Ascitic fluid analysis: the role of biochemistry and haematology

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In specific settings, biochemical and haematological analysis of ascitic fluid may provide answers to important clinical questions. This review seeks to outline the value and limitations of ascitic fluid analysis and the main clinical scenarios in which it may be useful.

Ascites refers to the accumulation of fluid in the peritoneal space. Its presence can usually be established by clinical examination, but in doubtful cases ultrasound may be helpful. Contributory pathogenetic factors include increased portal venous pressure, decreased plasma oncotic pressure, increased hepatic lymph formation and secondary hyperaldosteronism. The composition of ascites varies depending on the underlying cause, and this provides the basis for the role of laboratory investigations in the differential diagnosis of ascites. Biochemistry and haematology are also useful in the evaluation of suspected peritonitis in patients with ascites; a detailed account of the role of bacteriology is beyond the scope of this review. There follows a section on the diagnosis of malignancy-related ascites and the value of ascitic fluid analyses in establishing its pathogenesis. Finally, sample requirements for ascitic fluid analysis are outlined briefly.

DIFFERENTIAL DIAGNOSIS OF ASCITES

Transudate or exudate

Most clinicians will be familiar with the classification of ascites into transudates and exudates, based on the protein concentration of the accumulated fluid. Transudates have less protein than exudates, although the cut-off total protein concentration is arbitrary and varies. Most cut-offs lie between 20 and 30 g/litre. Inflammatory causes of ascites such as malignancy or infection are usually associated with exudates, while transudates more commonly reflect reduced plasma oncotic pressure or increased plasma hydrostatic pressure. However, the ability of the total protein concentration on its own accurately to reflect pathogenesis is limited. For example, ascites resulting from congestive cardiac failure (cardiac ascites) may have a high protein content, and conversely a low protein content may be seen in some cases of infective ascites. In any case, the normal peritoneal fluid protein concentration is in excess of 40 g/litre. It has therefore been suggested that the exudate-transudate concept should be discarded in the classification of ascites, in favour of the serum ascites albumin gradient (SAAG) (Runyon et al, 1992).

Serum ascites albumin gradient

The SAAG is defined as the serum albumin concentration minus the ascitic fluid albumin concentration. It is claimed that the SAAG correlates directly with the portal pressure. In their large series of 901 paired serum and ascitic specimens collected prospectively from 330 patients, Runyon et al found that all patients with SAAG of ≥11 g/litre had portal hypertension, whereas no patient with SAAG <11 g/litre had this disorder (Runyon et al, 1992).

Table 1 categorizes some of the commoner causes of ascites according to the SAAG. Sometimes causes of ascites that are normally associated with a narrow gradient will occur in patients with portal hypertension, in which case the gradient will be wide. In these situations additional analyses may help to narrow the differential diagnosis. For example, abnormalities of pH, lactate, glucose and/or lactate dehydrogenase (LDH) may point towards an inflammatory process; an ascitic lymphocytosis is found in tuberculosis, lymphomas or fungal infections of the peritoneum; and malignant cells are found in nearly all patients with peritoneal carcinomatosis.

The SAAG classification has been used to predict the presence of oesophageal varices (Torres et al, 1998). In this study of 31 patients with ascites, these authors found that 87.5% of patients with SAAG ≥14.35 g/litre had varices, and 66.7% of patients with SAAG <14.35 g/litre

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did not. (The size of the varices did not depend on the SAAG.) The SAAG has also been used to investigate the mechanism of ascites formation in patients with malignant ascites, and the response to dietary sodium restriction and diuretics (see below).

The SAAG should not be used indiscriminately. Its ability to predict accurately the presence or absence of portal hypertension may be affected by diuresis, very low serum albumin concentrations (<12 g/litre) or non-steady-state conditions. In addition, Hoefs (1990) has emphasized the importance of simultaneous sampling of blood and ascites; even a few hours difference may alter the gradient, making interpretation difficult or impossible. Within these limitations, the SAAG offers a useful physiological approach to the initial differential diagnosis of ascites.

PERITONITIS

Ten per cent or more of cirrhotic patients with ascites develop peritonitis. This usually occurs in the absence of an obvious focus of infection, in which case it is known as spontaneous bacterial peritonitis (SBP). Less commonly, an identifiable source of infection, such as a perforated viscus or intra-abdominal abscess, is responsible (secondary infection). Biochemical and haematological investigations can assist in three ways:

1. They may be used to predict who is likely to develop SBP
2. They may permit rapid detection of infection
3. They may help to differentiate SBP from secondary infection.

Risk stratification

It has been known for some time that a low protein concentration in ascitic fluid predisposes to SBP; the ability to phagocytose bacteria disappears below a total protein concentration of 10 g/litre (Runyon, 1986). More recently, prospective analysis of 109 cirrhotic patients with low ascitic fluid protein (<10 g/litre) has shown that a high bilirubin (>55 μmol/litre) or a low platelet count (<98x10^9/litre) identifies individuals who are at particularly high risk (Guarner et al, 1999).

Early detection

The need for laboratory investigations in the early detection of peritonitis arises from the inability to make the diagnosis reliably by clinical examination alone, and the length of time it takes for positive culture results to develop. Most episodes of SBP are associated with low concentrations of bacteria, which may not be detected using conventional culture techniques. The rate of detection can be improved by bedside inoculation of 10 ml ascites into each culture bottle followed by incubation for 5–7 days (Runyon et al, 1988a). For early detection of peritonitis, the neutrophil count in the ascitic fluid has been reported to be better than any biochemical test (or combination of tests) (Albillos et al, 1990); this is readily obtainable by doing a full blood count and differential on the ascitic fluid specimen. On the basis of this study and others, it has been concluded that patients with clinical peritonitis (regardless of the neutrophil count) or patients with a neutrophil count >0.5x10^9/litre should be treated for SBP; patients with counts between 0.25 and 0.5x10^9/litre should be treated or the paracentesis repeated within 12 hours (Hoefs, 1990).

SBP or secondary infection

Secondary peritonitis tends to be more severe than SBP, probably because of the heavier bacterial load. This severity is reflected in the ascitic fluid biochemistry. Both bacteria and host neutrophils consume glucose, concentrations of which are therefore reduced. Anaerobic metabolism of glucose, stimulated by the bacterial load, results in the production of lactate, which correlates inversely with pH. Lysis of stimulated neutrophils results in the release of LDH and other cellular proteins, with a consequent rise in the ascitic fluid total protein concentration. This combination of biochemical findings has been used to identify patients who have infected ascites as a result of perforation of an intra-abdominal viscus (Akriviadis and Runyon, 1990); it was found to be 100% sensitive but only 45% specific.

Although the mean neutrophil count tends to be higher in more severe infections, its variability limits its usefulness in the initial assessment

TABLE 1.
Serum ascites albumin gradient

Wide (≥11g/litre)	Chronic liver disease (cirrhosis)
	Veno-occlusive disease
	Massive hepatic metastases
	Congestive cardiac failure
	Spontaneous bacterial peritonitis
Narrow (<11g/litre)	Peritoneal carcinomatosis
	Reduced plasma oncotic pressure (e.g. nephrotic syndrome)
	Secondary peritonitis
	Tuberculous peritonitis

of the severity of infections. However, failure of the neutrophil count to fall after 48 hours in response to antibiotic treatment effectively excludes SBP (Akriviadis and Runyon, 1990).

Bacteriology

It will be evident from the above discussion that biochemical and haematological investigations have an important role to play in the evaluation of patients with peritonitis. However, they should be seen as additional to rather than instead of bacteriological investigations. The role for biochemistry and haematology stems partly from the delay in getting culture results back, but also from the particular problems presented by the low concentrations of bacteria commonly present in SBP. This is responsible for low rates of culture positivity (using conventional culture techniques) and also for the low rate of positive results for gram stains of ascitic fluid (10–50%), even where the neutrophil count in the ascites suggests the presence of infection (Hoefs and Runyon, 1985).

As indicated above, secondary infection is associated with a heavier bacterial load than SBP. The finding that the gram stain is positive for more than one organism, or that ascitic fluid cultures remain positive despite antibiotic treatment, points to secondary infection rather than SBP. The role of laboratory investigations in differentiating SBP from secondary infection is summarized in *Table 2*.

MALIGNANT ASCITES

Pathogenesis

Malignant ascites arises in various ways, and ascitic fluid analysis may be useful in establishing its pathogenesis. In one large prospective series of 448 patients, approximately 10% (45 patients) were found to have malignancy-related ascites (Runyon et al, 1988b). Thirty patients had peritoneal carcinomatosis, either on its own (24) or in association with massive liver metastases (6); smaller numbers had massive liver metastases alone (6), hepatoma on a background of cirrhosis (6), or chylous ascites (3). Nearly all patients with peritoneal carcinomatosis had malignant cells in the ascitic fluid.

Peritoneal carcinomatosis on its own was associated with a high ascitic fluid protein concentration and a narrow SAAG, but where massive liver metastases were also present the SAAG was wide. Patients with hepatoma (and cirrhosis) had negative cytology, low protein and a wide SAAG. Additional measurements which were found to be useful included serum alkaline phosphatase (elevated in massive liver metastases), ascitic and serum alpha-fetoprotein (elevated in hepatoma), and ascitic triglycerides (elevated above the serum concentration in chylous ascites).

Pockros and colleagues have examined the response to dietary sodium restriction and diuretics of patients with malignant ascites of different pathogenetic origin (Pockros et al, 1992). These authors found that ascites could be mobilized (by diuretics) in patients with massive hepatic metastases, but not in patients with peritoneal carcinomatosis or chylous ascites.

Malignant or benign

Some studies in this area have sought simply to identify in ascitic fluid general pointers towards malignancy. Others have examined the role for measurement of tumour markers in ascitic fluid in the diagnosis of malignancies of specific primary sites. Halperin et al (1999) found LDH and cholesterol to be higher in ascitic fluid in patients with ovarian cancer, regardless of histological type, than in patients with benign ovarian tumours. Greco et al (1995) found that both the total concentration of free fatty acids (FFA), and the ratio of unsaturated to saturated FFA, were higher in patients with malignant

TABLE 2.
Spontaneous bacterial peritonitis *vs* secondary infection

Ascitic fluid parameter	Spontaneous bacterial peritonitis	Secondary
Glucose (mmol/litre)	>2.8	≤2.8
Lactate dehydrogenase	≤ serum lactate dehydrogenase upper limit of normal	> serum lactate dehydrogenase limit of normal
Total protein (g/litre)	≤10	>10
Gram stain	Positive for ≤1 organism	Positive for >1 organism
Culture positivity after antibiotic therapy	Rarely remains positive despite antibiotic therapy*	Usually remains positive despite antibiotic therapy
Repeat neutrophil count after 48 hours antibiotics	Always less than initial neutrophil count	Less than initial neutrophil count in ~50% cases†

*Unless resistant organisms are present. †Failure of neutrophil count to fall in response in antibiotic treatment thus effectively excludes spontaneous bacterial peritonitis

ascites than in patients with non-malignant cirrhosis. It is unclear what advantage if any is served by measurement of FFA in ascites instead of cholesterol.

A similar principle applies to the measurement of tumour markers in ascitic fluid. If this is to be advocated, it is reasonable to ask what additional information, if any, is provided by such measurements over and above that which may be obtained by serum measurements of the same tumour markers, or indeed by other modalities of investigation, notably diagnostic imaging. In specific instances, this kind of evidence may be available. For example, it has been found that in patients with endometrial, cervical and ovarian cancers, measurement of total human chorionic gonadotrophin (hCG) in ascitic or tumour cyst fluids may provide greater diagnostic accuracy than serum hCG measurement (Grossmann et al, 1995).

However, in another study, serum and ascitic fluid levels of carcinoembryonic antigen (CEA), cancer antigen 125 (CA125), carbohydrate antigen 19-9 (CA19-9) and tissue polypeptide antigen were compared in terms of their ability to diagnose malignancy-related ascites (Chen et al, 1994); the sensitivity, specificity and accuracy of serum and ascitic fluid measurements were similar. A study of alpha-fetoprotein in serum and ascitic fluid produced similar findings (Miedouge et al, 1999).

Specific observations may be made about individual tumour markers. It is well-recognized that serum levels of CA125 are elevated in patients with ascites, irrespective of the cause; it may be less widely appreciated that CA125 in ascitic fluid is likewise a poor discriminant between benign and malignant pathologies (Ismail et al, 1994). Finally, ascitic fluid CEA has been found to be of prognostic value in two studies of patients with gastric cancer (Nishiyama et al, 1995; Irinoda et al, 1998), predicting peritoneal recurrence.

Cytology

The finding of malignant cells in ascitic fluid indicates the presence of malignancy, although not all patients with malignant ascites will have positive ascitic fluid cytology. Positive cytology per se provides clues to the likely pathogenesis of the ascites. As indicated above, nearly all patients with peritoneal carcinomatosis have positive cytology (Runyon et al, 1988b); by contrast, massive liver metastases, chylous malignant ascites and hepatocellular carcinoma superimposed on cirrhosis, are all characterized by negative cytology.

Other

Analysis of ascitic fluid in other clinical situations has not achieved widespread application, with the exception of chylous ascites, which may be diagnosed by comparing triglyceride concentrations in ascitic fluid and serum (Murphy, 1999).

SAMPLE REQUIREMENTS

Biochemistry

The biochemical analyses outlined above can be performed on ascitic fluid collected into a plain universal container (i.e. no preservative), unless the specimen is bloody, or grossly turbid. Junior doctors may find that their local laboratory may query some of their requests, especially for analyses that are performed (on ascitic fluid) relatively infrequently; this may reflect a failure to recognize the value of the analysis. Specimens for pH and lactate ideally require rapid processing. However, although low pH and high lactate in ascitic fluid correlate with the neutrophil count, they are not thought to provide any additional information (Runyon and Antillon, 1991).

Haematology

The full blood count and differential required for a neutrophil count can usually be performed on ascitic fluid collected into a universal container. However, for bloody specimens, it is advisable to collect an aliquot into a standard full blood count bottle in order to prevent clotting.

Bacteriology

Conventional culture techniques require only a universal container. However, if the technique of Runyon et al (1988a) is employed, it is recommended that the fluid be collected at the bedside into blood culture bottles; an additional specimen should be collected into a universal container so that a gram stain can be performed.

Cytology

A universal container is adequate for the routine cytological analysis. Although blood in an ascitic fluid specimen does not preclude cytology, clotting does. For similar reasons to those outlined above, therefore, it may be advisable to collect an aliquot into anticoagulant, e.g. a full blood count bottle.

CONCLUSION

Analysis of ascitic fluid has an important contribution to make to the investigation and management of patients with ascites. It has an established role in the differential diagnosis of ascites, the evaluation of patients with peritoni-

tis, and the diagnosis of malignancy. Focused use of laboratory investigations in these scenarios is likely to benefit patient care. HM

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Akriviadis EA, Runyon BA (1990) Utility of an algorithm in differentiating spontaneous from secondary bacterial peritonitis. *Gastroenterology* **98:** 127–33

Albillos A, Cuervas-Mons V, Millán I et al (1990) Ascitic fluid polymorphonuclear cell count and serum to ascites albumin gradient in the diagnosis of bacterial peritonitis. *Gastroenterology* **98:** 134–40

Chen SJ, Wang SS, Lu CW et al (1994) Clinical value of tumour markers and serum-ascites albumin gradient in the diagnosis of malignancy-related ascites. *J Gastroenterol Hepatol* **9:** 396–400

Greco AV, Mingrone G, Gasbarrini G (1995) Free fatty acid analysis in ascitic fluid improves diagnosis in malignant abdominal tumours. *Clin Chim Acta* **239:** 13–22

Grossmann M, Hoermann R, Gocze PM, Ott PM, Berger P, Mann K (1995) Measurement of human chorionic gonadotrophin-related immunoreactivity in serum, ascites and tumour cysts of patients with gynaecologic malignancies. *Eur J Clin Invest* **25:** 867–73

Guarner C, Sola R, Soriano G et al (1999) Risk of a first community-acquired spontaneous bacterial peritonitis in cirrhotics with low ascitic fluid protein levels. *Gastroenterology* **117:** 414–9

Halperin R, Hadas E, Bukovsky I, Schneider D (1999) Peritoneal fluid analysis in the differentiation of ovarian cancer and benign ovarian tumour. *Eur J Gynaecol Oncol* **20:** 40–4

Hoefs JC (1990) Diagnostic paracentesis: a potent clinical tool. *Gastroenterology* **98:** 230–6

Hoefs JC, Runyon BA (1985) Spontaneous bacterial peritonitis. *Dis Mon* **31:** 3–48

Irinoda T, Terashima M, Takagane A et al (1998) Carcinoembryonic antigen level in peritoneal washing is a prognostic factor in patients with gastric cancer. *Oncol Rep* **5:** 661–6

Ismail MA, Rotmensch J, Mercer LJ, Block BS, Salti GI, Holt JA (1994) CA125 in peritoneal fluid from patients with nonmalignant gynecologic disorders. *J Reprod Med* **39:** 510–12

Miedouge M, Salama G, Barange K, Vincent C, Vinel JP, Serre G (1999) Evaluation of alpha-fetoprotein assay in ascitic fluid for the diagnosis of hepatocellular carcinoma. *Clin Chim Acta* **280:** 161–71

Murphy M (1999) The role of laboratory investigations in identifying body fluids. *Hosp Med* **60:** 281–4

Nishiyama M, Takashima I, Tanaka T et al (1995) Carcinoembryonic antigen levels in the peritoneal cavity: useful guide to peritoneal recurrence and prognosis for gastric cancer. *World J Surg* **19:** 133–7

Pockros PJ, Esrason KT, Nguyen C, Duque J, Woods S (1992) Mobilisation of malignant ascites with diuretics is dependent on ascitic fluid characteristics. *Gastroenterology* **103:** 1302–6

Runyon BA (1986) Low-protein-concentration ascitic fluid is predisposed to spontaneous bacterial peritonitis. *Gastroenterology* **91:** 1343–6

Runyon BA, Antillon MR (1991) Ascitic fluid pH and lactate: insensitive and nonspecific tests in detecting ascitic fluid infection. *Hepatology* **13:** 929–35

Runyon BA, Canawati HN, Akriviadis EA (1988a) Optimisation of ascitic fluid culture technique. *Gastroenterology* **95:** 1351–5

Runyon BA, Hoefs JC, Morgan T (1988b) Ascitic fluid analysis in malignancy-related ascites. *Hepatology* **8:** 1104–9

Runyon BA, Montano AA, Akriviadis EA, Antillon MR, Irving MA, McHutchison JG (1992) The serum-ascites albumin gradient is superior to the exudate-transudate concept in the differential diagnosis of ascites. *Ann Intern Med* **117**: 215–20

Torres E, Barros P, Calmet F (1998) Correlation between serum-ascites albumin gradient and endoscopic parameters. *Am J Gastroenterol* **93:** 2172–8

KEY POINTS

- The serum ascites albumin gradient (SAAG) has replaced the transudate-exudate concept in investigating the differential diagnosis of ascites.
- The SAAG correlates directly with portal pressure; a wide gradient indicates portal hypertension.
- In patients with portal hypertension the SAAG will be wide even where the cause of ascites is normally associated with a narrow gradient.
- A low ascitic fluid total protein concentration predisposes to spontaneous bacterial peritonitis.
- The neutrophil count in the ascitic fluid is the best single test for early detection of peritonitis.
- Secondary peritonitis is usually associated with a heavier bacterial load than spontaneous bacterial peritonitis and this is reflected in the ascitic fluid biochemistry.
- Nearly all patients with peritoneal carcinomatosis have malignant cells in the ascitic fluid.
- With a few exceptions, measurement of tumour markers in ascitic fluid has not been shown to provide more information than that provided by serum measurement.