ASSESSMENT OF HIGH MOBILITY GROUP BOX 1 (HMGB1) IN ASCITIC FLUID FOR DIFFERENTIATION BETWEEN HEPATIC AND MALIGNANT ASCITES

Thesis

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INTRODUCTION

Ascites means pathological fluid accumulation within the abdominal cavity ***(Reynolds, 2000).*** It is a major complication of cirrhosis, occurring in 50% of patients over 10 years of follow up ***(Gines et al., 1987)***. The development of ascites is an important landmark in the natural history of cirrhosis as it is associated with a 50% mortality over two years ***(Liach et al., 1988).***

Major factors involved in the complex pathogenesis of ascites are portal and sinusoidal hypertension, arterial vasodilatation and neuro-humoral activation, all leading to sodium and water retention ***(Gentilini et al., 2002).***

The majority (75%) of patients who present with ascites have underlying cirrhosis, with the remainder being due to malignancy (10%), heart failure (3%), tuberculosis (2%), pancreatitis (1%), and other rare causes ***(Runyon, 1993).***

Malignant ascites is the condition in which fluid containing cancer cells collects within the abdomen. It is usually caused by ovarian, endometrial, breast, esophageal, gastric, colorectal, lung, pancreatic, hepatobilliary and primary peritoneal carcinomas ***(Kashani et al., 2008).*** Sometimes ascites is the sole manifestation of internal malignancies ***(Saif et al., 2009).***

The infiltrating tumour cells disrupt the normal regulation of fluid flow in the peritoneal cavity by simultaneously causing

a greater plasma inflow as well as a reduced lymphatic outflow. Generally, patients suffering from malignant ascites have a poor prognosis implying a median survival time of 1-4 months, depending greatly on the underlying type of tumour and its stage ***(Adam and Adam, 2004).***

The underlying cause of ascites is frequently obvious from the history and physical examination. However, it is important to exclude other causes of ascites. The essential investigations on admission include a diagnostic paracentesis with measurement of ascitic fluid albumin or protein, ascitic fluid neutrophil count, ascitic culture and ascitic fluid cytology should be requested when there is a clinical suspicion of underlying malignancy ***(Moore and Aithal, 2006).*** Only 7% of ascitic fluid cytologies are positive ***(Runyon et al., 1988).***

Ascitic fluid analysis is essential for the diagnosis of malignant ascites. Exudative or transudative ascities on the basis of total protein content (≥2.5 or <2.5 g/dL, respectively ***(Islam and Marino, 2001),*** is hampered by a large overlap between malignant and non-malignant ascites. The purpose of this subdivision is to help identify the cause of ascites. Thus "malignancy classically causes an exudative ascites and cirrhosis causes a transudate". The serum ascites-albumin gradient (SAAG) is far superior in categorising ascites with 97% accuracy ***(Runyon et al., 1992).***

In attempt to identify a reliable test to discriminate between malignant and non malignant ascites, various

biochemical markers in the serum and ascitic fluid were evaluated ***(Castaldo et al., 1994).***

High mobility group box 1 (HMGB1), also known as amphoterin or HMGB1, belongs to a group of chromatin associated non-histone proteins characterized by low molecular weight, acidic solubility and high content of charged amino acids ***(Goodwin et al., 1973 and Muller et al., 2001)***. Proteins of the HMGB family, comprising HMGB1, HMGB2, and HMGB3, are characterized by two DNA-binding domains called HMG boxes ***(Landsman and Bustin, 1993)***. Currently the best analyzed member of this group is HMGB1. HMGB1 is an intracellular protein, which can be secreted for example by activated monocytes, macrophages, and astrocytes and can be released by necrotic or damaged cells ***(Passalacqua et al., 1998; Wang et al., 1999 and Scaffidi et al., 2002).***

Recently, it was reported that HMGB1, a so-called danger signalling protein, was found to be highly expressed in human pleural and peritoneal effusions due to cancer and inflammation. Compared to transudates, the average level of HMGB1 was significantly higher in exudates ***(Winter et al., 2009).***

The differential diagnosis of ascites is a common clinical problem. Thus we aim in this study to measure the level of HMGB1 in ascites due to liver cirrhosis and malignant ascites to evaluate its role in differentiating the two conditions.

AIM OF THE WORK

This study aims to evaluate the role of ascitic fluid HMGB1 in differentiation between malignant ascites and ascites due to liver cirrhosis.

DEFINITION AND CLASSIFICATION OF ASCITES

Definition of ascites:

Ascites is defined as the presence of more than 25 ml of fluid in the peritoneal cavity. The normal hepato-splanchnic lymph production is approximately 1 ml/min. In patients with cirrhosis, this rate may increase up to 10 ml/min .When the production of lymphatic fluid exceeds the lymphatic transport capacity, ascites develops ***(Henriksen and Møller, 2005).***

Classification of ascites:

The International Ascites Club classifies ascites according to severity, complication, and response to diuretic treatment.

I. Classification of ascites according to severity:

- *Grade 1 (mild):* Ascites is only detectable by ultrasound examination.
- *Grade 2 (moderate):* Ascites causing moderate symmetrical distension of the abdomen.
- *Grade 3 (large):* Ascites causing marked abdominal distension.

(Arroyo et al., 1996).

II. Classification of ascites according to complication:

Ascites is classified into complicated and uncomplicated ascites ***(Arroyo et al., 1996).***

III. Classification of ascites according to response to diuretic therapy:

Ascites can be classified into responding to diuretic therapy and non responding to diuretic therapy which is defined as refractory ascites.

Refractory ascites:

Ascites that cannot be mobilized or early recurrence of ascites which cannot be satisfactorily prevented by medical therapy. The term refractory ascites includes the following two subtypes:

- **Diuretic resistant ascites:** Ascites that is refractory to dietary sodium restriction and intensive diuretic treatment (spironolactone 400 mg/day and furosemide 160 mg/day for at least one week, and a salt restricted diet of less than 90 mmol/day (2 g of salt /day) ***(Moore and Aithal, 2006).***
- **Diuretic intractable ascites:** Ascites that is refractory to therapy due to the development of complications that prevent the use of an effective diuretic dosage ***(Moore and Aithal, 2006).***

ETIOLOGY OF HEPATIC ASCITES

Many diseases can lead to the accumulation of fluid within the peritoneal cavity. The most common cause of ascites is cirrhosis, which accounts for 80% of cases; peritoneal malignancy (e.g., peritoneal metastases from GI tumors or ovarian cancer), heart failure, and peritoneal tuberculosis account for another 15% of cases ***(Hwangbo et al., 2007).***

There are many classifications for causes of ascites depending on different bases:

- Ascites with normal peritoneum and diseased peritoneum.
- Transudative and exudative ascites according to ascitic fluid total protein.
- High and low serum - ascites albumin gradient (SAAG).

I. Ascites with normal peritoneum and diseased peritoneum:

A) Causes of ascites with normal peritoneum:

1) Portal hypertension:

- Cirrhosis.
- Hepatic fibrosis.
- Congestive heart failure.
- Budd-Chiari syndrome.
- Portal vein occlusion.
- Constrictive pericarditis.

2) Hypoalbuminemia:

- Nephrotic syndrome.
- Protein energy malnutrition.
- Protein losing enteropathy.

3) Miscellaneous condition:

- Chylous ascites.
- Pancreatic ascites.
- Bile ascites.
- Nephrogenic ascites.
- Myxoedema.
- Urine ascites.

(Hwangbo et al., 2007)

B) Causes of ascites with diseased peritoneum:

1) Infection:

- Bacterial peritonitis.
- Tuberculous peritonitis.
- Fungal peritonitis.
- Parasitic diseases.

2) Malignancy.

3) Other causes:

- Familial Mediterranean fever.
- Vasculitis.
- Granulomatous peritonitis.

(Hwangbo et al., 2007)

II. Transudative and exudative ascites according to ascitic fluid total protein:

Before the 1980, the ascitic fluid total protein concentration was used to classify ascites as either exudative or transudative ***(Runyon, 2010).***

Low protein ascites with total protein concentration of less than 2.5 g/dl is called transudative ascites and usually occurs with portal hypertension or hypoalbuminaemia. A higher protein ascites with total protein concentration of more than 2.5 g/dl is called exudative ascites and is usually associated with tuberculosis, malignancy, pancreatitis, etc. However, a total protein concentration of greater than 2.5 g/dl has recently been shown to have an accuracy of only 56 % in detecting an exudate ***(Sood, 2008).***

The protein concentration in ascitic fluid in the setting of cirrhosis is determined almost entirely by the serum protein concentration and portal pressure. A patient with cirrhosis and a relatively high serum protein concentration will have a relatively high ascitic fluid protein concentration. Because of this relationship, almost 20% of ascitic samples in patients with cirrhosis will have a protein concentration greater than 2.5 g/dL ***(Runyon, 2010).***

III. High and low serum – ascites albumin gradient (SAAG):

Classification of ascites based on the serum – ascites albumin gradient (SAAG) has replaced the exudate–transudate concept and provides a reliable tool to determine whether ascites can be attributed to portal hypertension or has another etiology **(Table1)** ***(Saadeh and Davis , 2004).***

The SAAG is based on oncotic hydrostatic balance. Portal hypertension results in an abnormally high hydrostatic pressure gradient between the portal bed and ascitic fluid. A similarly large difference must exist between ascitic fluid and intravascular oncotic forces. Albumin exerts greater oncotic force per gram than that exerted by other proteins. Therefore, the difference between the serum and ascitic fluid albumin concentrations correlates directly with portal pressure ***(Runyon, 2010).***

The SAAG is calculated by subtracting the ascitic fluid albumin concentration from the serum albumin concentration obtained on the same day ***(Saadeh and Davis, 2004).***

If the SAAG is ≥1.1 g/dL, the patient has portal hypertension, with approximately 97% accuracy ***(Poonwala et al., 2000).*** Patients who have portal hypertension plus a second cause for ascites formation also have a SAAG ≥1.1 g/dL ***(Runyon, 2009)***. Also high gradient ascites (≥1.1 g/dL) present with heart-failure-induced ascites and other circumstances such as fulminant hepatic failure ***(Glickman and Rajapaksa, 2008).***

Low-gradient ascites (<1.1 g/dL) is associated with non cirrhotic causes, including malignancy, pancreatic disease, TB, and other infectious/inflammatory conditions ***(Lardizabal and Johnson, 2007).***

Approximately 5% of patients with ascites have “mixed” ascites (that is, two causes of ascites). Most of these patients have portal hypertension from cirrhosis as well as another cause of ascites, such as tuberculous peritonitis or peritoneal

carcinomatosis. The albumin gradient is high (1.1 g/dL or greater) in mixed ascites, as a reflection of the underlying portal hypertension ***(Runyon et al., 1992).*** Accuracy of SAAG is approximately 97% ***(Runyon, 2006).***

Table (1): Causes of ascites by SAAG level:

High gradient, SAAG ≥1.1 g/dL	**Low gradient, SAAG < 1.1 g/ dL**
Liver disease o Alcoholic hepatitis o Bludd-Chiari syndrome o Cirrhosis o Fatty liver of pregnancy o Fulminant hepatic failure o Hepatic congestion o Massive liver metastasis o Portal vein thrombosis **Cardiac disease** o Congestive heart failure o Constrictive pericarditis o Tricuspid insufficiency	**Infectious peritonitis** o HIV- associated, bacterial, tuberculous, or fungal infection **Malignancy** o Hepatocellular carcinoma. o Peritoneal carcinomatosis o Primary mesothelioma o Pseudomyxoma peritonei **Hypoalbuminemia** o Nephrotic syndrome o Severe malnutrition **Others** o Biliary ascites o Bowel infarction o Chylous ascites o Familial Mediterranean fever o Granulomatous or eosinophilic peritonitis o Pancreatic ascites o Vasculitis

(Sood, 2008)

Pathophysiology of Hepatic Ascites

The pathophysiology underlying the formation of ascites in cirrhosis is complex. There are many neuro-hormonal, renal and systemic vascular abnormalities. This is reflected in the number of theories. Three theories of ascites formation have been proposed: underfilling, overflow and peripheral arterial vasodilatation theories ***(Sherlock and Dooley, 2002).***

The underfilling theory suggests that the primary abnormality is inappropriate sequestration of fluid within the splanchnic vascular bed due to portal hypertension and a consequent decrease in effective circulating blood volume. This activates the plasma renin, aldosterone, and sympathetic nervous system, resulting in renal sodium and water retention ***(Shah and Field, 2009).***

The overflow theory suggests that the primary abnormality is inappropriate renal retention of sodium and water in the absence of volume depletion. This theory was developed in accordance with the observation that patients with cirrhosis have intravascular hypervolemia rather than hypovolemia**.** There is a primary renal change responding to a hepatic signal that leads to sodium retention (overfill theory) ***(Shah and Field, 2009).***

Several signals have been suggested. Reduced hepatic synthesis of a natriuretic agent, reduced hepatic clearance of a sodium-retaining hormone, or a 'hepato-renal reflex' of unknown aetiology could be responsible. The hypothesis

proposes that sodium and water retention lead to expansion of the plasma volume, an increase in cardiac output and a fall in systemic vascular resistance. The combination of portal hypertension and circulatory hypervolaemia lead to ascites ***(Sherlock and Dooley, 2002).***

It suggests that portal hypertension leads to vasodilation, which causes decreased effective arterial blood volume. As the natural history of the disease progresses, neuro-homoral excitation increases, more renal sodium is retained, and plasma volume expands. This leads to overflow of fluid into the peritoneal cavity. The vasodilation theory proposes that underfilling is operative early and overflow is operative late in the natural history of cirrhosis ***(Shah and Field, 2009).***

The abnormalities associated with the formation of ascites in patients with cirrhosis are:

- Portal hypertension
- Renal retention of sodium
- Splanchnic arterial vasodilatation
- Systemic vascular changes
- Increased splanchnic and hepatic lymph formation
- Hypoalbuminaemia

(Sherlock and Dooley, 2002)

The main factor contributing to the development of ascites in a patient with cirrhosis is the portal hypertension ***(Hernandez-Guerra et al., 2005).***

Portal and sinusoidal hypertension is a prerequisite for the development of ascites. The hydrostatic pressure within the hepatic sinusoids favours transudation of fluid into hepatic lymphatics and the peritoneal cavity. Moreover, the formation of ascites depends on the balance between the increased local transvascular filtration and augmented lymph drainage ***(Henriksen and Moller, 2005).*** Thus, the amount of ascitic fluid produced is governed by increased trans-sinusoidal filtration of protein and fluid and by accelerated trans-peritoneal hydrostatic and oncotic dynamics ***(Henriksen et al., 2001).***

Splanchnic vasodilatation develops as persistent portal hypertension results in local overproduction of vasodilators such as nitric oxide (NO), calcitonin gene–related peptide, substance P, carbon monoxide ***(Iwakiri and Groszmann, 2007)***.

Splanchnic arteriolar vasodilation and consequent pooling of blood in the splanchnic circulation causes a decrease in effective arterial blood volume and arterial pressure. In response to these changes, baroreceptor-mediated activation of the sympathetic nervous system (SNS), renin-angiotensin-aldosterone system (RAAS), and antidiuretic hormone (ADH) cause avid renal water and sodium retention in order to restore homeostasis ***(Cardenas et al., 2006).***

A side from changes in the splanchnic hemodynamics, patients with ascites develop a hyperdynamic state characterized