



Pathogenesis of malignant ascites in ovarian cancer patients

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ABSTRACT

Peritonitis carcinomatosa, indicating the presence of malignant cells in the peritoneal cavity, is a well-known complication of malignant disease. The collection of intraperitoneal fluid in a patient with ovarian cancer is most likely due to intraperitoneal spread of disease. The recognition of small quantities of intraperitoneal fluid may have staging and prognostic significance, while symptomatic large collections may reflect end-stage disease, which permits only palliative therapeutic options. In this paper, we discussed the pathogenesis of malignant ascites in ovarian cancer patients and suggested potential new treatment approaches.

KEY WORDS: Ascites; Ovarian Neoplasms; Neovascularisation, Pathologic; Endothelial Growth Factors; Capillary Permeability

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INTRODUCTION

Epithelial ovarian cancer is the sixth most frequent form of cancer in women worldwide and the fourth most frequent cause of cancer death among women in both the United States and the United Kingdom. At the same time it is the second most common gynecologic malignancy and the most frequent cause of death from gynecologic cancer in the developed countries (1,2). At the time of diagnosis the majority of patients will present with advanced disease (FIGO stage III-IV) because the disease is often asymptomatic in its early stage (3). Following primary surgical cytoreduction, the current standard treatment for patients with advanced ovarian cancer involves the systemic administration of a paclitaxel and platinum-containing chemotherapy regimens.

Despite the fact that it is one of the most chemosensitive cancers, with response rate to platinum-containing regimens of greater than 60% (4) and intravenous paclitaxel greater than 80% (5), the prognosis remains poor with a 5-year survival rate of approximately 15%-20% in stage III and less than 5% in stage IV patients (6). The largely unchanged mortality rate from ovarian cancer reflects its late clinical appearance. Two-thirds of the patients are diagnosed with stage III or IV disease, commonly associated with the accumulation of ascitic fluid in the peritoneal cavity (7).

Many diseases are complicated by the accumulation of free fluid within the peritoneal cavity i.e. the onset of ascites. The most common cause of ascites is liver cirrhosis, but in about 20% of cases there is an extrahepatic cause. Runyon and colleagues (8) reported that parenchymal liver diseases are the most common cause in about 80%, then malignancy in 10%, heart failure in 5%, tuberculosis 2%, and other causes in the rest 3% of cases.

Ascites is a common and distressing complication of human abdominal cancer, including ovarian cancer (9,10). The collection of intraperitoneal fluid in a patient with ovarian cancer is most likely due to intraperitoneal spread of disease and if neoplastic cells are identified, the term malignant ascites is used. This finding has multiple implications: (1) the recogni-

tion of small quantities of intraperitoneal fluid may have staging and prognostic significance, (2) symptomatic large collections are a sign of disseminated carcinomatosis and may reflect end-stage disease which permits only palliative therapeutic options, and (3) the presence of malignant ascites may be part of a clinical picture amenable to curative efforts. In such cases strategies aimed at obtaining regression of tumor and prolongation of survival should be considered.

SYMPTOMS AND SIGNS

Abdominal distension and changes in abdominal girth are the classic symptoms of ascites. Signs of ascites include dullness to percussion, shifting dullness, and fluid wave. These may be totally absent if effusions are 100 ml or less. Smaller effusions, which are not clinically evident, may be diagnosed incidentally during the workup of malignancy by radiological techniques (ultrasonography, computed tomography, or magnetic resonance imaging) (11-13). The ascitic fluid should be evaluated for various chemistry values (8,14,15). Some biochemical, cytological and microbiological analysis of ascitic fluid and serum, used alone or in combination, can help in differential diagnosis of ascites.

MORPHOLOGIC CHARACTERISTICS OF THE PERITONEAL MEMBRANE

In physiological conditions, a basic principle of capillary fluid hemodynamics is the relative capillary impermeability to proteins while fluid and solutes are able to pass the membrane relatively easily. As a consequence, differences in protein concentration across the capillary membrane are present and oncotic pressure differences are created. Those differences in oncotic pressure limit net capillary fluid-filtration and prevent edema formation due to reabsorption fluid from the interstitial space.

The microscopic picture of peritoneal membrane shows, apart from the capillary endothelium and basement membrane, three distinct barriers which prevent the loss of proteins into the peritoneal cavity: the interstitial stoma, the mesothelial basement membrane, and the mesothelial cells lining the peritoneum (Figure 1).

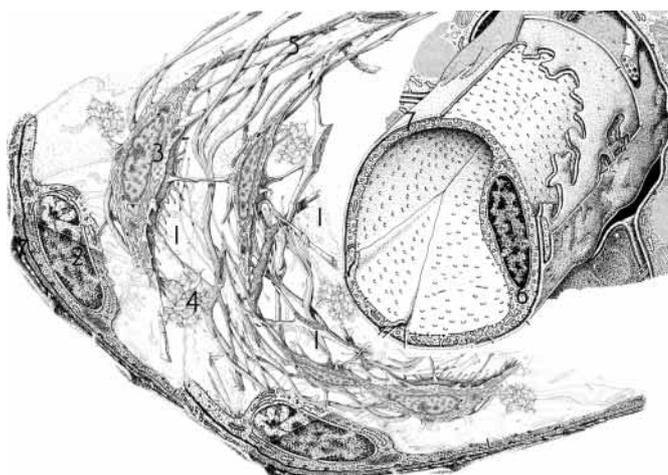


Figure 1. Schematic presentation of peritoneal interstitium 1. Interstitial space, 2. Mesothelial cells, 3. Fibrocyte, 4. Proteoglycans, 5. Collagen fibers, 6. Endothelial cell, 7. Basal lamina

Endothelial cells present the first barrier following the route from the intravascular to the intraperitoneal space. Those cells have an extraperitoneal glycocalyx with fixed anionic charges, which is difficult to pass for albumin. Albumins, as anionic macromolecules, considerably contribute to plasma oncotic pressure (16). Peritoneal endothelial cells are linked with tight junctions, so transendothelial transport is through the intracellular pores (17). Endothelial basement membrane separates endothelial cells from the interstitial space. Proteoglycans present in the basement membrane constitute a negative charge reticulum, which again is a selective barrier for anionic proteins. The interstitial space consists of loose connective tissue composed of fibroblasts, collagen, hyaluronic acid, and negatively charged macromolecules. Hyaluronic acid binds a considerable amount of water. The interstitial space acts as a filter and reduces or blocks diffusion of macromolecules. The submesothelial basement membrane is a continuous layer at the interstitial site of the mesothelial cells. Negatively charged glycosaminoglycans are also present at this site. Mesothelial cells present the last barrier to be passed. The mesothelium consists of a monolayer of flat cells with a total estimated surface of approximately two square meters. Mesothelial cells are functionally similar to endothelial cells. They have glycocalyx containing anionic charges and transcellular channels for macromolecular transport. In summary, the presence of tight junctions between the endothelial cells in the peritoneal capillaries and the presence of negatively charged macromolecules at several extracellular sites produce an effective barrier against leakage of negatively charged molecules such as albumin from plasma to the peritoneal cavity. Those anatomic structures prevent excessive fluid-filtration from the capillaries to the peritoneum. The peritoneal lymphatic system collects fluid, proteins, other macromolecules and cells and returns them to systemic circulation. The lymphatic capillary net is organized as a plexus along the submesothelial surface and drains to lymph vessels. Those have smooth muscle cells and are innervated. Contractions of lymph vessels are generated by myogenic stimuli, and are influenced at least by activation of α -adrenovasoactive peptides. The anatomic features of peritoneal lymphatic system are the so-called stomata. The stomata serve for open communications between the abdominal cavity and the submesothelial diaphragmatic lymphatics. They play a major role in peritoneal lymphatic drainage, since most intraperitoneal fluid is absorbed at this site (16). What are the mechanisms involved in lymph formation? Those mechanisms are still unclear. A hydraulic pressure theory was proposed in the early 1930's of the last century (18). Normally, the interstitial pressure is negative, thus an increase in intraabdominal pressure leads to increased lymph production (19). Another hypothesis has focused on osmotic forces as a dominant factor. This theory postulates a protein concentrating mechanisms

at the initial lymphatics. The necessary osmotic force can be created by active transendothelial transport of albumin (20).

CHARACTERISTICS OF MALIGNANT ASCITES

Malignant ascites is characterized by positive cytology of malignant cells, large number of white blood cells and a higher lactate dehydrogenase level (14, 21). Interestingly, the main ascitic fluid protein-levels are high in patients with peritonitis carcinomatosa, as are ascites albumin concentrations (21).

The data show intraperitoneal protein and albumin accumulation in malignant ascites. What are the reasons for impaired drainage or increased production?

Fluid accumulation occurs if lymphatic drainage of peritoneal cavity is compromised or if net filtration is increased, overwhelming the lymphatic capacity. In malignant ascites, fluid accumulation is the result of filtration minus drainage (Figure 2).

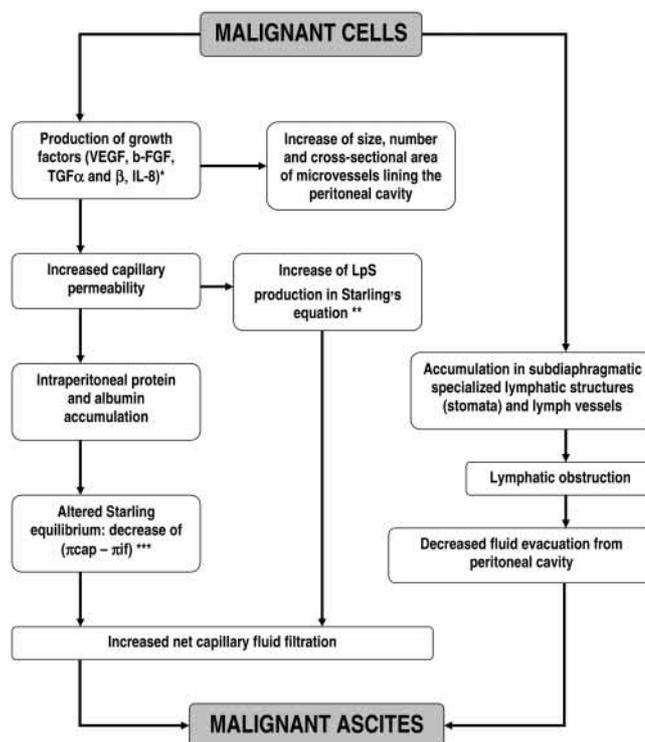


Figure 2. Proposed pathogenesis of malignant ascites. * VEGF - vascular endothelial growth factor; b-FGF - basic-fibroblastic growth factor; TGF α and β - transforming growth factor α and β ; IL-8 - interleukin-8; ** Starling's law of capillary hemodynamics: $LpS[(P_{cap} - P_{if}) - s(\pi_{cap} - \pi_{if})]$; ***Changes in the balance between the capillary and interstitial oncotic forces ($\pi_{cap} - \pi_{if}$)

There is evidence of impaired lymphatic drainage in peritonitis carcinomatosa, especially alterations in diaphragmatic and retrosternal lymph vessels (22). Decreased lymphatic drainage is a contributing factor in the pathogenesis of malignant ascites (23). In addition to impaired lymphatic drainage, there is evidence of six-to sixteen-fold increased fluid production (9). According to the Starling's law of capillary hemodynamics, exchange of fluid between the plasma and the interstitium is determined by the hydraulic and oncotic pressure in each compartment (24).

Net filtration = $LpS(\delta \text{ hydraulic pressure} - \delta \text{ oncotic pressure}) = LpS [(P_{cap} - P_{if}) - s(\pi_{cap} - \pi_{if})]$; Lp - unit permeability or porosity of the capillary wall; S - surface area available for filtration; P_{cap} and P_{if} - capillary and interstitial fluid hydraulic pressures; π_{cap} and π_{if} - capillary and interstitial fluid oncotic pressures; s - reflection coefficient of proteins across the capillary wall with values ranging from 0, if completely permeable, to 1 if completely impermeable (24).

An increase of net filtration and ascitic fluid accumulation is a result of (1) increased capillary permeability, (2) increased surface area available for filtration, (3) increased hydraulic pressure difference, and (4) decreased oncotic pressure difference, or a combination of these factors.

Increased capillary permeability

In peritonitis carcinomatosa, increased permeability to proteins and new capillaries were observed. Inhibition of angiogenesis with locally administered protamine prevents new capillaries from developing and also prevents the occurrence of ascites in experimental models (25). It has generally been considered that factors, which are produced by tumor cells and which increase vascular permeability and induce angiogenesis, are present in malignant ascitic fluid and contribute to its development (26). Angiogenesis starts from stimulation of the endothelium, resulting in hyperpermeability of the endothelial membrane and degradation of the basement membrane and underlying stroma. The migration and proliferation of endothelial cells is the next step, and the formation of new blood vessels and capillaries is the second one. Vascular endothelial growth factor (VEGF) is not the only one out of the most potent and specific angiogenic factors, but it also stimulates vascular permeability (27,28).

VEGF has been identified in ovarian tumor cells (29) and increased VEGF gene expression is seen in neoplastic human ovaries (30). Nagy et al. (31), using a mouse model showed that carcinoma cells injected into the peritoneal cavity resulted in VEGF induced peritoneal capillary permeability and leakage of plasma proteins, including albumin and fibrin(ogen), from newly developed capillaries. Other factors that stimulate tumor cells growth, which may also induce angiogenesis, have been identified in malignant ascites and include basic fibroblast growth factor (bFGF) and angiogenin (29), transforming growth factors α and β (TGF- α and β) (32) and interleukin-8 (33). Epidermal growth factor (EGF) and TGF- α have been shown to be produced by some tumor cell/types and these factors promote ascites formation in mice (34). Richardson et al. (35) observed a marked loss of capillary vessels, consistent with the possibility that malignant ascites fluid contains cytokines with apparently opposing effect. The authors identified angiostatin by SDS-PAGE / Western blot analysis in human ovarian and gastric derived ascites and demonstrated its biological activity. The conclusion was that proteases produced by ovarian cancer cells grown *in vitro* are capable of converting plasminogen to angiostatin. The origin of angiostatin in malignant ascites fluid is not certain. Circulating angiostatin has been implicated in the suppression of secondary tumor growth (36), although the precise mechanism by which it is elaborated *in vivo* by the primary tumor has not been defined (37). But *in vitro*, it is generated by the cleavage of plasminogen by proteases including pancreatic elastase, urinary-type plasminogen activator (uPA), and macrophage-derived metalloproteinase (MMP)-12 and MMP-9 (38). O'Mahony et al. (39) showed that human pancreatic cancer cells produce uPA, which is capable of degrading plasminogen to angiostatin. Cystic fluid from patients with ovarian cancer contains uPA and plasminogen activator inhibitor-1. uPA production by ovarian cancer cells but not by normal ovarian epithelium has been clearly recognized (40). Ovarian cancer cells also produce MMP-9 (41). However, these enzymes had been implicated in the breakdown of extracellular tissue via the production of plasmin, thereby increasing the metastatic potential rather than a possible breakdown of plasminogen to angiostatin. On the other side, Westphal et al. (42) showed that the conditioned medium for human ovarian epithelial carcinoma cells *in vitro* will degrade human plasminogen to angiostatin and Buick et al. (43) confirmed this observation using SFCM from HEY cells, an ovarian epithelial cancer cell line. It is therefore likely that the angiostatin in malignant ascites is generated from plasminogen, which has been degraded by proteases produced by malig-

nant ovarian epithelial cells. It is not known whether the angiostatin concentration in whole malignant ascites fluid is potentially effective as an anti-angiogenic agent. However, low incidence of extraperitoneal metastatic disease in ovarian cancer patients (approximately 16% present with stage IV disease) is related to suppression of angiogenesis. Hari et al. (44) referred that angiostatin induces mitotic cell death of proliferating endothelial cells as the targets. But, there is a possibility that once angiogenesis is established in patients, naturally occurring angiostatin is no longer effective. The administration of an antiangiogenic agent may be less effective than prevention of action of pro-angiogenic agents such as VEGF, thereby promoting the activity of the naturally occurring anti-angiogenic agents. These data suggest that the progression of the tumor and the development of ascites may depend on a balance between the production of pro- and anti- angiogenic factors. The more complete understanding of the relative contributions of these factors will promote the development of improved treatment. Malignant ascites production, but not tumor growth, was completely inhibited in mice when treated with function-blocking VEGF antibodies (45) and developed again within two weeks after the treatment was stopped. These positive experimental results have been confirmed by others using anti-VEGF antibodies, VEGF tyrosine kinase receptor inhibitors or exogenous soluble human VEGF receptor (46).

Increased surface area for filtration

After intraperitoneal tumor cell injection in mice, size and number of peritoneal lining microvessels and subsequently cross sectional area increased (31). The site of production of malignant ascites is the tumor-free omentum small bowel surface and tumor surface. Hirabayashi and Graham (9) concluded, "undoubtedly fluid exuded from the tumor surface but the lion's share came from the disease-free peritoneum". In human subjects, tumor-free peritoneal surface is able to produce surplus of fluid in malignant ascites.

Increased hydraulic pressure difference

Hirabayashi and Graham (9) reported a minor increase in portal vein pressure in ovarian cancer patients with ascites.

Decreased oncotic pressure difference

In physiologic conditions, albumin is known to be an effective osmol that contributes to intravascular oncotic pressure, necessary to reabsorb fluid from the interstitial space. If the oncotic pressure difference decreases, reabsorption decreases and interstitial fluid accumulation results. In peritonitis carcinomatosa, protein degradation to smaller peptides and amino acids contribute to intra-abdominal oncotic pressure and fluid may be filtrated into the peritoneal cavity.

In conclusion, ascites is a common and distressing complication of ovarian cancer. The source of malignant ascitic fluid is likely the non-cancer-bearing peritoneal surface rather than the tumor. The increasing net capillary fluid-production is due to an increase of overall capillary membrane surface, increased capillary permeability and subsequent increase of intraperitoneal protein concentration, leading to increased intraperitoneal oncotic pressure. It has generally been considered that factors which are produced by tumor cells (VEGF and b-FGF) and which increase vascular permeability and induce angiogenesis are present in malignant ascites fluid and contribute to its development. Interference with these mediators may serve as a target in future therapeutic strategies.

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