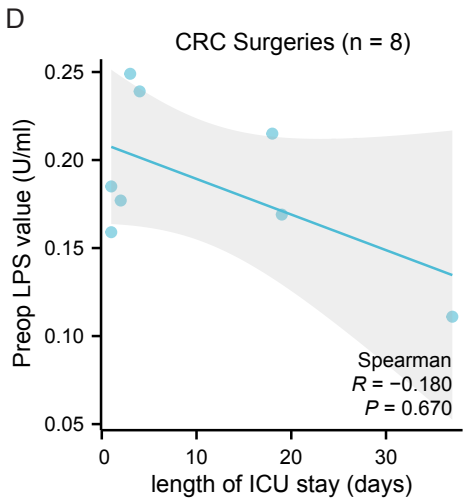


Figure 12: Preoperative serum LPS in patients undergoing CRC resection surgeries.

A) We divided all the postoperative CRC resection patients into two groups according to their Clavien-Dindo grade：those with Clavien-Dindo grade = II and those with Clavien-Dindo grade > II. There was no significant difference in preoperative serum LPS between the Clavien-Dindo grade > II group and the Clavien-Dindo grade = II group (p = 0.6742). The analysis was conducted on a sample size of 15.

B) In all CRC resection surgeries, there was no significant difference between preoperative serum LPS and the length of hospital stay (p = 0.894, r = -0.038). The analysis was conducted on a sample size of 15.

C) We divided the postoperative CRC resection patients into two groups according to whether they had been in the ICU. The two groups had no significant difference in preoperative serum LPS value (p = 0.8216). The analysis was conducted on a sample size of 15.

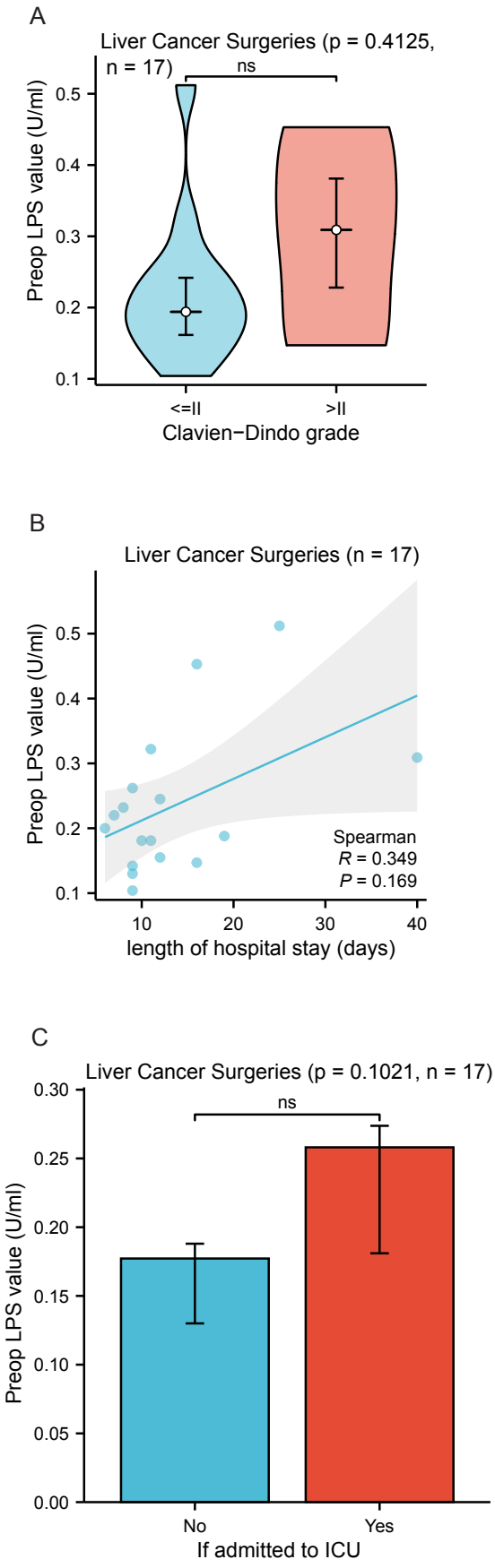
D) In the postoperative CRC resection patients in the ICU, there was no significant relationship between preoperative serum LPS and the length of ICU stay (p = 0.670, r = -0.180). The analysis was conducted on a sample size of 8.

(Abbreviations: preop: preoperative; LPS: lipopolysaccharides; CRC: colorectal cancer; ns: insignificant.)

3.8.3 Preoperative Serum LPS in Patients undergoing Liver Cancer Resection Surgeries

Within liver cancer patients undergoing surgery, we found no significant difference in LPS values between the Clavien-Dindo grade >II group and the Clavien-Dindo grade <=II group (Clavien-Dindo grade >II vs Clavien-Dindo grade <=II: 0.309 (0.228, 0.381) vs 0.194 (0.1615, 0.2418) U/ml, p = 0.4125) (refer to Figure 13A).

Furthermore, we investigated the role of preoperative serum LPS in liver cancer surgeries concerning its association with hospital stay and ICU admission/stay as surgical outcomes. Our analysis revealed no correlation between hospital stay and preoperative serum LPS (r = 0.349, p = 0.169) (Figure 13 B). When categorizing participants based on ICU admission post-liver cancer surgeries, no significant difference in preoperative serum LPS levels was found between the two groups (ICU vs. No ICU: 0.142 (0.13, 0.188) U/ml, p = 0.1021) (Figure 13C). Moreover, no significant linear correlation was observed between the length of ICU stay and preoperative serum LPS (r = 0.421, p = 0.173) (Figure 13D).


A
Liver Cancer Surgeries (p = 0.4125, n = 17)
ns
Preop LPS value (U/ml)
0.5
0.4
0.3
0.2
0.1
<=II
>II
Clavien−Dindo grade
B
Liver Cancer Surgeries (n = 17)
Preop LPS value (U/ml)
Spearman
R = 0.349
P = 0.169
10
20
30
40
length of hospital stay (days)
C
Liver Cancer Surgeries (p = 0.1021, n = 17)
ns
Preop LPS value (U/ml)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
No
Yes
If admitted to ICU

-

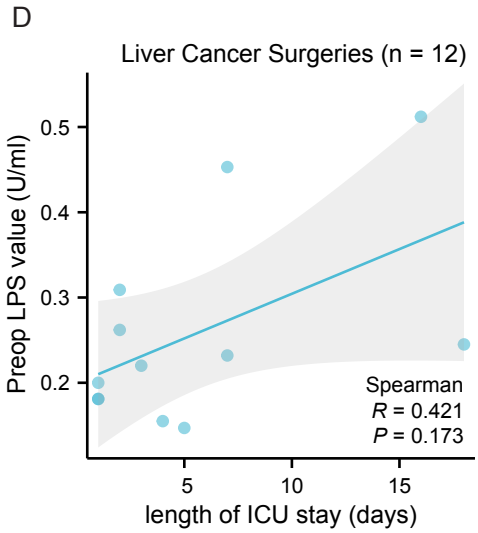


Figure 13: Preoperative serum LPS in patients undergoing liver cancer resection surgeries.

A) We divided all the postoperative liver cancer resection patients into two groups according to their Clavien-Dindo grade: those with Clavien-Dindo grade <= II and those with Clavien-Dindo grade > II. There was no significant difference in preoperative serum LPS between the Clavien-Dindo grade > II group and the Clavien-Dindo grade <= II group. (p = 0.4125). The analysis was conducted on a sample size of 17.

B) In all liver cancer resection surgeries, there was no significant difference between preoperative serum LPS and the length of hospital stay (p = 0.169, r = 0.349). The analysis was conducted on a sample size of 17.

C) We divided the postoperative liver cancer resection patients into two groups according to whether they had been in the ICU. The two groups had no significant difference in preoperative serum LPS value (p = 0.1021). The analysis was conducted on a sample size of 17.

D) In the postoperative liver cancer resection patients in the ICU, there was no significant relationship between preoperative serum LPS and the length of ICU stay (p = 0.173, r = 0.421). The analysis was conducted on a sample size of 12.

(Abbreviations: preop: preoperative; LPS: lipopolysaccharides; ns: insignificant.)

4. Discussion

The intestinal microbiota is a key source of circulating LPS, with elevated LPS levels often reflecting microbial dysbiosis or compromised intestinal permeability [71, 72]. This makes serum LPS a promising noninvasive biomarker for evaluating gut microbiota health and gut barrier integrity. IAP plays an essential role in maintaining and restoring intestinal homeostasis, partly by detoxifying bacterial LPS through dephosphorylation of lipid A [13, 73, 74]. By doing so, IAP prevents the activation of inflammatory pathways, promotes the development of a healthy microbiota, and supports the integrity of the intestinal barrier [50, 51].

Our study builds on previous findings, reinforcing the significant negative correlation between preoperative stool IAP and preoperative serum LPS levels [75]. This correlation was consistently observed in our larger cohort, which includes a broader range of diseases, further validating the role of IAP in regulating LPS levels. The negative relationship between stool IAP and serum LPS suggests that higher intestinal IAP activity may help reduce circulating LPS, potentially reflecting better gut barrier integrity and microbial homeostasis. These results align with previous animal studies, where increased stool IAP activity through IAP treatment or IAP enhancers significantly reduced serum LPS levels [76]. This highlights the potential of stool IAP as a biomarker for gut health and its therapeutic implications for managing conditions associated with elevated serum LPS [77].

The strong correlation between preoperative stool IAP activity and serum IAP levels suggests that serum IAP may be a reliable biomarker for assessing intestinal health and inflammation. This finding is significant, as it indicates that serum IAP levels could reflect changes in gut integrity and function, mirrored by alterations in stool IAP activity [75]. Previous studies have shown that intravenous IAP administration in animal models significantly reduces LPS-induced inflammatory responses, and intraperitoneal IAP injections have been found to lower proinflammatory cytokines, such as TNF-α, IL-6, and IL-1β [78, 79]. These results underscore the role of serum IAP in modulating systemic inflammation, regulating gut barrier integrity, and its potential as a therapeutic target for inflammatory conditions and gut microbial disturbances.

Our study revealed important associations between preoperative serum IAP levels and key clinical parameters, further supporting its role in systemic inflammation and gut health. The negative correlation between preoperative serum IAP and the inflammatory marker IL-6 suggests that IAP may modulate systemic inflammation [80]. Additionally,

the positive correlation between serum IAP and albumin levels points to a potential link between gut health and nutritional status [81]. The negative association between serum IAP and CRP reinforces the idea that serum IAP could serve as a marker for systemic inflammation [81]. While these findings provide valuable insights, further research is needed to understand the mechanisms underlying these correlations and explore IAP's therapeutic potential in managing inflammation and maintaining gut integrity.

Our study provides valuable insights into the potential role of preoperative serum IAP as a biomarker for cancer diagnosis and monitoring. As previously discussed, IAP plays a crucial role in maintaining intestinal homeostasis by dephosphorylating LPS, thus reducing inflammation and preserving gut barrier integrity [74, 82, 83]. Both endogenous and exogenous IAP have been shown to inhibit LPS absorption in animal studies, further supporting its anti-inflammatory effects [27]. Since chronic inflammation and gut barrier dysfunction are key drivers of cancer development, IAP's ability to modulate both inflammation and gut barrier integrity suggests it could play an essential role in cancer prevention and progression [84-86]. Our findings of elevated preoperative serum IAP levels in cancer patients, compared to healthy controls, further support the hypothesis that preoperative serum IAP may be associated with malignancies—either due to its production by certain cancer cells or as part of a systemic response to tumorigenesis [48, 87-89]. These findings position serum IAP as a promising biomarker for cancer detection and monitoring, given its dual role in regulating gut barrier integrity and influencing cancer progression. Further research is required to clarify the mechanisms linking IAP, gut barrier function, and cancer to assess its potential clinical value in cancer diagnostics and therapeutic strategies.

We observed that serum IAP levels were significantly elevated in patients with pancreatic cancer and CRC compared to healthy controls, underscoring a potential association between elevated IAP and these specific malignancies. However, this pattern was not seen in liver cancer patients, suggesting that serum IAP levels may vary across different cancer types due to distinct biological mechanisms or tumor microenvironmental factors [47, 90, 91]. This discrepancy may also result from insufficient data, underscoring the need for further research to clarify these findings.

Additionally, stratifying specific cancer patients according to UICC stages revealed no significant differences in preoperative serum IAP levels across different stages. The lack of stage-specific variation in serum IAP levels implies that IAP might not be a reliable marker for assessing cancer progression or determining the stage of the disease. Future

research should focus on identifying other biomarkers that might provide more stage-specific information or on combining IAP with other markers to enhance its prognostic value [92].

However, the lack of significant differences in serum IAP levels between cancer patients and non-cancer cohorts introduces complexity as standalone diagnostic tools. This finding implies that while IAP might be involved in cancer-related processes, they are not exclusive to malignancy and may be influenced by other physiological or pathological conditions [93, 94]. This complexity highlights the need for more comprehensive studies involving more extensive and diverse control cohorts to understand better the variability of these biomarkers across different cancer types and stages.

Despite differences in serum LPS levels between cancer patients and healthy controls or non-cancer groups, it's important to note that previous studies have shown higher serum LPS levels in pancreatic cancer patients compared to non-cancer patients [75]. This discrepancy could be due to variations in sample size, patient populations, or the context-dependent nature of LPS elevation influenced by factors such as disease severity, treatment status, or variations in the tumor microenvironment. Further studies with larger sample sizes are necessary to explore the role of LPS in cancer fully.

However, previous studies have proved the molecular mechanism of LPS-promoted cancer. LPS modulates TNF-related apoptosis-inducing ligand (TRAIL)- which induces apoptosis in pancreatic cancer cells by altering the expression of TRAIL receptors and osteoprotegerin levels, thereby promoting apoptosis resistance in TRAIL-sensitive cell lines [95]. LPS also downregulates miR-181a, a microRNA that targets two tumor suppressors, PTEN and MAP2K4, leading to their inhibition [96]. Moreover, LPS increases the expression of TLR4 in pancreatic cancer cells and tissues, which in turn enhances cancer cell migration [96]. LPS also promotes pancreatic cancer progression by upregulating trefoil factor 2, which activates the β-catenin pathway, enhancing cell proliferation and inhibiting apoptosis [97]. Besides, LPS activates the NLRP3 inflammasome in pancreatic cancer cells, which increases cell proliferation by enhancing caspase-1 activity and IL-1β production [97]. Additionally, LPS activates the NOD-like receptor family pyrin domain containing 3 inflammasomes in pancreatic cancer cells, which increases cell proliferation by enhancing caspase-1 activity and IL-1β production [97].

Besides, LPS promotes CRC cell migration and invasion by upregulating the expression of the long non-coding RNA LINC00152 in human colon cells, which induces histone

acetylation on the LINC00152 promoter and reduces the binding of the repressor YY1 [58]. LPS also promotes CRC progression by inducing macrophage infiltration, which secretes C-C motif chemokine ligand 5, stabilizing PD-L1 on cancer cells and facilitating immune escape by inhibiting T-cell-mediated killing [98]. Besides, LPS promotes CRC progression by inducing inflammation through the TLR4/MyD88/NF-κB signaling pathway and disrupting the gut microbiota, contributing to tumorigenesis [99]. Moreover, LPS contributes to colitis-associated colorectal tumorigenesis by promoting inflammation through the TLR4/NF-κB p65/IL-6/p-STAT3 signaling pathway, disrupting gut microbiota and compromising intestinal barrier function [56]. LPS also promotes CRC progression by upregulating miR-140, which suppresses TRAF6 [100]. This suppression modulates inflammatory cytokine secretion, driving CRC progression [100].

Furthermore, LPS contributes to liver cancer by inducing inflammation and ferroptosis, driven by excessive reactive oxygen species production and the release of inflammatory cytokines like IL-1β, TNF-α, and IL-6, which together exacerbate liver damage and promote cancer progression [101]. LPS also promotes liver cancer and fibrosis in chronic liver congestion by inducing capillarization of liver sinusoidal endothelial cells (LSECs), which increases sphingosine-1-phosphate production, thereby driving the progression of hepatocellular carcinoma and fibrosis [60]. Moreover, LPS promotes liver cancer metastasis by enhancing the formation of neutrophil extracellular traps, which trap hepatocellular carcinoma cells, activate the TLR4/9-COX2 signaling pathway, and increase cell death resistance and invasiveness [102]. Besides, LPS promotes liver cancer by interacting with galectin-3 to activate rapamycin complex 1 via Rag GTPases and Ragulator on lysosomes, thereby enhancing glycolysis through upregulation of genes like glucose transporter member 1, hexokinase 2, and pyruvate kinase M2, with high galectin-3 expression correlating with poor prognosis [103].

Our evaluation of preoperative serum IAP and LPS levels in patients undergoing abdominal surgeries provides vital insights into predicting surgical outcomes and understanding postoperative complications. Utilizing the Clavien-Dindo classification, which grades complications by postoperative management measures, we discerned a significant difference in preoperative serum IAP levels between lower and higher Clavien-Dindo grade groups [104]. This suggests a potential association between preoperative IAP levels and the severity of postoperative complications. A 27.289 ng/ml cutoff value for preoper-

ative serum IAP emerged as a pivotal threshold, demonstrating its ability to stratify patients likely to experience favorable surgical outcomes versus those prone to severe postoperative complications [105, 106]. Patients exhibiting higher preoperative serum IAP and albumin levels, indicative of severe systemic inflammation and nutritional status, were associated with more favorable surgical outcomes [78, 79, 81], aligning with the Clavien-Dindo grading system.

Our study's findings suggest a complex relationship between preoperative serum IAP levels and postoperative outcomes in all abdominal surgeries. Although we did not find a direct correlation between serum IAP levels and the overall length of hospital stay in all abdominal surgery, it is notable that previous research has linked higher Clavien-Dindo grades—indicative of more severe complications—to longer hospital stays [104]. Our data, however, indicate that abdominal surgery patients with elevated serum IAP levels tend to have more favorable outcomes, including lower Clavien-Dindo grades [107]. This suggests that serum IAP may be indirectly associated with shorter hospital stays. However, the absence of a significant correlation in our study might be due to the limited sample size, potentially leading to a false-negative result [108]. Moreover, while no direct link was observed between serum IAP levels and ICU admission in all abdominal surgeries, the negative correlation between serum IAP and the length of ICU stay in those abdominal surgery patients admitted to the ICU points to the possibility that higher IAP levels may reflect less severe or shorter critical care needs following abdominal surgeries [109]. These findings highlight the importance of further research with larger cohorts to validate these observations and to explore the prognostic potential of serum IAP levels in surgical outcomes.

In cancer resection surgery, patients in the Clavien-Dindo grade > II group had lower preoperative serum IAP values than patients in the Clavien-Dindo grade ≤ II group, indicating a potential correlation with the severity of postoperative complications. Additionally, a negative correlation was found between hospital stay and preoperative IAP, further emphasizing the role of IAP in predicting surgical outcomes. Among post-surgery cancer patients in the ICU, a negative linear correlation was observed between the length of ICU stay and preoperative IAP, further underscoring the role of preoperative serum IAP in predicting the outcome of cancer resection surgeries.

However, our findings also point to variability in the predictive value of preoperative serum IAP levels across different surgeries. Notably, in pancreatic cancer and CRC surgeries, we did not observe a significant relationship between preoperative serum IAP levels and surgical outcomes. This discrepancy suggests that the role of IAP may be more context-dependent than initially anticipated, varying according to the type of surgery. These results underscore the need for further research to explore the factors influencing IAP levels and their association with surgical outcomes across different cancer types. Moreover, expanding the sample size in future studies is essential to rule out the possibility of false-negative results due to insufficient data.

Interestingly, in liver cancer resection surgeries, we observed a negative correlation between hospital stay and preoperative IAP levels, as well as a difference in IAP levels between ICU and non-ICU groups. This suggests that higher preoperative IAP levels may be associated with a more favorable surgical outcome and a reduced need for intensive care. These findings indicate the potential utility of IAP as a prognostic marker in specific surgical contexts, warranting further investigation to validate its broader application in clinical practice.

In this study, we observed a significant decrease in postoperative serum LPS levels compared to preoperative levels in both abdominal and cancer resection surgeries, suggesting that surgical intervention may influence systemic LPS levels. LPS, a marker for intestinal microbiota composition, gut barrier integrity, and systemic inflammation, displayed dynamic fluctuations in response to surgical procedures. Several factors could account for this result. During gastrointestinal decompression, stomach acid is removed, likely reducing the acidity of material entering the duodenum. This and the perioperative fasting period reduce gastric acid secretion and discharge into the duodenum, potentially leading to a higher duodenal pH. Increased duodenal surface pH may enhance IAP activity, which is known to neutralize LPS and result in lower postoperative serum LPS levels. Since preoperative samples were collected before decompression and fasting, the preoperative LPS levels were higher. Additionally, antibiotics before surgery likely reduced the number of Gram-negative bacteria, the primary source of LPS, contributing to the observed decrease in postoperative LPS. Removing tumor or inflammatory tissue during surgery could also reduce LPS levels by alleviating local inflammation and gut barrier dysfunction.

However, in our previous study, we observed an increase in serum LPS levels following pancreaticoduodenectomy (PD) and other major abdominal surgeries, contrasting with the findings of decreased LPS levels in the current study. This discrepancy may be attributed to the role of IAP, which is highly concentrated in the duodenum but present in low levels or absent in other parts of the gastrointestinal tract, such as the stomach, pancreas, ileum, and colon. The extent of IAP reduction correlates with the length of duodenal resection, with PD leading to a substantial decrease in IAP activity. Since IAP is crucial for maintaining gut homeostasis and preventing intestinal dysbiosis, its deficiency can result in gut barrier dysfunction, facilitating the translocation of LPS into the bloodstream, which contributes to elevated serum LPS levels after PD. Additionally, postoperative factors such as inflammation and dietary changes may further disrupt intestinal integrity, promoting LPS translocation and triggering a systemic inflammatory response. This mechanism may explain why serum LPS levels increase following major abdominal surgeries.

Our study demonstrated that preoperative serum LPS levels were not significantly different between patients with lower and higher Clavien-Dindo grades, nor were they associated with other surgical outcomes such as hospital stay, ICU admission, or ICU stay. These findings suggest that preoperative LPS levels may have limited utility as a predictive biomarker for postoperative complications in abdominal or cancer surgery. This challenges its role as a direct preoperative indicator of surgical risk.

However, our prior research has shown that postoperative LPS levels may be more relevant in predicting outcomes in pancreatic cancer patients, where a positive correlation was observed between postoperative LPS levels and prolonged hospital stays. This suggests that LPS may play a more significant role in the postoperative phase, potentially reflecting ongoing inflammatory processes or complications that arise after surgery. Moreover, the increase in blood LPS levels indicates gut dysbiosis or an impaired gut barrier, which could be associated with increased postoperative complications such as anastomotic leakage and postoperative pancreatic fistulae [110]. Moving forward, it is crucial to conduct studies with larger sample sizes and implement dynamic monitoring of serum LPS levels at multiple time points before and after surgery. This would allow for a more comprehensive understanding of how LPS levels fluctuate in response to surgical interventions and complications. Such studies could clarify the role of LPS in predicting

postoperative outcomes and support its potential as a biomarker for postoperative management.

5. Conclusion

Our study highlights the potential role of preoperative serum IAP as a biomarker for predicting surgical outcomes in abdominal and cancer resection surgeries. The negative correlation between preoperative serum IAP levels and postoperative complications, as classified by the Clavien-Dindo grading system, suggests that higher IAP levels may indicate more favorable surgical outcomes. In contrast, preoperative serum LPS levels did not show significant predictive value for surgical outcomes, indicating that LPS may play a more prominent role in the postoperative phase, particularly in cases of complications such as gut dysbiosis or impaired gut barrier function.

The significant decrease in postoperative serum LPS levels compared to preoperative levels suggests that surgical interventions can influence systemic inflammation and gut integrity. This was especially observed in surgeries involving the gastrointestinal tract, where IAP plays a crucial role in neutralizing LPS. Furthermore, the study underscores the complex and context-dependent nature of IAP and LPS as biomarkers, as their associations with clinical outcomes appear to vary across different types of surgeries and cancer contexts.

In conclusion, preoperative serum IAP levels show promise as a prognostic marker for surgical outcomes, while further research is needed to fully understand the role of LPS in both the preoperative and postoperative phases. Larger cohort studies and dynamic monitoring of LPS and IAP levels over time could enhance the clinical utility of these biomarkers in predicting and managing postoperative complications.

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Affidavit

Li, Qiang

Surname, first name

I hereby declare, that the submitted thesis entitled

> Interplay Between Gut Homeostasis-Regulating Enzyme Intestinal Alkaline Phosphatase and Bacterial Lipopolysaccharides in Patients undergoing Abdominal Surgery for Cancer

is my own work. I have only used the sources indicated and have not made unauthorised use of services of a third party. Where the work of others has been quoted or reproduced, the source is always given.

I further declare that the dissertation presented here has not been submitted in the same or similar form to any other institution for the purpose of obtaining an academic degree.

Shanghai 02.07.2025

Place, Date

Qiang Li

Signature doctoral candidate

Confirmation of congruency between printed and electronic version of the doctoral thesis

Doctoral candidate: Qiang Li

I hereby declare that the electronic version of the submitted thesis, entitled

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is congruent with the printed version both in content and format.

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Place, Date

Qiang Li

Signature doctoral candidate

List of Publications

1. **Li, Qiang,** Viktor von Ehrlich-Treuenstätt, Josefine Schardey, Ulrich Wirth, Petra Zimmermann, Joachim Andrassy, Alexandr V. Bazhin, Jens Werner, and Florian Kühn. "Gut Barrier Dysfunction and Bacterial Lipopolysaccharides in Colorectal Cancer." *Journal of Gastrointestinal Surgery : Official Journal of the Society For Surgery of the Alimentary Tract* 27, no. 7 (2023): 1466-72. https://dx.doi.org/10.1007/s11605-023-05654-4. (IF: 2.2)

2. **Li, Qiang,** Jutta Weitz, Chao Li, Josefine Schardey, Lena Weiss, Ulrich Wirth, Petra Zimmermann, Alexandr V. Bazhin, Jens Werner, and Florian Kühn. "Smoking as a Risk Factor for Colorectal Neoplasms in Young Individuals? A Systematic Meta-Analysis." *International Journal of Colorectal Disease* 38, no. 1 (2023): 114. https://dx.doi.org/10.1007/s00384-023-04405-w. (IF: 2.5)

3. Ye, Zeng, **Qiang Li,** Yuheng Hu, Haifeng Hu, Junfeng Xu, Muzi Guo, Wuhu Zhang, Xin Lou, Yan Wang, Heli Gao, Desheng Jing, Guixiong Fan, Yi Qin, Yue Zhang, Xuemin Chen, Jie Chen, Xiaowu Xu, Xianjun Yu, Mingyang Liu, and Shunrong Ji. "The Stromal Microenvironment Endows Pancreatic Neuroendocrine Tumors with Spatially Specific Invasive and Metastatic Phenotypes." *CANCER LETTERS* 588 (2024): 216769. https://dx.doi.org/10.1016/j.canlet.2024.216769. (IF: 9.1)

4. Ye, Zeng, Yan Zhou, Yuheng Hu, **Qiang Li,** Zijin Xu, Xin Lou, Wuhu Zhang, Di Zhu, Cao Xie, Qingtong Zhou, Jing Gao, Hu Zhou, Dehua Yang, Yi Qin, Xiaowu Xu, Jie Chen, Shunrong Ji, Ming-Wei Wang, and Xianjun Yu. "Single-Cell Sequencing Reveals the Heterogeneity of Pancreatic Neuroendocrine Tumors under Genomic Instability and Histological Grading." *IScience* 27, no. 9 (2024): 110836. https://dx.doi.org/10.1016/j.isci.2024.110836. (IF: 4.6)