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BRIEF ARTICLE

Procalcitonin, and cytokines document a dynamic inflammatory state in non-infected cirrhotic patients with ascites

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Abstract

AIM: To quantitate the simultaneous serum and ascitic fluid levels of procalcitonin and inflammatory markers in cirrhotics with and without ascites.

METHODS: A total of 88 consecutive severe cirrhotic patients seen in a large city hospital liver clinic were studied and divided into two groups, those with and without ascites. Group 1 consisted of 41 cirrhotic patients with massive ascites, as demonstrated by necessity for therapeutic large-volume paracentesis. Group 2 consisted of 47 cirrhotic patients without any clinically documented ascites to include either a recent abdominal computed tomography scan or ultrasound study. Serum and ascitic fluid levels of an array of inflammatory markers, including procalcitonin, were measured and compared to each other and a normal plasma panel (NPP).

RESULTS: The values for inflammatory markers assayed in the serum of Groups 1 and 2, and ascitic fluid of the Group 1. The plasma levels of the inflammatory cytokines interleukin (IL)-2, IL-4, IL-6, IL-8, interferon gamma (IFNγ) and epidermal growth factor (EGF) were all significantly greater in the serum of Group 1 as compared to that of the serum obtained from the Group 2 subjects (all $P < 0.05$). There were significantly greater serum levels of IL-6, IL-8, IL-10, monocyte chemoattractant protein-1, tumor necrosis factor-α, vascular endothelial growth factor and EGF when comparing Group 2 to the NPP. There was no significant difference for IL-1A, IL-1B, IL-2, IL-4 and IFNγ levels between these two groups. Serum procalcitonin levels were increased in cirrhotics with ascites compared to cirrhotics without ascites, but serum levels were similar to ascites levels within the ascites group. Furthermore, many of these cytokines, but not procalcitonin, demonstrate an ascites-to-serum gradient. Serum procalcitonin does not demonstrate any significant difference segregated by liver etiology in the ascites group; but ascitic fluid procalcitonin is elevated significantly in car-

diac cirrhosis/miscellaneous subgroup compared to the hepatitis C virus and alcoholic cirrhosis subgroups.

CONCLUSION: Procalcitonin in the ascitic fluid, but not in the serum, differentiates between cirrhotic subgroup reflecting the dynamic interplay of ascites, bacterial translocation and the peri-peritoneal cytokine.

Key words: Ascites; Bacterial translocation; Inflammatory markers; Procalcitonin; Cirrhosis

Core tip: Procalcitonin received much attention as a serum marker in differentiating sepsis from systemic inflammatory response syndrome and bacterial sepsis. Procalcitonin significance in assessing ascitic fluid inflammation and/or infection is less well characterized. This study demonstrates that non-infected cirrhotics with ascites *vs* those without ascites manifest peri-peritoneal based immune response mediated by a constellation of pro- and anti-inflammatory protein markers and procalcitonin. This peri-peritoneal response is distinct from the systemic immune response to the underlying hepatic disease process. Its recognition can potentially determine the likelihood for future adverse events like spontaneous bacterial peritonitis, the hepato-renal syndrome and impending death.

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INTRODUCTION

Cirrhosis is characterized by an extensive fibronodular replacement of the hepatic parenchyma resulting in both synthetic dysfunction and portal hypertension. This process is driven by hepatic inflammatory processes in the setting of an inciting agent, such as hepatitis C virus (HCV), alcohol (ETOH) abuse or a metabolic derangement in a host with a particular genetic constitution[1-4]. Decompensated cirrhosis is identified by the presence of portal hypertensive clinical sequelae, namely hepatic encephalopathy, varices, and most commonly, ascites. Ascites is associated with two other serious complications such spontaneous bacterial peritonitis (SBP) and hepatorenal syndrome (HRS), both of which increase morbidity and can lead to death[4,5].

Bacterial translocation (BT) is the migration of enteric flora into mesenteric lymph nodes or other extra-intestinal sites[6-8]. It is well known that cirrhotics, with and without ascites, have many factors which predispose to enhanced BT including: malnutrition, altered enteric flora species and bacterial overgrowth, increased bowel stasis, altered gut permeability, and decreased mucosal defense mechanisms[8-12]. In particular, portal hypertension can lead to enteric mucosal edema and microvascular stasis, both of which further alter gut permeability[11,12]. Consistent with these facts, it known that oral medications such as cisapride and trimethoprim-sulfamethoxazole can attenuate bacterial overgrowth, enhance mucosal function, and increase intestinal transit, all leading to reduced rates of BT[13-15].

Even in the absence of overt infection, there is a significant inflammatory response in cirrhotic patients occurring as a result of a complex interaction of pro- and anti-inflammatory cytokines consisting of numerous interleukins, tumor necrosis factor-alpha (TNF-α), interferon gamma (IFNγ) and complement proteins[16-18]. This concerted cytokine response has both temporal and site specific properties in cirrhotic patients with portal hypertension[16,17]. One particular inflammatory marker is procalcitonin (PCT), a 116 amino acid protein made in multiple sites of the body including liver and intestine. PCT has received much attention as a possible discriminatory serum marker in differentiating sepsis from systemic inflammatory response syndrome (SIRS) and bacterial sepsis from non-bacterial sepsis in multiple populations including cirrhotic patients[19-27]. Its significance in measuring ascitic fluid inflammation and/or infection, *e.g.*, SBP, is less well characterized[28].

It has been shown that many of these same cytokines are generated by activated macrophages, neutrophils, NK cells and lymphocytes in peri-peritoneal tissues in cirrhotic patients[29-34]. These immune cells are activated upon sensing appropriate bacterial antigens, such as bacterial DNA or lipopolysaccharide, either in the intestinal mucosa or after BT. These interactions can also activate the adaptive immune system beyond innate infection cellular control mechanisms[33-35]. As the enteric mucosa is continuously exposed to bacterial antigens, which is enhanced strain in the setting of cirrhosis and portal hypertension, this immune response is continuously engaged and tightly regulated.

Thus, the aims of the present investigation were to: (1) quantitate the simultaneous serum and ascitic fluid levels of PCT and an array of inflammatory markers in non-infected cirrhotic patients with and without ascites; and (2) further segregate serum and ascitic fluid PCT with respect to different etiologies of liver disease.

MATERIALS AND METHODS

Subjects

A total of 88 consecutive severe cirrhotic patients seen in a large city hospital liver clinic were studied and divided into two groups. The inclusion criteria consisted of age > 18 years or > 65 years, cirrhosis documented by clinical examination, computed tomography (CT), or liver biopsy. The only exclusion criteria was the presence of any clinical infection. Group 1 consisted of 41

Table 1 Characteristics of the 88 patients studied

Primary liver disease	ETOH	NASH	HCV	Cardiac/miscellaneous	Total
Patients					
Ascites (+)	21	0	8	12	41
Ascites (–)	13	11	23	0	47
Age (yr)					54 ± 11
Ascites (+)	50 ± 1	58 ± 5	54 ± 10	55 ± 11	
Ascites (–)	48 ± 5	56 ± 8	53 ± 9	56 ± 12	
Gender (M/F)					66/22
Ascites (+)	11/9	N/A	14/9	9/8	
Ascites (–)	8/3	3/8	4/4	N/A	
MELD score					9.6 ± 3.5
Ascites (+)	9.6 ± 2.1	9.3 ± 1.6	10.3 ± 5.0	8.6 ± 2.5	
Ascites (–)	9.4 ± 2.2	9.2 ± 2.0	10.0 ± 4.6	8.6 ± 3.1	
CTP score					5.6 ± 1.2
Ascites (+)	5.8 ± 1.5	5.3 ± 0.6	5.6 ± 1.5	5.4 ± 0.8	
Ascites (–)	5.6 ± 2.0	5.3 ± 0.8	5.6 ± 1.2	5.3 ± 1.2	

Data given as mean ± SE. ETOH: Alcohol; NASH: Non-alcoholic steatohepatitis; HCV: Hepatitis C virus; MELD: Model for End-stage Liver Disease; CTP: Childs-Turcotte-Pugh.

cirrhotic patients with massive ascites, as demonstrated by necessity for therapeutic large-volume paracentesis (LVP) because of tense abdominal distension resulting in difficulty ambulating and performing the activities of normal daily living. In each case a minimum of 2 liters of ascites was removed and the maximum volume removed from any patient was 6 liters. No patient received antibiotics before the paracentesis. Group 2 consisted of 47 cirrhotic patients without any clinically documented ascites to include either a recent abdominal CT scan or ultrasound study. No patient demonstrated symptoms or clinical evidence of active infection, and no ascitic fluid from the LVP performed on Group 1 patients met criteria for SBP. SBP is diagnosed when the ascitic fluid absolute polymorphonuclear cell count is 250 cells/mL or more in the absence of recent surgery. SBP occurs in 30% of patients with ascites and has a 20% mortality rate[36].

Investigative procedures

Procalcitonin (PCT) was assessed utilizing the Bio Merieux Inc. Vidas Assay (Lombard, IL, United States) in the serum of both groups and in the ascitic fluid of Group 1. A panel of cytokines consisting of interleukin (IL)-1A, IL-1B, IL-2, IL-4, IL-6, IL-10, monocyte chemotactic protein (MCP)-1, TNF-α, IFNγ, vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF) were assayed in the serum of patients in Groups 1 and 2 and in the ascitic fluid of those in Group 1 utilizing biochip platforms obtained from Randox Life Sciences (Boston, MA, United States). Values for each assay were compared to the results obtained using a commercially available normal plasma panel (NPP) obtained from Bioreclamation, LLC (Liverpool, NY, United States).

Regulation

All 88 subjects studied agreed to be studied after reading and signing an informed written consent which was approved by the Institutional Review Board (IRB) of the Cook County Health and Hospitals system, Chicago, Illinois, United States. Additional funding was provided by a grant from the Rush-Cook County joint research endeavor program.

Statistical analysis

All data are reported as mean values ± standard error of the mean + SE). Unpaired *t* tests, χ^2 and ANOVA were used to compare the values between groups and against the NPP utilizing a P value < 0.05 as the measure of statistical significance.

RESULTS

The 41 patients in Group 1, *i.e.*, cirrhotics patients with large-volume ascites, included 21 with ETOH cirrhosis, 12 with cardiac cirrhosis/miscellaneous, 8 with extrahepatic malignancy, and 8 with HCV cirrhosis. Of this latter group, 3 also had a hepatoma (Table 1). The 47 patients of Group 2, *i.e.*, cirrhotic patients without ascites, consisted of 23 with HCV cirrhosis, 13 with ETOH cirrhosis, and 11 with non-alcoholic steatohepatitis (NASH) cirrhosis (Table 1). Further data as regards to age, gender, Model for End-stage Liver Disease (MELD) score, and Childs-Turcotte-Pugh (CTP) score are also listed in Table 1. In regards to cirrhotic etiology, the only differences were the finding of NASH patients in Group 2 compared to none in Group 1, and patients with malignancy and cirrhosis in Group 1 not present in Group 2. There were no significant differences in age, MELD score or CTP score between the two groups.

The biochemical characteristics of both groups are shown in Table 2. Pertinently, Group 1 demonstrated significantly greater prothrombin times and the international normalized ratio (INR). Group 2 demonstrated significantly higher levels of serum alanine transaminase and albumin. The characteristics of the ascitic fluid in the Group 1 are shown in Table 2. All 41 of these patients were culture negative and had significantly lower

Table 2 Biochemical data of the 88 patients

Parameter	Ascitic Group 1 (n = 41)	Non-ascitic Group 2 (n = 47)	*P* value
Hemoglobin (g/dL)	10.8 ± 0.4	10.4 ± 1.7	NS
WBC (cells × 10^3/μL)	8.3 ± 1.3	6.7 ± 2.8	NS
Platelets (cells × 10^3/μL)	132 ± 13	157 ± 66	NS
Prothrombin time (s)	20.5 ± 1.2	17.7 ± 1.1	< 0.05
INR	1.8 ± 0.1	1.5 ± 0.1	< 0.05
BUN (mg/dL)	13.3 ± 7.2	21 ± 4	NS
Creatinine (mg/dL)	1.0 ± 0.7	0.9 ± 0.2	NS
Total bilirubin (mg/dL)	5.9 ± 1.0	4.7 ± 1.9	NS
AST (IU/L)	110 ± 40	98 ± 15	NS
ALT (IU/L)	66 ± 8	210 ± 111	< 0.05
Albumin (g/dL)	2.2 ± 0.1	2.6 ± 0.1	< 0.05
Ascitic fluid WBC (cells × 10^3/μL)	417.7 ± 172.5	N/A	N/A
Ascitic fluid PMN (cells × 10^3/μL)	196.9 ± 144.2	N/A	N/A
Ascitic fluid albumin (g/dL)	0.90 ± 0.14	N/A	N/A
Ascitic fluid culture	All negative	N/A	N/A

Data given as mean ± SE. INR: International normalized ratio; BUN: Blood urea nitrogen; AST: Aspartate transaminase; ALT: Alanine transaminase; PMN: Polymorphonuclear cells; NS: Non-significant; WBC: White blood cells.

Table 3 Serum and ascitic fluid levels of inflammatory markers in the 88 patients studied

	Ascites Group 1			Non-ascitic Group 2	Control		*P* value	
	Serum	Ascitic fluid	*P* value	Serum	NPP	*P* value	Group 1 (serum) *vs* Group 2	Group 1 (serum) *vs* NPP
IL-1A (ng/mL)	0.21 ± 0.08	0.21 ± 0.04	NS	0.17 ± 0.07	0.09 ± 0.01	NS	NS	< 0.05
IL-1B (ng/mL)	6.15 ± 2.83	3.10 ± 0.48	NS	3.56 ± 1.11	2.38 ± 0.62	NS	NS	< 0.05
IL-2 (ng/mL)	5.61 ± 3.49	1.31 ± 0.35	< 0.05	0.96 ± 0.01	1.01 ± 0.40	NS	< 0.05	< 0.05
IL-4 (ng/mL)	2.51 ± 0.79	9.48 ± 1.21	< 0.05	1.25 ± 0.07	1.21 ± 0.39	NS	< 0.05	< 0.05
IL-6 (ng/mL)	94.43 ± 29.58	1240.5 ± 365.64	< 0.05	7.27 ± 1.93	0.64 ± 0.16	< 0.05	< 0.05	< 0.05
IL-8 (ng/mL)	111.83 ± 37.01	335.08 ± 60.0	< 0.05	50.19 ± 18.84	2.22 ± 0.79	< 0.05	< 0.05	< 0.05
IL-10 (ng/mL)	1.47 ± 0.31	57.15 ± 22.32	< 0.05	0.95 ± 0.23	0.62 ± 0.08	< 0.05	NS	< 0.05
MCP-1 (ng/mL)	141.23 ± 23.39	456.35 ± 42.03	< 0.05	200.69 ± 18.19	57.11 ± 12.59	< 0.05	< 0.05	< 0.05
TNF-α (ng/mL)	5.26 ± 1.18	26.32 ± 7.13	< 0.05	9.89 ± 3.54	1.61 ± 0.80	< 0.05	NS	< 0.05
IFNγ (ng/mL)	1.08 ± 0.38	1.61 ± 0.41	NS	0.51 ± 0.04	0.47 ± 0.34	NS	< 0.05	NS
VEGF (ng/mL)	38.31 ± 18.73	234.82 ± 103.11		55.94 ± 11.37	7.14 ± 4.06		NS	< 0.05
EGF (ng/mL)	72.42 ± 14.95	1.78 ± 0.36		45.70 ± 7.38	3.12 ± 0.99		< 0.05	< 0.05

Data given as mean ± SE. IL: Interleukin; MCP: Monocyte chemotactic protein; TNF: Tumor necrosis factor alpha; IFN: Interferon gamma; VEGF: Vascular endothelial growth factor; EGF: Epidermal growth factor; NPP: Normal plasma panel; NS: Non-significant.

albumin levels in the ascitic fluid compared to serum. The mean serum-ascites albumin gradient (SAAG) was > 1.1 g/dL in Group 1.

The serum PCT levels of Group 1 (n = 41) were significantly greater than in Group 2 (n = 47) and the NPP (0.42 ± 0.19 ng/mL *vs* 0.10 ± 0.01 ng/mL, P < 0.05); approximately × 4 greater than each group. There was not a significant difference between the serum and ascitic fluid PCT levels of Group 1 (0.42 ± 0.19 ng/mL *vs* 0.27 ± 0.13 ng/mL, P > 0.05). The ascitic fluid PCT in Group 1 was significantly greater than the serum PCT level of Group 2 and the NPP (n = 12) (0.27 ± 0.13 ng/mL *vs* 0.10 ± 0.01 ng/mL, P < 0.05); approximately × 3 greater than each group. The PCT levels between Group 2 and the NPP were not significantly different (0.10 ± 0.01 ng/mL *vs* 0.09 ± 0.01 ng/mL, P > 0.05).

Table 3 reports the values for inflammatory markers assayed in the serum of Groups 1 and 2, and ascitic fluid of the Group 1. The plasma levels of the inflammatory cytokines IL-2, IL-4, IL-6, IL-8, IFNγ and EGF were all significantly greater in the serum of Group 1 as compared to that of the serum obtained from the Group 2 subjects (all P < 0.05). However, IL-1A, IL-1B, IL-10, MCP-1, TNF-α, and VEGF serum levels were not significantly different when comparing the serum values of Groups 1 and 2 for these measures. There were significantly greater levels of IL-2, IL-4, IL-6, IL-8, IL-10, MCP-1, TNF-α, VEGF and EGF in the ascitic fluid compared to serum of Group 1. There was no significant difference for IL-1A, IL-1B, and IFNγ between these groups. There were significantly greater levels of IL-1A, IL-4, IL-6, IL-8, IL-10, MCP-1, TNF-α, VEGF and EGF in Group 1 ascites compared to NPP. IL-1B and IL-2 levels were not significantly different when comparing Group 1 ascites to the NPP (not included in Table 3). There were significantly greater levels for all inflammatory markers, except IFNγ when comparing Group 1 serum levels to the NPP. There were significantly greater serum levels of IL-6, IL-8, IL-10, MCP-1, TNF-α, VEGF and EGF when comparing Group 2 to

Table 4 Serum and ascitic procalcitonin levels segregated by liver disease

	Serum	Ascites	*P* value
EtOH (ng/mL)	0.38 ± 0.22	0.22 ± 0.13	NS
HCV (ng/mL)	0.44 ± 0.27	0.13 ± 0.12	NS
Cardiac/miscellaneous (ng/mL)	0.95 ± 0.74	0.77 ± 0.07	NS
P value	NS	< 0.05[a]	

[a]*P* < 0.05 cardiac/misc *vs* non-cardiac diseases only. Data given as mean ± SE. EtOH: Alcohol; HCV: Hepatitis C virus; NS: Not significant.

Table 5 The serum-ascitic fluid (P-A) gradient for Group 1

Serum-ascitic fluid gradient	Value
IL-1A (ng/mL)	-0.0008 ± 0.097
IL-2 (ng/mL)	4.30 ± 3.53
IL-4 (ng/mL)	-6.96 ± 1.48
IL-6 (ng/mL)	-1146.11 ± 362.15
IL-8 (ng/mL)	-223.24 ± 58.54
IL-10 (ng/mL)	-55.68 ± 22.18
MCP-1 (ng/mL)	-315.13 ± 39.24
TNF-α (ng/mL)	-21.06 ± 7.33
IFNγ (ng/mL)	-0.52 ± 0.48
VEGF (ng/mL)	196.52 ± 105.60
EGF (ng/mL)	70.64 ± 14.74
PCT (ng/mL)	-0.134 ± 0.12

Data given as mean ± SE. IL: Interleukin; MCP: Monocyte chemotactic protein; TNF: Tumor necrosis factor α; IFN: Interferon gamma; VEGF: Vascular endothelial growth factor; EGF: Epidermal growth factor; PCT: Procalcitonin.

the NPP. There was no significant difference for IL-1A, IL-1B, IL-2, IL-4 and IFNγ levels between these two groups.

The serum and ascitic fluid PCT levels segregated as to the etiology of the liver disease are shown in Table 4. The cardiac cirrhosis/miscellaneous group had the greatest PCT levels in serum being × 2-3 that present in the other three disease subgroups. The ETOH and HCV subgroups each had PCT serum levels approximately half that of the cardiac cirrhosis/miscellaneous group. These differences were not statistically significant amongst each other. All PCT serum levels in each cirrhosis subgroup were significantly greater than that in the NPP ($P < 0.05$), data not shown. The greatest ascitic fluid PCT levels were present in the cardiac cirrhosis/miscellaneous group, with the levels for the HCV and ETOH cirrhosis subgroups being 17% and 29% of that group ($P < 0.05$). The ascitic fluid PCT levels in all of the cirrhosis subgroups were greater than in the NPP ($P < 0.05$), data not shown.

Table 5 displays the mean inflammatory marker gradient (serum minus ascitic fluid) for Group 1 patients. Gradients with positive values reflect inflammatory markers in which the serum level is greater than the ascitic fluid level. Gradients with negative values reflect inflammatory markers in which the ascitic fluid level is greater than the serum level. Thus, the IL-4, IL-6, IL-8, IL-10, MCP-1, and TNF-α levels were greater in the ascitic fluid than the in serum. The IL-1A and PCT serum and ascitic fluid levels were approximately equal. The IL-2, VEGF and EGF levels were greater in the serum than in the ascitic fluid.

Utilizing linear regression analysis, no relationship between the serum or ascitic PCT levels were observed when the PCT values was analyzed against either the whole blood or ascitic fluid total white blood cell counts, number and percentage of either the monocytes or lymphocytes as well as the number and percentage of monocytes plus lymphocytes in whole blood (data not shown).

DISCUSSION

Cirrhotic patients with portal hypertension commonly develop ascites, which itself is associated with SBP, HRS and even death[1-5]. Ascitic fluid promulgation further involves the dysregulation of the renin-angiotensin-aldosterone axis and the resultant alterations in vascular tone and volume control[4,5]. These processes are known to affect enteric mucosal permeability and enhance BT. In a recursive way, BT itself and the resultant host inflammatory response can not only alter enteric mucosal permeability, but exacerbate ascites and in certain instances lead to SBP[7-9,11,12].

In infected ascites, the cellular and cytokine inflammatory response is expectantly activated. The cellular aspect is so sensitive and reliable that an ascitic fluid neutrophil count of $\geqslant 250 \times 10^3$ cells/μL on diagnostic paracentesis, even in the absence of symptoms, can provide a provisional diagnosis of SBP while awaiting ascitic fluid culture results[5]. Furthermore, in those patients not clinically responding to SBP treatment, repeat paracentesis with cell count and differential studies may be performed to provide a rapid prognostic tool[5]. Other non-cellular inflammatory markers in SBP have been studied as well, including ascitic fluid lactoferrin, with productive results[37,38].

Interestingly though, even in non-infected cirrhotic patients with portal hypertensive ascites, there seems to be a significant degree of cellular and cytokine inflammation[16-18]. Multiple studies have documented a number of pro- and anti-inflammatory markers in the serum and ascitic fluid of such patients[17,18]. Furthermore, an immune response, both innate and adaptive, can be generated and exacerbated by a network of immunocytes sampling bacterial antigens in the enteric mucosa or after BT[29-37]. These processes are in a dynamic equilibrium, wherein a particular arrangement of inflammatory markers, both in the serum and ascitic fluid, interacts with a critical load of bacterial antigen. In certain instances, for reasons not entirely clear, this immune defense mechanism can be overwhelmed and lead to enhanced BT or even SBP. Whereas in other instances, these same defense mechanisms are successful in identifying and eliminating or sufficiently suppressing the bacteria load.

Serum procalcitonin levels have been shown to be

significantly increased above the level of 0.5 ng/mL in 88% of patients with decompensated cirrhosis and proven bacterial infection. About 50% of these patients present with extremely high serum PCT levels of greater than 5 ng/mL, correlating with high rates of in-hospital mortality[21]. However, a similar increase in serum procalcitonin levels was observed in 46% of patients who presented with acute alcoholic hepatitis and underlying cirrhosis with no evidence of bacterial infection. Thirty-one percent of patients with acute viral hepatitis were also found to have an elevated serum procalcitonin. A normal level of PCT (< 0.5 ng/mL) was observed in all patients presenting with uncomplicated cirrhosis regardless of the etiology of cirrhosis. These data suggest that a serum PCT > 0.5 ng/mL is not sufficient to differentiate between liver disease patients who have a bacterial infection from those without it[21].

In contrast, Connert *et al*[22] demonstrated that serum PCT levels above 0.58 ng/mL is a valid marker of bacterial infection in decompensated cirrhotic patients with a sensitivity of 92% and specificity of 78%. Patients who present with such levels of serum PCT were associated with 50% mortality in the first two months. Interestingly, serum levels of IL-6, TNF-α, and C-reactive protein failed to discriminate the presence or absence of an associated bacterial infection[22].

A higher cut-off value of serum PCT was use by Viallon *et al*[23] to diagnose SBP in cirrhotic patients. Serum procalcitonin levels of 0.75 ng/mL and higher were diagnostic of SBP with a sensitivity of 95% and a specificity of 98%. Importantly, ascitic fluid to serum ratio of TNF-α and IL-6 was greater than 2 in all 21 patients presenting with SBP. In contrast, the ascitic fluid to serum ratio of PCT was < 1 in all SBP cases suggesting that procalcitonin is not produced intraperitoneally. Thus, serum PCT determination may play a role as a non-invasive test in the diagnosis of SBP[23].

Su *et al*[28] reviewed the current evidence on the diagnostic value of serum procalcitonin levels in identifying SBP. They included three qualifying studies consisting of 181 episodes of suspected infection with 27% being confirmed as SBP. Serum PCT levels demonstrated moderate to high accuracy for PCT as a helpful marker for SBP. However, further large and prospective studies are needed to clarify these findings[28].

In this study, an array of inflammatory markers including PCT, a family of ILs, MCP-1, TNF-α, IFNγ, VEGF and EGF were analyzed in the serum and ascites of cirrhotic patients in Group 1 and the serum of cirrhotic patients without ascites in Group 2. The PCT level was significantly greater in the serum and ascites of Group 1 patients as compared to Group 2 patients. Additionally, the serum levels of IL-2, IL-4, IL-6, IL-8, MCP-1, IFN-γ and EGF were all significantly elevated in cirrhotic patients with ascites compared to those without ascites. Furthermore, this array of inflammatory markers, except for PCT and IFNγ, but with the addition of IL-10, TNF-α and VEGF demonstrated a significant difference between the levels present in ascites and serum. Such differences define a concentration gradient in which the majority of these inflammatory markers are greater in the ascitic fluid as compared to the serum. Such a gradient suggests enhanced peri-peritoneal production and promulgation of these inflammatory markers compared to the response arising from more systemic processes.

A second aim of the study was to characterize the inflammatory response segregated by liver etiology, and for this only serum and ascitic PCT levels in cirrhotic patients with ascites were evaluated. The serum PCT level was significantly elevated compared to NPP levels, but was not statistically different compared to the ascitic fluid levels. Additionally, there was a significant difference in ascitic fluid, but not serum, PCT levels when comparing the cardiac cirrhosis/miscellaneous subgroup to other subgroups. This study also demonstrated that PCT levels have no relationship with serum or ascitic fluid total leukocyte or monocyte levels, supporting data of its inflammatory expression pattern in multiple non-hematologic organ sites[19,20]. As there was no cohort group with infection studied, no conclusion as to its discriminatory capacity between sepsis and SIRS can be made. In particular, the role of PCT as a diagnostic marker of the severity of SBP has received little investigation.

The serum-ascites gradients of these inflammatory markers lends further support to the complex peri-peritoneal interplay of BT and immunocyte activation in both non-infected and infected cirrhotic patients with ascites[16-20,29]. It is interesting to speculate on the particular importance of ascites itself. This study documents the finding in patients with requiring LVP. However, the frequency of LVP, the amount of fluid removed, and background use or failure of diuretic therapy was not documented. A number of studies have reported that the manner in which ascitic fluid is reduced, *e.g.*, either by LVP or by diuretic therapy can have significant impact upon ascitic fluid complement, immunoglobulin, and opsonic concentrations[39,40]. It is reasonable to infer that certain concentrations and combinations of these factors in the setting of BT are crucial to an appropriate inflammatory response and in controlling infection. These studies suggest that diuretic therapy maintains higher concentrations of these key proteins and might be beneficial in preventing SBP compared to LVP, with or without combined diuretics[39,40].

The patients in the present study absent any clinical infection, and specifically those with ascites had demonstrated sterile ascitic fluid documented by culture. It was not documented however as to whether these patients had suffered any recent gastro-intestinal illnesses, change in bowel habits, or change in medications or diet. Such events are known to contribute both to enteric mucosal function and integrity, bacterial burden and distribution of species[4,6,13-15]. These alterations might then have effects upon the magnitude of BT and the pattern and in-

tensity of the local inflammatory response that have not been identified in the present report.

Additionally, despite the addition of ascites to Group 1, as referenced in Table 1, these two groups of patients had very similar clinical and biochemical backgrounds, including their MELD scores, which is a logarithmic equation including serum total bilirubin, creatinine and INR[41]. In these two groups only the INR was significantly different in patients with ascites. It might be commented that in so far as ascites represents decompensated liver disease, one might expect a worse MELD score, itself a marker for 90 day mortality without liver transplant[4,5,42,43]. Yet, the MELD score, while effective, is still quite imperfect in capturing the total biology of cirrhosis. Thus, the provisional addition of "exception points" for certain patients, *e.g.*, those with hepatocellular carcinoma, who have particularly good post-transplant outcomes that would not be predicted by the traditional MELD score has been accepted[44]. Further, is the constant interest in modifying the MELD score equation itself with a serum sodium (Na) component; the so called "MELDNa" score[45]. It is likely that serum Na and albumin, in addition to volume status have a role to play in capturing quantitatively the biological essence and clinical relevance of ascites to treatment and overall clinical outcome, but this remains an area of continued research.

Based on the present study, the serum level of PCT as well as the serum level of other inflammatory cytokines assessed in this study could be used to determine the likelihood for reduced life span or future adverse events such as the development of spontaneous bacterial peritonitis or the hepatorenal syndrome in those with ascites. This however remains to be proven in longitudinal studies[28].

In summary, this study demonstrates that non-infected cirrhotic patients with ascites as compared to those without ascites manifest a unique underlying immune response as mediated by a constellation of pro- and anti-inflammatory protein markers. This immune response functions in a gradient fashion, suggesting a peri-peritoneal predominance compared to systemic origin. From this data, it is reasonably inferred that the biology of ascites has a strong role in the interplay between BT and the cellular and cytokine inflammatory response. Further studies might examine the arrangement of these inflammatory markers in response to ascitic fluid management and/or particular concentrations of enteric flora.

COMMENTS

Background

Serum procalcitonin (PCT) level has received much attention as a possible discriminatory serum marker in differentiating sepsis from systemic inflammatory response syndrome and bacterial sepsis from non-bacterial sepsis in multiple populations including cirrhotic patients.

Research frontiers

It is well known that cirrhotics, with and without ascites, have many factors which predispose to enhanced bacterial translocation (BT). The research hot spot is to quantitate the simultaneous serum and ascitic fluid levels of PCT and several inflammatory markers in non-infected cirrhotic patients with and without ascites, and whether these markers differ with respect to etiologies of liver disease.

Innovations and breakthrough

The cardiac cirrhosis/miscellaneous group had the greatest serum PCT levels. The alcohol and hepatitis C virus subgroups each had PCT serum levels approximately half that of the cardiac cirrhosis/miscellaneous group. This study demonstrates that non-infected cirrhotic patients with ascites as compared to those without ascites manifest a unique underlying immune response as mediated by a constellation of pro- and anti-inflammatory protein markers. This immune response functions in a gradient fashion, suggesting a peri-peritoneal predominance compared to systemic origin. From this data, it is reasonably inferred that the biology of ascites has a strong role in the interplay between BT and the cellular and cytokine inflammatory response. Further studies might examine the arrangement of these inflammatory markers in response to ascitic fluid management and/or particular concentrations of enteric flora.

Applications

This study results suggest that the serum level of PCT as well as the serum level of other inflammatory cytokines assessed could be used to determine the likelihood for reduced life span or future adverse events such as the development of spontaneous bacterial peritonitis or the hepatorenal syndrome in those with ascites. This however remains to be proven in longitudinal studies.

Terminology

Decompensated cirrhosis is identified by the presence of portal hypertensive clinical sequelae, namely hepatic encephalopathy, varices, and most commonly, ascites. Ascites is associated with two other serious complications such spontaneous bacterial peritonitis and hepatorenal syndrome, both of which increase morbidity and can lead to death.

Peer review

This is an article of importance in its field. This study is an innovated research with pretty good presentation and readability of the article. The authors reported an interesting study about findings of several biomarkers in cirrhotic patients with and without ascites.

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