Prevalence and Characteristics of Spontaneous Bacterial Peritonitis in Hospitalized Patients with Ascites due to Liver Cirrhosis.

Thesis submitted for partial fulfillment of the MSc. degree in Internal Medicine

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32□

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Abstract

Spontaneous bacterial peritonitis (SBP) is a bacterial infection of ascitic fluid without any intra-abdominal surgically treatable source of infection. SBP is a serious potentially fatal complication in patients with cirrhosis that requires early recognition and effective antibiotic therapy.

This study was conducted on 100 cirrhotic, ascitic patients presented to the Internal Medicine Department, Kasr Elaini hospital. The aim of this study was to determine the prevalence and characteristics of spontaneous bacterial peritonitis in hospitalized patients with ascites due to liver cirrhosis. They were divided into two groups :group **I:** included 57 patients (57%) with the PMN > 250 cells/ mm^3 and group **II:** included 43 patients (43%) with ascitic fluid PMN< 250 cells/ mm^3**.** The clinical findings of SBP were extremely variable ranging from a silent subclinical to a severe fatal illness. In our study 14 patients (24.5%) with SBP had no signs or symptoms of infection. Abdominal pain and tenderness were commonly elicited features among patients with SBP occurring in 48% of them. Fever and encephalopathy are other presenting features that were detected only in patients with SBP (51% and 39%, respectively). Gastrointestinal bleeding is present in 23 patients (40.4%). Renal impairment was present in 28 patients (48.3%) with SBP.

In SBP patients, PMN count in ascitic fluid was significantly higher than in patients with non-infected ascitic fluid "control group", at the time of diagnosis. However positive culture of ascitic fluid detected bacterial growth in only 12 patients (21.05 %) of the group I and in one patient (2.3%) of the group II according to the laboratory results. Organisms isolated in our study were E. coli in 8 patients (13.8%)), Klebsiella in 2 patients (3.4%) and Gram positive organism in 2 patients (3.4%).

Key words

- Spontaneous bacterial peritonitis (SBP)
- Ascites.
- Liver cirrhosis.

Table of contents

Page number

List of abbreviations

µL	Micro liter
µm	Micro meter
µmol	Micro mol
A	Angestron
aa	Amino acids
AASLD	American Association for the Study of Liver Diseases'
ADA	adenosine deaminase
ADH	antidiuretic hormone
AFB	acid fast bacillus
A-FN	fibronectin in the ascitic fluid
AFP	Alpha fetoprotein
AIDS	Acquired immunodeficiency syndrome
ALD	alcohol-induced liver disease
APACHE II	Acute Physiology and Chronic Health Evaluation II
ARDS	Adult respiratory distress syndrome
BA	bacterascites
BMT	bone marrow transplantation
BPI	Bactericidal Permeability Increasing Factor
C3	third component of complement system
C4	Fourth component of complement system
CD3	
CNNA	culture-negative neutrocytic ascites
CO2	Carbon dioxide
CSF	cerebrospinal fluid
CT	Computerized topography
CTP	Child-Turcotte-Pugh
dl	Deci Liter
DNA	Deoxyribonucleic acid
E coli	*Escherichia coli*
ELISA	Enzyme linked immune sorbent assay
EPH	extrahepatic portal hypertension
ERCP	Endoscopic retrograde cholangiopancreatography

ESLD	End Stage Liver Disease
EVS	endoscopic variceal sclerotherapy
FMLP	formyl-methionyl-leucyl-phenylalanine
g	Gram
G-CSF	granulocyte-colony stimulating factor
GE	Granulocyte elastease
GFR	glomerular filtration rate
GI	Gastro-intestinal
gM	Immunoglobulin M
GM-CSF	Granulocyte macrophage-colony stimulating factor
GNB	gram-negative bacilli
GRO	growth related oncogene
GVN	gentamycin-vancomycin-nystatin
h	hour
HBsAg	Hepatitis B surface antigen
HCC	hepatocellular carcinoma
HCG	human chorionic gonadotropin
HIV	Human immunodeficiency virus
HRS	Hepatorenal syndrome
HVPG	hepatic vein pressure gradient
ICAM-l	intercellular adhesion molecule-I
ICU	Intensive Care Unit
IFN-γ	interferon-γ
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IHLs	intrahepatic lymphocytes
IL-1	interleukin 1
IL-10	interleukin 10
IL-12	interleukin 12
IL-1β	interleukin 1beta
IL2	interleukin 2
IL-6	interleukin 6
IL-8	interleukin 8
INR	International normalized ratio
kD	Kilo Dalton

L	Litre
LAC	Lactulose
LBP	lipopolysaccharide binding protein
LDH	lactate dehydrogenase
LE	leucocyte esterase
LPS	Lipopolysaccharide
LTB4	leukotriene B4
LXA4	lipoxin A4
MAN	mannitol
MCP-1	monocyte chemotactic protein-1
MELD	Model End Stage Liver Disease
mg	Milligram
MGSA	melanoma growth stimulatory activity
MIP-1a	monocyte inflammatory protein a
mm3	Cubic milli-meter
MMC	migrating motor complex
mmHg	milli-meter mercury
MNB	Monomicrobial non-neutrocytic bacterascires
mRNA	Messenger RNA
NAP-2	neutrophil activating peptide 2
NCN	neomycin-colistin-nystatin
NIEC	North Italian Endoscopic Club
NK cells	Natural killer cells
NO	nitric oxide
PAF	platelet activating factor
PAMPs	pathogen associated molecular patterns
PCR	polymerase chain reaction
P-FN	fibronectin in plasma
PGD	primary graft dysfunction
PGE_2	prostaglandin E_2
PMC	peritoneal mesothelial cells
PMN	polymorph nuclear neutrophils
PNB	Polymicrobial non-neutrocytic bacterascites
PRA	plasma renin activity
PSA	prostate specific antigen

QR-GNB	quinolone-resistent gram-negative bacilli
RAAS	renin-angiotensin-aldosterone system
RBC	Red Blood Cells
RES	Reticulo endothelial system
RNA	Ribonucleic acid
SA	sterile ascites
SAAG	serum-ascites albumin gradient
SAI	spontaneous ascitic infection
SBP	spontaneous bacterial peritonitis
SBP-RI	Renal impairment in the course of SBP
Scr	Serum creatinine
SID	Selective intestinal decontamination
SLPI	Secretory leukoprotease inhibitor
SNS	sympathetic nervous system
SOT	sodium overload test
T.N.F- α	Tumor Necrosis Factor alfa
TB	Tuberculous
Tbil	Total bilirubin
TGFb1	transforming growth factor beta-1
TGF-beta	transforming growth factor beta
TIPS	transjugular intrahepatic portosystemic shunts
TMP-SMX	trimethoprim-sulfamethoxazole
TXA2	thromboxane A2
US	United states
UTI	Urinary tract infection
VCAM-l	and vascular cell adhesion molecule-1
VOD	Venoocclusive disease
WBC	white Blood Cell

List of figures

List of tables

The Peritoneum

The peritoneum, the largest and most complexly arranged serous membrane, is an empty and intricately folded sac, lining the abdomen (parietal part) and reflected over the viscera (visceral part). The total area of the peritoneum is approximately 1.8 m^2. Both layers are in contact, the space between them is the peritoneal cavity, which consists of a main region, the greater sac and the lesser sac (behind the stomach and the adjoining structures), both communicate via the epiploic foramen. In males, the peritoneum forms a closed sac, while in females; it is continuous with the mucus membranes of the fallopian tubes **(Hiyama and Bennion, 1997).**

The parietal peritoneum is thicker than the visceral peritoneum and contains a richer capillary network that ramifies extensively within the peritoneal lining; this vascular supply allows being freely dissected from the deeper structures without altering its viability **(Hiyama and Bennion, 1997).**

Histology

The peritoneum is a single layer of mesothelial cells (about 3 µm thick), which contains a well developed cytoskeleton of intermediate filaments, endoplasmic reticulum, Golgi apparatus and abundant smooth surfaced and coated vesicles indicative of active trasnsmembrane transport **(Pronk *et al.*, 1993).** The peritoneal mesothelial cells (PMC) contain mechanisms that allow for easy gliding of the opposing peritoneal surfaces, like type 2 pneumocytes, PMCs contain characteristic lamellar bodies that produce surfactant that act as a lubricant within the peritoneal space **(Beavis *et al.*, 1994)**

Numerous microvilli, about 3 µm in length project from the apical surface of the PMCs to minimize the shear between both surfaces. These microvilli greatly increase the surface area of the mesothelial cells. The relative density of microvilli differs throughout the peritoneal cavity **(Nakatani *et al.*, 1996).**

Also there is a strong structural integrity between neighboring PMCs, they are joined by tight junctions, desmosome-like junctions, intracellular canaliculi, and monofilament like filaments. Tight intra-mesothelial cell junctions generally connect these cells, although there are also large intracellular gaps between these lining cells. Alterations in mesothelial cell metabolism and cellular swelling may influence the ability of substances to diffuse across this cell layer

PMCs have the same mesothelial origin as the endothelial cells lining blood vessels that release important biological modifiers such as nitric oxide and endothelines, this raise the possibility that PMCs may be the source of effector mechanisms during peritonitis **(Takahashi *et al.*, 1991)**

Beneath the mesothelial cells is a basement membrane composed of a loose network of type IV collagen **(Gotloib and Shostack, 1987)**. The basement membrane offers little resistance to diffusion of most molecules smaller than 30,000 KD. The basement membrane overlies a complex connective tissue layer which includes; collagen and other connective tissue proteins, elastic fibers, fibroblasts, adipose cells, endothelial cells, mast cells, eosinophils, macrophages, and lymphocytes. **(Takahashi *et al.*, 1991)**

Peritoneal Fluid

It is appreciated since the late 1870s that the peritoneum acts as a bidirectional semi - permeable membrane. It normally contains about 100 ml serous fluid that resembles plasma ultra filtrate and contains less than 3 gm/dl proteins. Fluid is absorbed by the peritoneal mesothelial lining cells and subdiaphragmatic lymphatics. Mesothelial cells also absorb solutes during the continuous process of endocytosis.

Splanchnic blood flow and factors that alter membrane permeability affect the efficiency of fluid exchange. Peritoneal permeability is markedly increased by intraperitoneal inflammation. **(Topley and Williams, 1994)**

While the entire peritoneum acts as a semi permeable membrane for fluid and solutes, the passage of particulate matter such as bacteria is restricted to certain areas under normal conditions. Particulate matter can be absorbed through stomata between the mesothelial cells of the diaphragmatic peritoneum directly into specialized lymphatic channels called lacunae which run parallel to the muscle fibers of diaphragm **(Nakatani *et al.*, 1996)**, from here the lymph flows into networks under the diaphragmatic pleura and then into the main lymphatic ducts via the substernal lymph nodes. These stomata are elastic and vary in size which is regulated by the actin found in the processes of P.M.C.s, it increases during inflammation. These stomata allow the passage of particles up to 10 µ in diameter, which include bacteria 0.5-2 µ in diameter **(Li and Jiang, 1993)**.

Peritoneal fluid has the properties of lymph, and secreted by the peritoneal serosa. Diaphragmatic lymphatic channels act like valves and suck synchronous with respiration peritoneal fluid through the thoracic ducts into the venous circulation. Lymphatic capillaries are distributed in the subperitoneal connective tissue of the diaphragm. During inspiration the stretching of the diaphragm causes a rapid flow into the lacunae, while during expiration the contraction of the diaphragm forces the fluid into the lymphatics. This mechanism affords a rapid initial clearing of bacteria from the peritoneal cavity **(Nakatani *et al.*, 1996)**. A reverse process can be observed during shock and in the presence of severe inflammation of the peritoneum, i.e. abscesses. Under these conditions the normal peritoneum becomes permeable to bacteria, which then translocate from the bowel lumen into the peritoneal cavity or into abscesses. **(Hau, 1990)**

An inflammatory process of peritonitis causes a rapid shift from the intravascular space to the interstitial space and in the peritoneal cavity. The ileus, which always accompanies peritonitis, causes additional fluid shifts by losses into the bowel lumen and lack of reabsorption of proximal secretions. The loss of fluid into the interstitial space, the peritoneal cavity and the bowel lumen results in hypovolemia and increased intra-abdominal pressure. The normal intraperitoneal pressure is under 10 mmHg. An elevation above 10 mmHg is called abdominal compartment syndrome. The relationship between volume and pressure in the peritoneal cavity is not linear. When a certain critical volume has been reached small additional increases in volume will lead to a disproportionate increase in pressure: **(Schein *et al.*, 1995)**

- Mildly elevated intra abdominal pressure (10-20 mmHg) results in impaired visceral blood flow and a mechanical embarrassment of pulmonary function.
- Moderately elevated intra abdominal pressure (20-40 mmHg) leads in addition to a decrease in venous return and thus to impairment of myocardial function and oliguria.

- Finally, intra abdominal pressure above 40 mmHg will lead to anuria

The peritoneum acts as functional exchange surface of approximately $1m^2$, this enables using the peritoneal cavity in renal dialysis and drainage procedures as ventriculoperitoneal shunts.

Electrically charged proteoglycans and polyanionic chondroitin sulfate molecules trapped in the interstitium, influence the passage of materials in and out of the peritoneal cavity, decreasing its permeability **(yung *et al.*, 1995)**.

Under normal circumstances, about 1\3 of the fluid draining from the peritoneal cavity pass through the diaphragmatic lymphatics, and the remainder exits through the parietal peritoneum **(Flessner *et al.*, 1983)**. In experimental animals 1\2 of the bacteria placed in the peritoneal cavity appeared in the thoracic duct within 6 minutes **(Tsilbary and wissig, 1993).**

The milky spots

The omentum is the only site other than the diaphragmatic stomata which can absorb particles from the peritoneal cavity. But unlike stomata, it contains potent local effector mechanisms mediated by macrophages and B- lymphocytes. These aggregates of cells were referred to as "Taiches laiteuse" or "milky spots" by the French anatomist Ranvier in 1874 **(Vanvugt *et al.*, 1996)**.

Milky spots contain precursors of mononuclear phagocyte system which are the prime source of peritoneal macrophages **(Wijffels *et al.*, 1992)**. They consist mainly of macrophages and lymphocytes surrounding profuse and characteristic capillary convolutions (omental glumeruli) that lie directly under the mesothelium. These cells are supported by a delicate network of reticular fibers and are infiltrated by non myelinated nerve fibers, which showed dopamine immunoreactivity suggesting being a site of immune-neuroendocrine interaction **(Shimotsuma *et al.*, 1993)**.

The number of milky spots is the highest in infancy and gradually decreases with age but they become prominent during intra peritoneal infections, macrophages tends to form clusters near the peritoneal surface of the milky spots and are oriented toward the peritoneal cavity, clusters are mainly periarteriolar. Migration of macrophages from milky spots into the peritoneal cavity is facilitated by the absence of a basal lamina in the submesothelial connective tissue. Macrophages are present in high numbers at the peritoneal surface **(Cranshaw and leak, 1995)**.

It has been suggested that peritoneal associated lymphoid tissue (milky spots, lymph nodes, and lymphocytes in the peritoneal fluid) may function as intestinal thymus. Cell population differ from those present in peripheral blood and contain 45% macrophages, 42 % $CD2^+$ T cells, 2% $CD22^+$ B cells, and 2% dendritic cells, plus a range of other cell types **(Holub *et al.*, 1990)**.

B-lymphocytes bearing cluster of differentiation 5 (CD5) phenotype are rare in peripheral blood, spleen, and lymph nodes but are common in the peritoneum **(Murakami and Honjo, 1995)**.Such cells develop from non conventional lineage, not from thymus or bone marrow. The bulk of IgM secretion is attributed to peritoneal $CD5^+$ B cells **(Lue *et al.*, 1994)**.