

Diagnostic Accuracy of Ascitic Fluid Lactoferrin in Diagnosis of Spontaneous Bacterial Peritonitis

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Abstract:

SBP is a frequent and severe complication in patients with cirrhosis and ascites. diagnostic paracentesis is used commonly to investigate the presence of SBP. The aim of this study was to evaluate the role of ascitic fluid lactoferrin as a reliable biomarker for the presence of PMNs and detection of SBP in patients with cirrhosis. Ascitic fluid lactoferrin level is much higher among patients with SBP compared to those without SBP. The usefulness of ascitic fluid lactoferrin is still uncertain to rule out SBP because of the small number of cases.

Introduction

Spontaneous bacterial peritonitis (SBP) is a frequent and severe complication in patients with cirrhosis and ascites. SBP is a bacterial infection that occurs in the absence of an evident intraabdominal and surgically treatable source of infection (Enomoto and Nishiguchi, 2015). Cirrhotic ascites forms as a result of development of portal hypertension, hypoalbuminemia and reduced plasma oncotic pressure which favor the extravasation of fluid from the plasma to the peritoneal fluid (Sheeran and Runyon, 2005). Some patients with SBP have symptoms or signs clearly suggestive of peritoneal infection where as others may be completely asymptomatic or with minor symptoms only. Therefore, diagnostic paracentesis is used commonly to investigate the presence of SBP (Ribeiro et al., 2007). The diagnosis of SBP is based on a polymorphonuclear leukocyte (PMN) count in ascitic fluid of ≥ 250 cells/mm³, irrespective of whether the ascitic fluid indicates positive results on bacterial culture (Fernandez and Acevedo, 2015). SBP leads to hospitalization of 10%-30% of cirrhotic patients, and the mortality rate in this group approaches 30%

(Huang et al., 2015). Lactoferrin is an iron binding protein that is found mainly in external secretions and in PMNs and is released on degranulation (Boone et al., 2013). The measurement of ascitic fluid lactoferrin could provide a reliable biomarker for the presence of PMNs and detection of SBP in patients with cirrhosis as lysis of the PMNs during transport to the laboratory may lead to false negative results (Khalifa et al., 2013).

Our study included 6 original articles, 2 responses and editorials to the first published original article, 2 review articles, one conference abstract and one systematic review.

Objectives:

Collect and analyze multiple research studies to assess the possible role of lactoferrin in diagnosis of SBP.

Strategy and methods of the research:

The strategy of this systematic review was based upon raising some research questions addressing the possible role of lactoferrin in diagnosis of SBP and put a plan to find the best available answers for each. This was done by looking in the literature and critically appraising the available researches in this field. Good quality researches had

been selected to reach a conclusive answer for each question.

The research questions are:

- 1- Is there an evidence-based value of lactoferrin in diagnosis of SBP?
- 2- Can cell count using haemocytometer be replaced by lactoferrin in diagnosis of SBP?
- 3- Is there any evidence about the role of lactoferrin in diagnosis of hepatocellular carcinoma in absence of SBP?

Selection criteria of the available researches:

a) Type of studies:

Randomized clinical trials (RCT) are be the gold standard to obtain the evidence, but such trials are non applicable for our research questions,

Results of the study

In this systematic review, we searched for researches fulfilling our conditions in the internet as mentioned in the "Materials and methods" section. A total of 23 papers found in the last 10 years. However, we found that; 11 of these papers did not fulfil all of our conditions, either because they used lactoferrin for other purposes than diagnosis of SBP, or they used other methods for diagnosis of SBP than ascitic fluid lactoferrin. These 11 papers were then excluded from our study, and only 12 papers included.

The included 12 papers were then analysed and we found that they belong to 6 types of researches (Figure 1):

These 6 papers were then thoroughly analysed in our systematic review as follows:

as there is no intervention done for the cases. So, we searched for comparative and screening researches. Available systematic reviews had been searched for.

b) Time of studies: English language Studies published during last ten years in the following medical databases:

c) Sites visited: Cochrane library, Pubmed, Medline, Sciencedirect, Any other site containing useful information.

d) Keywords used: Lactoferrin, SBP, Liver cirrhosis, Ascites.

e) The topic of interest: Diagnostic accuracy of ascitic fluid lactoferrin in diagnosis of Spontaneous Bacterial Peritonitis.

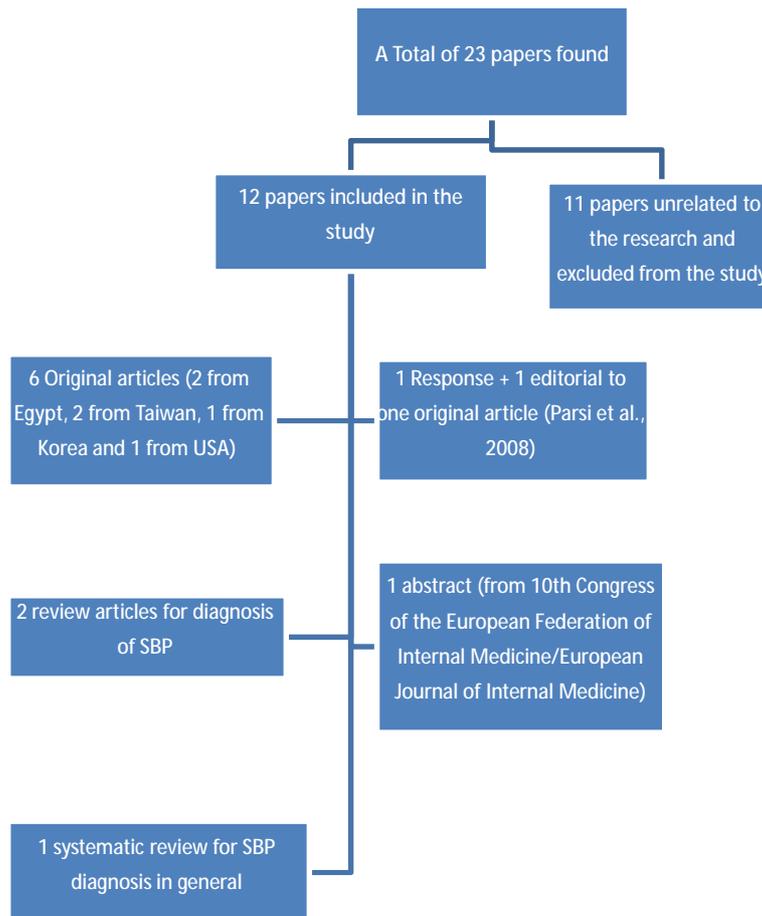


Figure 1. Papers included in our study

Table 1. Types and basic data of the included papers in this systematic review

Serial	Authors	Country	Year	Type of the study
1	Parsi et al	USA	2008	Original article
2	Khalifa et al	Egypt	2013	Original article
3	Feng et al	Taiwan	2014	Original article
4	Wu et al	Taiwan	2015	Original article
5	Abulseoudet al	Egypt	2016	Original article
6	Lee et al	Korea	2016	Original article
7	Thanellas et al	Greece	2011	Conference abstract
8	Riggio et al	Italy	2009	Response to: Parsi et al., 2008
9	Kallwitz	USA	2008	Editorial to: Parsi et al, 2008
10	Deveret al	USA	2015	Review article
11	Shizuma	Japan	2016	Review article
12	Riggio et al	Italy	2009	Systematic review

Table 2. Original articles used in this systematic review

Serial	Authors	Country	No. of cases	No. of ascetic samples	Cut off value	Sensitivity	Specificity	Level of evidence
1	Parsi et al,2008	USA	148	218	242 ng/ml	95.5%	97%	III
2	Khalifa et al,2013	Egypt	70	70	270 ng/ml	96%	95%	III
3	Feng et al,2014	Taiwan (and China)	7	8	-	-	-	IV
4	Wu et al., 2015	Taiwan	22	22	-	-	-	III
5	Abulseoudet al, 2016	Egypt	60	60	255 ng/ml	100%	88.9%	III
6	Lee et al,2016	Korea	102	102	63 ng/ml	91.7%	78.2%	III

The 6 original articles were as follows (in chronological order):

1. Parsi et al (2008) from USA
2. Khalifa et al (2013) from Egypt
3. Feng et al (2014) from Taiwan
4. Wu et al (2015) from Taiwan

5. Abulseoud et al (2015) from Egypt
6. Lee et al (2016) from Kore

Table 3. Meta-analysis of accuracy and cut-off level of AFLAC in the 4 papers with ROC curve analysis (No.1,2,5,6).

Article	Parsi et al	Khalifa et al	Abulseoud et al	Lee et al	Meta-analysis
No. of samples	218	70	60	102	450
Cut off value	242	270	255	63	207.52
True positive	21	48	30	22	121
True negative	187	19	26.67	61	293.67
False positive	6	1	3.33	17	27.33
False negative	1	2	0	2	5
Sensitivity	95.45%	96.00%	100.00%	91.67%	96.03%
Specificity	96.89%	95.00%	88.90%	78.21%	91.49%
Positive predictive value	77.78%	97.96%	90.01%	56.41%	81.57%
Negative predictive value	99.47%	90.48%	100.00%	96.83%	98.33%
Accuracy	96.17%	95.50%	94.45%	84.94%	93.76%
AUC	0.98	0.995	0.98	0.898	0.964

Discussion

Ascites is the pathological accumulation of free fluid in the peritoneal cavity. The fluid accumulates because of conditions directly involving the peritoneum (infection, malignancy), or due to other diseases remote from the peritoneum (i.e., liver disease, heart failure, hypoproteinaemia) (*Muhammed et al., 2012*). Ascites is primarily a transudative fluid with poor opsonic activity which provides a favourable environment for growth of bacteria. SBP rarely occurs without cirrhosis, but cardiac, renal, malignancy, portal vein thrombosis and autoimmune, related infection of ascites has been reported (*Scarpellin et al., 2010*). SBP is a frequent and severe complication in patients with cirrhosis and ascites. It is a bacterial infection that occurs in the absence of an evident intra-abdominal and surgically treatable source of infection (*Enomoto and Nishiguchi, 2015*). Some patients with SBP have symptoms or signs clearly suggestive of peritoneal infection

where as others may be completely asymptomatic or with minor symptoms only. Therefore, diagnostic paracentesis is used commonly to investigate the presence of SBP (*Ribeiro et al., 2007*). A variety of factors are associated with the development of SBP including the pathophysiological hallmark: bacterial translocation in an immunocompromised host (*Wiest, 2005*). Lactoferrin is a red iron-binding whey protein present mainly in external secretions such as breast milk and in polymorphonuclear neutrophils. The presence of lactoferrin in body fluids is proportional to the flux of neutrophils and its assessment can provide a reliable biomarker for inflammation (*Sugiyama et al., 2002*). In gastrointestinal diseases increased fecal lactoferrin is a sensitive and specific surrogate marker for inflammatory bowel diseases in patients with chronic diarrhea and pain, and ascites lactoferrin can also provide a promising and reliable

biomarker for bacterial peritonitis (*Martins et al., 1995*). The measurement of ascitic fluid lactoferrin could provide a reliable biomarker for the presence of PMNs and detection of SBP in patients with cirrhosis as lysis of the PMNs during transport to the laboratory may lead to false negative results (*Khalifa et al., 2013*). The rationale of our study was to review and assess our knowledge about the value of ascitic fluid lactoferrin level as a diagnostic tool to identify SBP in patients with ascites caused by cirrhosis. Our study included 6 original articles, 2 responses and editorials to the first published original article, 2 review articles, one conference abstract and one systematic review. All of the 6 original articles included in this systematic review showed that ascitic fluid lactoferrin level is much higher among patients with SBP compared to those without SBP (*Parsi et al., 2008, Khalifa et al., 2013; Feng et al., 2014; Wu et al., 2015; Abulseoud et al., 2016 and Lee et al., 2016*). Four of the 6 original articles used ROC curve analysis to establish a suitable cut-off value for lactoferrin, above which SBP should be suspected. From these 4 articles, 3 gave similar results, with cut off values ranged from 242-270 ng/ml (*Parsi et al., 2008, Khalifa et al., 2013; and Abulseoud et al., 2016*); while the fourth article showed much lower cut-off value of only 63 ng/ml; with much lower specificity and no better specificity (*Lee et al., 2016*). The meta-analysis of these 4 articles showed that the cut-off value may be adjusted to around 207 ng/ml, with a sensitivity of over 96% and a little lower specificity of 91.5% and an overall accuracy of 93.8%.

Conclusion

The measurement of ascitic fluid lactoferrin could provide a reliable biomarker for the presence of PMNs and detection of SBP in patient with cirrhosis. Ascitic fluid lactoferrin level is much higher among patients with SBP compared to those without SBP.

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