Analysis of Ascitic Fluid Lactoferrin Levels in the Diagnosis of Spontaneous Bacterial Peritonitis after Systemic Antibiotic Treatment

1. Department of Gastroenterology and Hepatology, Linkou Medical Center, Chang Gung Memorial Hospital, Taoyuan, Taiwan
2. Chang Gung University, College of Medicine, Graduate institude of Clinical Medical Sciences Taoyuan, Taiwan

Chen Tsung-Hsing

Background

* Patients in advanced stages of liver cirrhosis tend to develop bacterial peritonitis without evident source of infection, a form of infection which has been termed spontaneous bacterial peritonitis (SBP) in 1963

  Ann Intern Med 1964; 60: 568-580

* SBP is a frequent, life-threatening bacterial infection in patients with liver cirrhosis and ascites.

* Portal hypertension leads to increased bacterial translocation from the intestine

* New diagnostic tests which can be carried out quickly at the patient's site and provide additional prognostic information would be helpful

Background

* The diagnosis of SBP is based on a polymorphonuclear leukocyte (PMN) count in ascitic fluid of ≥250 cells/mm3,

  →however; a differential leukocyte count of the ascites can be obtained only in some clinical settings

* SBP leads to the hospitalization of 10–30% of cirrhotic patients, and the mortality rate in this group approaches 30%

  high mortality associated with SBP, ranging between 16% and 52%

J Gastroenterol 2013; 108: 1473-1479
Clin Gastroenterol Hepatol 20119: 260-265

Background

* Owing to the high mortality, patients with SBP should be started on empiric, broad-spectrum antibiotics immediately.

* According to the 2012 American Association for the Study of Liver Disease (AASLD) guidelines, in patients with suspected SBP, empiric therapy should be initiated promptly to maximize patient survival

Background

Other tools for SBP diagnosis:

* urinary dipstick that is calibrated especially to ascites
* Calprotectin, a protein secreted by neutrophils, is another candidate for a bedside test

J Hepatol 2010; 53:477-483
World J Gastroenterol 2013; 19: 2028-2036

* **Lactoferrin**

Background

* Lactoferrin is an iron-binding protein found in human mucosal secretions as well as in the specific granules of PMNs

* Neutrophils after degranulation were observed to be the main source of lactoferrin in blood plasma (Iyer and Lonnerdal, 1993).

Background

* It is also found in most mucosal secretions such as uterine fluid, vaginal secretion, seminal fluid, saliva, bile, pancreatic juice, small intestine secretions, nasal secretion, and tears

Masson et al., 1966; Baker, 1994; Levay and Viljoen, 1995; Lonnerdal and Iyer, 1995; Kikuchi et al., 2003; Baker and Baker, 2005

* The concentration of lactoferrin in the blood increases during infection, inflammation (Birgens, 1985), excessive intake of iron, or tumor growth (Levay and Viljoen, 1995)

Background

* Biological functions of lactoferrin
  →Lactoferrin is considered to be a part of the innate immune system

1. Lactoferrin and iron metabolism
2. Antimicrobial activity
3. Antibacterial activity
4. Antiviral activity
5. Antiparasitic activity
6. Lactoferrin and host defense
7. Lactoferrin and tumor growth
8. Lactoferrin and cell proliferation and differentiation

Background

* A previous study: detection of intestinal inflammation
  →Lactoferrin possesses various biological functions, including roles in iron metabolism, cell proliferation and differentiation, and antibacterial, antiviral, and antiparasitic activity

* measurement of ascitic fluid lactoferrin levels is a reliable biomarker for the presence of PMNs and detection of SBP in patients with cirrhosis

Parsi MA, Saadeh SN, Zein NN, et al. Ascitic fluid lactoferrin for diagnosis of spontaneous bacterial peritonitis. Gastroenterology 2008; 135: 803-807. 9.

Background

Reasons for this study

* Patients with advanced cirrhosis are prone to the development of bacterial infections other than SBP, such as pneumonia or urinary tract infection, for which may require antibiotic treatment
* SBP develops in up to 3.5% of patients that are treated as outpatients, its prevalence is as high as 12% in hospitalized patients

Aliment Pharmacol Ther 2014; 40: 105-112
Hepatology 2003; 37: 897-901
World J Hepatol 2013; 5: 104-108

→In the present study, we aimed to identify the lactoferrin level after systemic antibiotic treatment for SBP and other systemic infections

Materials and Methods

* inclusion criteria were a known diagnosis of cirrhosis and the presence of ascites

* Exclusion criteria : rupture of hepatocellular carcinoma, peritoneal carcinomatosis, and hemorrhagic ascites.

Materials and Methods

* A total of 22 ascites samples were evaluated.
* Thirteen patients received antibiotics before paracentesis (13/22, 59%).
* Cirrhosis was related to:
  1. chronic viral hepatitis alone in 54.5% (HBV = 8, HCV = 4)
  2. alcoholism alone in 18.2% (n = 4),
  3. combination of alcoholism and chronic viral hepatitis in 18.2% (HBV = 2, HCV = 2)
  4. 9.1% (n = 2) of patients, cirrhosis was caused by other factors

Materials and Methods

* 22 patients enrolled
* diagnosis of SBP: 1st paracentesis, based on the PMN count
* All the ascites samples collected during the second paracentesis, performed for symptom relief

  →cell counts, bacterial culture, and lactoferrin concentration

Materials and Methods

* Patients were classified into 3 groups:

→9 patients with an ascitic fluid PMN count of <250 cells/mm³ who did not initially receive systemic antibiotic treatment (group A);

→9 patients with an ascitic fluid PMN count of <250 cells/mm³ who initially received antibiotic treatment for reasons other than SBP (group B);

→4 patients with an ascitic fluid PMN count of ≥250 cells/mm³ (group C) : culture positive and negative

Lactoferrin Kits

* The lactoferrin levels in ascitic samples were quantified using a human lactoferrin ELISA kit according to the manufacturer's instructions (Bethyl Laboratories Inc)

Table 1: Baseline characteristics of patients with liver cirrhosis (N = 22)

	Group A (n = 9)	Group B (n = 9)	Group C (n = 4)
Age (years)	55 ± 10.5	63 ± 17.7	56 ± 11.0
Sex (M:F)	6:3	8:1	1:3
Child-Pugh score	9 ± 2.0	10 ± 1.7	10 ± 2.8
Fever	0% (0/9)	11.1% (1/9)	50% (2/4)
Abdominal pain	0% (0/9)	0% (0/9)	50% (2/4)
Encephalopathy	11.1% (1/9)	22.2% (2/9)	50% (2/4)

Group A: ascitic fluid PMN count of <250 cells/mm³ who did not initially receive systemic antibiotic treatment

Group B: ascitic fluid PMN count of <250 cells/mm³ who initially received antibiotic treatment for reasons other than SBP

Group C: ascitic fluid PMN count of ≥250 cells/mm³

Result

Table 2: Laboratory characteristics of study groups

	Group A	Group B	Group C	Group Cp
Leucocyte count (/mm^3)				
Total count	43.1 ± 17.7	125 ± 151.7	653.5 ± 603.5	863.7 ± 556.1
PMN	3.72 ± 3.39	39.5 ± 42.8	285.8 ± 188.6	386.7 ± 106.2
Albumin level (mg/dL)				
Ascitic	0.84 ± 0.66	0.49 ± 0.50	0.39 ± 0.40	0.49 ± 0.42
Serum	2.85 ± 0.45	2.71 ± 0.39	2.58 ± 0.30	2.67 ± 0.31
SAAG	2.20 ± 0.45	2.09 ± 0.56	2.19 ± 0.61	2.15 ± 0.71
Ascitic lactoferrin level (ng/mL)	19.64 ± 6.32	23.64 ± 9.53	197.7 ± 167.7*	261.69 ± 145.5

*High variance may relate to the difference lactoferrin between positive ascitic culture and negative ascitic culture

Result

Table 3: Laboratory data and bacterial culture results of the patients with Spontaneous bacterial peritonitis

	PMN count ($/mm^3$) Baseline	PMN count ($/mm^3$) after antibiotic treatment	Lactoferrin level (ng/mL)	Bacterial culture result/causative organism
1	662.4	1.4	6.057	Negative
2	2925	263.4	184.996	*Enterococcus faecalis*
3	10450	522.5	134.665	*Staphylococcus lugdunensis*
4	496.8	356.2	465.397	*Escherichia coli*

PMN, polymorphonuclear leukocyte

Result

* The lactoferrin concentration was also significantly elevated (261.69 ± 145.5 ng/mL) in the cases with positive results on bacterial culture compared with the cases without SBP, in both group A ($p = 0.002$) and group B ($p = 0.001$).
* There was no difference between the lactoferrin concentration in the negative culture ascites (6.057 ng/mL) of group A and group B.

Discussion

* According to a previous study, SBP with a positive result on ascitic fluid bacterial culture is associated with a higher mortality than CNNA(culture-negative neutrocytic ascites)

Kamani L et al. Outcomes in culture positive and culture negative ascitic fluid infection in patients with viral cirrhosis: cohort study. BMC Gastroenterol 2008; 8: 59- 64

* In the present study, ascitic fluid lactoferrin levels were assessed in patients with cirrhosis after systemic antibiotic treatment

Discussion

* We noted that patients who received antibiotic treatment for conditions other than SBP, such as gastrointestinal bleeding, did not show significantly elevated lactoferrin levels compared to those who did not receive antibiotic treatment and did not have SBP

* The current study confirmed that ascitic fluid lactoferrin concentration remains elevated in SBP patients with a positive result on bacterial culture, even after systemic antibiotic treatment

Conclusion

* elevated lactoferrin levels in the ascites of cirrhotic patients with SBP and positive ascitic bacterial culture appears to be a promising diagnostic predictor, even after systemic antibiotic treatment.

* What is next step in the future?
  →more sample
  →precise diagnosis

Thanks for your attension