Published in final edited form as:

Nat Rev Cancer. 2013 April ; 13(4): 273–282. doi:10.1038/nrc3432.

Meeting the challenge of ascites in ovarian cancer: new avenues for therapy and research

Emma Kipps,

The Institute of Cancer Research/Royal Marsden Hospital, Medicine, Downs Road, Sutton SM2 5PT, UK.

David S. P. Tan, and

The Drug Development Program, Princess Margaret Hospital, 610 University Avenue, Toronto M5G 2M9, Canada.

Stan B. Kaye

The Drug Development Unit, Sycamore House, Royal Marsden NHS Foundation Trust, Downs Road, Sutton SM2 5PT, UK.

Abstract

Malignant ascites presents a considerable clinical challenge to the management of ovarian cancer, but also provides a wealth of opportunities for translational research. The accessibility of ascitic fluid and its cellular components make it an excellent source of tumour tissue for the investigation of prognostic and predictive biomarkers, pharmacodynamic markers and for molecular profiling analysis. In this Opinion article, we discuss recent advances in our understanding of its pathophysiology, the development of new methods to characterize its molecular features and how these findings can be used to improve the treatment of malignant ascites, particularly in the context of ovarian cancer.

Ascites is the excess accumulation of fluid in the peritoneal cavity. Derived from the Greek word *askites*, meaning bag-like, it is caused by many pathologies; the most common is hepatic cirrhosis, which accounts for 81% of cases¹. Others causes include heart failure (3%) and tuberculosis (2%), and a substantial minority (10%) is associated with malignancy (TABLE 1). The composition of ascitic fluid is dependent on the disease with which it is associated (BOX 1).

The most common primary site of cancer that is associated with ascites is ovarian, accounting for 38% of malignant ascites occurring in females². Patients with other abdominal epithelial malignancies, including pancreatobiliary and gastric cancer, develop ascites in 21% and 18.3% of cases, respectively³. Malignant ascites can also develop secondary to extra-abdominal tumours, such as breast and lung cancer, as well as lymphoma.

Correspondence to S.B.K. stan.kaye@rmh.nhs.uk.

Competing interests statement

The authors declare no competing financial interests.

In many forms of malignancy, ascites is a sign of advanced disease and poor prognosis^{3,4}, with only 11% of patients surviving longer than 6 months². Epithelial ovarian cancer (EOC) is the exception. The majority of women with ovarian cancer present with advanced disease (stage III or stage IV) that may include ascites. In these patients, treatment with surgery together with combination chemotherapy has resulted in a median progression-free survival of 16–22 months and a 5-year survival rate of 27%⁵, although better results are now being reported with improvements in therapy. One study has suggested that ascites at presentation is an independent prognostic factor for time to relapse⁶ and, overall, more than one-third of women with ovarian cancer develop ascites during the course of their disease³, and this is not limited to any specific histological subtype. Although some oncologists consider that the incidence of ascites may be reducing with the widespread use of combination chemotherapy that incorporates taxanes, there are no published supporting data.

Ascites can cause debilitating symptoms as a substantial volume of fluid can cause pain, early satiety and respiratory compromise⁷. The gastrointestinal and urinary systems can also be affected, resulting in significant morbidity (TABLE 1).

In patients with ovarian cancer, ascites is essentially treated through the treatment of the underlying disease; that is, using platinum-based intravenous chemotherapy. However, once chemoresistant disease has developed, intractable ascites can be a major problem and the majority of patients are subjected to frequent paracentesis to temporarily alleviate symptoms. Newer strategies to control ascites, including anti-vascular endothelial growth factor (VEGF) therapy, are proving promising, but there is an urgent need to further understand the pathophysiology of ascites so that more effective therapy can be developed. In this Opinion article, we discuss recent findings on the biology of ascites. Ascitic fluid provides a rich source of tumour cells that can be used to study various biological aspects of the underlying tumour, including drug resistance and mechanisms of tumour progression. Consequently, these studies have the potential to enhance our insight into effective therapeutic strategies to prevent the formation of ascites and to overcome platinum resistance in ovarian cancer.

Pathophysiology of ascites

The transition of epithelial cells to a more mesenchymal phenotype (epithelial to mesenchymal transition (EMT)) is associated with motility and, in cancer biology, with invasive growth⁸. Epithelial ovarian cells convert to a mesenchymal phenotype as part of a normal physiological process, which increases the motility and proliferation of these cells to allow repair of the surface epithelium when an oocyte is released⁹. Therefore, it is not surprising that EOC is frequently associated with the early invasion of the surrounding peritoneum. Adhesion to the mesothelin and hyaluronic acid expressed by mesothelial cells, which line the peritoneal cavity, is facilitated by CD44 (REFS 10,11), β -integrins¹² and CA125 (REFS 13,14) expressed on the ovarian cancer cell surface. Once attached to the peritoneal surface, cells proliferate and invade the mesothelium (the outer layer of the peritoneal membrane). It has been suggested that transcoelomic metastasis (the seeding of tumour cells onto the surface of the peritoneum) is directly associated with ascites production. During embryogenesis, surface epithelial cells of the ovary originate from the

coelomic mesothelium⁹, which might partly explain why ovarian cancer disseminates through the transcoelomic route. Reduction in peritoneal tumour bulk as a result of surgery and chemotherapy is mostly associated with a reduction in ascites; supporting the concept that transcoelomic metastases are involved in ascites production.

Anatomy of the peritoneal membrane

The peritoneal membrane consists of five layers and covers the visceral organs, as well as the abdominal and pelvic cavities (FIG. 1). The first layer of the peritoneal membrane comprises endothelial cells that line the intravascular space of capillaries. These cells have an extracellular glycocalyx and fixed anionic charge that makes it difficult for large blood plasma proteins, such as albumin, to pass through. Tight junctions link endothelial cells together, and so intracellular pores provide transport through the layer¹⁵. Separating the endothelial cells from the interstitial space (the third layer) is the endothelial basement membrane. Proteoglycans anchored in the basement membrane also exert a negative charge, which constitutes a selective barrier for albumin. Loose connective tissue in the interstitial space, comprised of fibroblasts, collagen and hyaluronic acid, blocks the diffusion of macromolecules before the submesothelial basement membrane. The final layer consists of mesothelial cells. Bound by tight junctions and secreting surface glycosaminoglycans (hyaluronan)¹⁶ into the abdominal space, mesothelial cells provide an effective anti-adhesive surface and a protective barrier against physical damage. In response to injury or insult mesothelial cells release chemokines, such as CCL2 (also known as MCP1) and interleukin-8 (IL-8; also known as CXCL8) and they also increase the expression of intracellular adhesion molecule 1 (ICAM1) and vascular cell adhesion protein 1 (VCAM1), to which leukocytes attach¹⁷.

However, in malignancies, such as ovarian cancer, this balance of fluid production and reabsorption is disrupted and pathological volumes of fluid — that is, ascites — accumulate in the peritoneal cavity. The pathophysiological mechanisms for this have been attributed to impaired fluid drainage and/or to increased net filtration.

Impaired drainage from the peritoneal cavity

The peritoneal membrane is rich in lymphatic tissue. The lymphatic system circulates fluid, proteins and other macromolecules back into the vascular circulation. Lymphatic stomata, first described by von Recklinghouse in 1863 (REF. 18), are triple-layered structures comprising mesothelium, a loose network of connective tissue and endothelium that permit the flow of fluid between the peritoneal surface and the submesothelial lymphatics. These lymphatic portals are particularly abundant on the omental and sub-diaphragmatic peritoneal surfaces¹⁹, and their obstruction by tumour cells has been suggested as a mechanism for ascites. This hypothesis was first proposed almost 50 years ago²⁰. Experiments showed that intraperitoneal-injected Indian ink was not transported in the lymphatic system in mice with malignant ascites, suggesting obstruction. More substantial evidence was published in 1972 (REF. 21) when intraperitoneal-injected radioactive erythrocytes were not found in the peripheral bloodstream within 5 hours in mice that were inoculated with ovarian tumour cells that result in ascites. A similarly designed study using radioactive albumin also demonstrated reduced efflux from the peritoneum following tumour injection²².

Although the involvement of the lymphatic system in the pathogenesis of ascites is not disputed, it is unlikely to be a major contributing factor as there are indirect effects of malignancy on the anatomy and physiology of the peritoneal membrane. In mouse models²³, ascites formation can occur in the absence of a tumour obstructing the draining lymphatics of the peritoneal cavity, and high concentrations of protein are evident in malignant ascites, indicative of alterations in peritoneal membrane permeability. In addition, in the clinic, ascites formation can be seen in the absence of tumour bulk, suggesting a prominent role for non-obstructive mechanisms, including those which involve cytokines and chemokines that are secreted by the tumour cells and the associated cells that constitute the tumour microenvironment.

Increased filtration into the peritoneal cavity

In physiological conditions, the difference in oncotic pressure (a form of osmotic pressure exerted by proteins, such as albumin, in blood plasma that increases water uptake) across the peritoneal membrane (high at the endothelial layer and low at the mesothelial layer) limits capillary fluid filtration and prevents oedema owing to water reabsorption into the capillaries from the interstitial space (FIG. 1). In patients with tumours in the abdominal cavity, the cross-sectional area of microvessels that line the peritoneal cavity is increased²² (discussed below), and this results in an increased filtration surface for fluid. In addition, malignant ascites has a high protein concentration that is secondary to increased capillary permeability. This decreases the plasma to peritoneal oncotic pressure difference, so that the direction of fluid flow is into the peritoneal cavity, obeying Starling's hypothesis of capillary haemodynamics. As only a minor increase in portal vein pressure in patients with ovarian cancer with or without ascites has been observed, increased hydraulic pressure is not thought to contribute to the pathogenesis of ascites in ovarian cancer²⁴.

Ascitic fluid that is generated as a result of malignancy profoundly affects microvascular permeability²⁵, and in 1986 it was shown to cause the leak of proteins from a healthy omentum⁴. VEGF was subsequently identified as being instrumental in altering the permeability of the peritoneal membrane, and therefore in the pathogenesis of ascites. VEGF is a glycosylated mitogen that specifically acts on endothelial cells, inducing cell proliferation, migration and inhibition of apoptosis. Moreover, VEGF induces angiogenesis and the permeabilization of blood vessels²⁶.

VEGF is crucial for normal ovarian function²⁷ and overexpression of VEGF in patients with EOC has been shown to correlate with the development of malignant ascites^{28,29} and a poorer prognosis^{30,31}. VEGF selectively accumulates in ascites secondary to EOC at much higher levels than in ascites resulting from non-malignant causes³², and it is predominantly associated with the advanced stages of ovarian cancer³² and higher grade disease³³. Preclinical experiments have demonstrated that overexpression of VEGF can induce ascites production from normal ovarian epithelium³⁴ and enforced expression of VEGF by ovarian carcinoma cells has been shown to dramatically reduce the time to onset of ascites formation³⁵. In animal models, VEGF inhibition has been shown to suppress the formation and re-accumulation of ascites in mice with intraperitoneal ovarian tumours^{36,37}.

One mechanism by which VEGF increases peritoneal permeability is through the downregulation of the tight junction protein claudin 5 in the peritoneal endothelium^{38,39}. Claudin 5 is a transmembrane protein in the tight junction that, together with occludin⁴⁰, mediates adhesion by promoting homophilic interactions along the cell border^{41,42}. Studies in transgenic mice have shown that the absence of tight junction proteins affects the control of vascular permeability to fluids⁴³. Angiogenesis is known to be associated with increased vessel permeability⁴⁴, and a further mechanism contributing to this is tyrosine phosphorylation of cadherin–catenin complexes, which is mediated by VEGF⁴⁵. The resulting complexes cause decreased junctional strength and increased permeability⁴¹.

There are several factors that are thought to influence VEGF production by ovarian cancer cells, including hypoxia⁴⁶, lysophosphatidic acid (LPA)⁴⁷, tumour necrosis factor (TNF)⁴⁸, endothelin 1 (REF. 49), cyclooxygenase 1 (REF. 50), IL-1 β ⁵¹, matrix metalloproteinases (MMPs)⁵², insulin-like growth factor 1 (REF. 53), epidermal growth factor (EGF)⁵⁴, platelet-derived growth factor⁵⁵ and transforming growth factor- β (TGF β)⁵⁶. Blockade of TGF β inhibits ascites production through the inhibition of VEGF expression in orthotopic human ovarian carcinoma models⁵⁶. Interestingly, peritoneal drainage of ascites was also shown to increase secondary to the normalization of lymphatic vessel morphology and a reduction in lymphangiogenesis.

LPA is a biologically active phospholipid that is present in high levels in ascites and in serum from patients with ovarian cancer^{57,58}, and a LPA-induced gene expression signature has been shown to correlate with a worse prognosis⁵⁹. Produced extracellularly by the phosphodiesterase autotaxin (ATX)⁶⁰, LPA signals through G protein-dependent cell-surface receptors, resulting in the production of unsaturated fatty acyl chains. These G protein-dependent receptors are upregulated during the malignant transformation of ovarian surface epithelial cells⁶¹. The effects of LPA are diverse and include increased mRNA levels and transcriptional regulation⁵⁸ of VEGFA, urokinase plasminogen activator (UPA), IL-6 and IL-8. This results in increased endothelial permeability and in the inhibition of gap junctional communication between adjacent cells⁶² and, therefore, in ascites production.

Additional properties of ascites

There is evidence to suggest that EOC cells, including those in ascitic fluid, might have evolved to evade immunological surveillance⁷. Ascites-derived epithelial ovarian cancer cells have been shown to constitutively release microvesicles that contain CD95 ligand (CD95L; also known as FAS ligand) rather than expressing CD95L on their cell surface. CD95L expression or secretion can result in the apoptosis of immune cells that express CD95 (REF. 63). High concentrations of complement C1 complex inhibitor and the alternative complement pathway inhibitors factor H and factor H-like protein 1 have been noted on isolated metastatic EOC cells in ascitic fluid, suggesting a mechanism for the evasion of complement-induced immune destruction⁶⁴. Furthermore, regulatory T cells that suppress tumour-specific T cell immunity promote tumour growth *in vivo*, and a correlation between the presence of regulatory T cells in ascitic fluid and reduced survival has also been found⁷. In addition, a ganglioside, GD3, has recently been identified in malignant ascites as

an inhibitory factor that prevents the innate immune activation of natural killer T (NKT) cells⁶⁵.

Despite these immune suppressive effects, ascites is rich in lymphocytes, cytokines and chemokines⁶⁶. Cytokines, including IL-1 β , IL-6, IL-8 and IL-10 (REFS 67–69), are present at concentrations that are two to three logs greater than serum concentrations⁶⁹, indicating that ascites provides an environment that is conducive to cell growth. It is interesting that the expression of IL-6 is significantly higher in ascites secondary to ovarian cancer when compared with ascites resulting from other primary tumours⁷⁰. Therefore, cytokine expression may generate a specific microenvironment that enables the characteristic pattern of metastatic spread in ovarian cancer. In animal models, the expression of IL-8 is associated with increased tumorigenicity and ascites formation^{71,72}. IL-6 has been shown to promote growth⁷⁰, chemoresistance, tumour invasion⁷³ and angiogenesis^{74,75}. High levels of IL-6 (REFS 69,76,77) and IL-10 (REF. 68) expression in ascites have been shown to be associated with shorter progression-free survival⁷⁷, poor overall survival, poor initial response to chemotherapy⁶⁹ and drug resistance. As well as the secretion of these cytokines by tumour and stromal cells that are present in the ascites microenvironment, Naldini *et al.*⁷⁸ showed that healthy donor peripheral blood mononuclear cells cultured in the presence of ascitic fluid from patients with ovarian cancer significantly increased the release of cytokines and chemokines, such as IL-6 and IL8, which are known to support tumour progression, and inhibited the production of anti-inflammatory cytokines, such as interferon- γ and IL-12. In addition, MUC16 expressed on the surface of ovarian cancer cells has been shown to provide immune protection by inhibiting the interaction of ovarian cancer cells with natural killer cells⁷⁹. Further characterization of cytokine profiles may provide a greater understanding of the role of ovarian cancer cells in the pathogenesis of ascites, as well as the role of ascites in ovarian cancer tumour progression.

Other chemokines also have a role in the pathophysiology of ascites. CXCR4, the receptor for CXCL12, seems to be selectively expressed on EOC cells⁸⁰ and contributes to the proliferation⁸¹ and migration⁸² of EOC. The level of chemokine ligand CXCL4 is increased in ascites from patients with EOC and, together with CXCL6, CXCL8, CXCL14 and CCL13, CXCL4 has been shown to be upregulated in peritoneal specimens that lack detectable cancer cells from patients with ovarian cancer compared with patients with benign ovarian disease⁸³, suggesting the upregulation of the chemokine network throughout the peritoneum. Proteinases, including MMPs, are crucial for invasive cancer growth and the pathogenesis of ascites. MMPs, mainly MMP9, have a role in the release of biologically active VEGF and consequently in the formation of ascites⁵² and have been shown to be an independent predictor of decreased survival⁸⁴.

In vitro and *in vivo* research has suggested that the activation of the EGF receptor may partly induce a pro-inflammatory microenvironment⁷⁶ through the upregulation of nuclear factor- κ B (NF- κ B). The NF- κ B transcription factor is constitutively active in >50% of ovarian cancers and results in the upregulation of multiple genes that include *IL6* and *IL8*. Inhibition of NF- κ B signalling significantly inhibits the expression of VEGF and IL-8, resulting in decreased vascularization of lesions, decreased formation of malignant ascites and prolonged survival in mice⁷¹. Autocrine stimulation of the IL-6 receptor on ovarian cancer

cells facilitates the activation of signal transducer and activator of transcription 3 (STAT3) through Janus kinase 2 (JAK2), resulting in the inhibition of apoptosis and the induction of angiogenesis^{85,86}.

The activation of the PI3K pathway is deregulated in up to 70% of ovarian cancers⁶¹ through mechanisms that include amplification of *PIK3CA* and AKT or inactivating mutations of *PTEN*. Activation of AKT^{87,88} and ERK1 or ERK2–ELK1 signalling⁸⁹ by survival factors in ascitic fluid has been shown to promote resistance to TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis, highlighting the contribution of the tumour microenvironment to cell survival in ovarian cancer. AKT is also functionally required for bone morphogenetic protein (BMP) signalling in tumour spheroids and the adhesion and dispersion⁹⁰ of epithelial ovarian cancer cells. The mechanisms underlining the interaction between AKT and BMP pathways, however, remain to be fully elucidated.

The treatment of ascites

Pharmacological

At presentation, patients with ovarian cancer and ascites are treated with surgery together with first-line combination chemotherapy of carboplatin and paclitaxel. There is an increasing trend for surgery to be deferred until at least three cycles of chemotherapy have been given, with the aim of reducing the problems associated with surgery, such as bowel resection. The clinical response to first-line chemotherapy exceeds 60%. However, the majority of patients relapse and the addition of a third cytotoxic drug, such as gemcitabine, topotecan, caelyx or epirubicin, to platinum and paclitaxel has yielded disappointing results^{91–93}. The VEGF-binding antibody bevacizumab has been added in Phase III trials (Gynecologic Oncology Group 218 study (GOG218; clinical trial ID: NCT00262847) and ICON7 (NCT00483782)) to standard treatment during and up to 15 months after chemotherapy, with reports of improved progression-free survival^{94,95}, as well as increased overall survival in high-risk subgroups (patients with stage IV disease or suboptimally debulked stage III disease)⁹⁶. Randomized trials have indicated that bevacizumab, concurrent with and following chemotherapy, can also provide benefit in terms of delaying further recurrence in both platinum-sensitive (OCEANS trial (NCT00434642)⁹⁷) and platinum-resistant (AURELIA trial (NCT00976911)⁹⁸) disease. Although in these trials bevacizumab was not introduced specifically to treat ascites, its increasing utility should have an effect. However, resistance to anti-VEGF therapy will remain a key problem, and efforts to understand the underlying mechanisms and to develop further anti-angiogenic strategies are a high priority⁹⁹.

For women with relapsed ovarian cancer, resistance to conventional chemotherapy is also a crucial factor and it develops in almost all cases. If this resistance is associated with ascites, pharmacological therapeutic options specific to the control of ascites are limited. Randomized controlled studies are few, with the majority of treatments having been studied in a small series; including the use of diuretics, octreotide, intraperitoneal therapy, immunological modulators, metalloproteinase inhibitors and anti-angiogenics.

Diuretics are frequently used in the treatment of ascites that has arisen as a result of non-malignant disease¹⁰⁰. However, no randomized trials have assessed the efficacy of diuretics in malignant ascites¹⁰¹ and evidence for their use is weak¹⁰². Phase II data suggest that they are effective in approximately one-third of patients with malignancy¹⁰³, and side effects of treatment include nausea, hypotension and electrolyte disturbance. Efficacy is thought to decline with tumour progression¹⁰⁴ and, interestingly, patients with portal hypertension² or massive hepatic metastases¹⁰³ are most likely to respond to diuretics, which may account for the lack of efficacy in relation to ascites arising in patients with ovarian cancer. Octreotide, a safe, well-tolerated somatostatin analogue increases the glomerular filtration rate and the sodium and water excretion of patients with non-malignant ascites, mainly through the suppression of an activated renin–aldosterone axis¹⁰⁵. In a pilot study¹⁰⁶, monthly intramuscular injections of long-acting octreotide were shown to delay the need for repeat paracentesis from a mean of 14 days to 28 days in patients with malignant ascites. Although there may have been a degree of clinical benefit, this delay was not significant ($P = 0.17$).

The pharmacological model for intraperitoneal chemotherapy was published more than 30 years ago¹⁰⁷. High concentrations of chemotherapy drugs in the peritoneum resulted in a prolonged exposure to these agents compared with intravenous administration. More recently, intraperitoneal chemotherapy has been explored for the first-line treatment of optimally debulked women with stage III ovarian cancer¹⁰⁸. Despite a 16-month survival advantage, the complication rate, 34% of which was due to catheter-related problems, was significantly greater in the experimental group, and only 42% of women in the trial completed the recommended six cycles of intraperitoneal chemotherapy with cisplatin. Patients in the experimental group also suffered from more pain and fatigue, as well as higher rates of haematological, gastrointestinal and metabolic toxicities. The dose of cisplatin used in the experimental group (100 mg per m²) has not been widely used for these reasons.

Although the use of first-line intraperitoneal chemotherapy remains controversial, it has been the route of choice in many early phase trials in which patients with malignant ascites from any primary tumour were recruited to study chemotherapy in the control of ascites. Intraperitoneal administration of bleomycin^{109,110}, mitoxantrone^{110,111} or fluorouracil in combination with cisplatin¹¹² have all been reported to improve the control of ascites. However, there are no standardized criteria for assessment and further studies would therefore be required for full evaluation.

Evidence to suggest that an immunological approach to treat malignant ascites in EOC may be effective has previously been observed in small studies of intraperitoneal triamcinolone¹¹³, a long-acting synthetic corticosteroid, intraperitoneal interferon¹¹⁴ and TNF¹¹⁵. More recently, a prospective randomized Phase II/III trial was conducted using intraperitoneal catumaxomab (Removab; Fresenius Biotech)¹¹⁶. This is a trifunctional monoclonal antibody with two different antigen-binding sites (one that binds to tumour cells via the epithelial cell-adhesion molecule and another to T cells through CD3), as well as a functional Fc domain that activates Fcγ-receptor I-, Fcγ-receptor IIa- and Fcγ-receptor III-positive immune cells. Therefore, different immune effector cells are simultaneously recruited and activated, resulting in tumoricidal activity through different immunological

killing mechanisms. The study showed that, at appropriate doses, treatment with catumaxomab led to significantly longer paracentesis-free survival and improved palliation following intraperitoneal administration in patients with malignant ascites compared with controls. These data led to the approval of catumaxomab for the treatment of malignant ascites by the European regulatory authorities in 2009; this was the first therapeutic agent to be specifically approved for the treatment of malignant ascites. Ott *et al.*¹¹⁷ demonstrated a strong correlation between humoral response to catumaxomab and clinical outcome by measuring human antimouse antibodies (HAMAs) 8 days after the fourth treatment, suggesting that HAMA development (with associated symptoms of cytokine release) may be a biomarker for catumaximab response. The effective harnessing of the immune system by catumaxomab suggests that this treatment may be beneficial to patients with earlier stage disease.

MMPs have a crucial role in tumour invasion and angiogenesis. Preclinical research in this area led to the development of batimatstat, an MMP inhibitor. Patients with ovarian cancer were included in a Phase I study of intraperitoneal batimatstat¹¹⁸ that found the main adverse effect to be nausea and vomiting. Interestingly, five of the 23 patients neither reaccumulated ascites nor died up to 112 days after dosing, and agents of this type may warrant further study.

There is strong preclinical evidence for the dependence of ascites formation on abnormal tumour vascularity and permeability. Therefore, research on VEGF inhibitors has specifically studied their use to palliate the symptoms of ascites in heavily pretreated patients. Numnum *et al.*¹¹⁹ reported that repeated paracentesis could be discontinued because of dramatically reduced levels of ascites after the initiation of therapy with bevacizumab (15 mg per kg intravenously, every 3 weeks). Intraperitoneal administration of bevacizumab has also been explored. The largest series only included nine patients with refractory ascites, which resulted from colorectal, breast, uterine or ovarian cancer¹²⁰. However, in all the patients, the ascites resolved without reaccumulation or repeat paracentesis over a median observation period of >2 months. In addition, no grade 2–5 adverse events were observed. Despite these results, and other positive case reports^{121,122}, no randomized trials using bevacizumab specifically for the palliation of ascites have yet been reported.

Composed of entirely human sequences, aflibercept (also known as VEGF-Trap) binds to and neutralizes VEGFA, VEGFB and placental growth factor¹²³. Preclinical models found that aflibercept prevented ascites accumulation and inhibited the growth of disseminated cancer³⁵. A pilot study was carried out in 12 patients in whom time to repeat paracentesis was increased following treatment with aflibercept (4 mg per kg, every 2 weeks)¹²⁴, and the effectiveness of VEGF blockade using aflibercept was confirmed in a Phase II randomized, double-blind trial conducted by Gotlieb *et al.*¹²⁵ in patients with ovarian cancer. However, the authors acknowledge that the limitation of this treatment is the risk of significant morbidity associated with bowel perforation, which was higher in the treatment group. Therefore, the advantages of aflibercept over bevacizumab in this context are unclear.

Non-pharmacological

Paracentesis via percutaneous drainage remains the most regularly used intervention for providing immediate short-term palliation of symptoms. Complications of paracentesis include continuous leakage from the drainage site and, occasionally, bowel perforation. Procedures often require a hospital stay and may have to be frequently repeated. To reduce the need for multiple procedures, and so admissions, peritoneovenous shunts (PVSs) are used in the treatment of ascites^{126,127} to direct ascitic fluid through a one-way valve into the vena cava, palliating symptoms in up to 70% of patients¹⁰². Encouragingly, the primary patency of shunts averages 87 ± 57 days¹²⁸. However, major complications that occur in 6% of patients include pulmonary oedema, pulmonary embolus and infection¹⁰².

Catheter drainage is an alternative form of management. Catheters, made from flexible silicone with a polyester cuff, are tunnelled subcutaneously towards the peritoneal cavity¹²⁹ and are easy to self-drain, thus enhancing patient autonomy while negating the need for repeated paracentesis. A review assessing the safety and efficacy of indwelling intraperitoneal catheters (Tenckhoff and PleurX) for the management of refractory malignant ascites reported a safe and effective palliative strategy despite an 11% infection rate¹³⁰. A recent study¹³¹ reported a 100% technical success rate for the insertion of a PleurX drain with an associated low complication rate, thus advocating their use as a first-line approach in patients with refractory malignant ascites. On the basis of this evidence, the UK National Institute for Clinical Excellence have recommended that the PleurX peritoneal catheter drainage system should be considered for use in patients with treatment-resistant, recurrent malignant ascites¹³².

Bridging the translational gap

As is evident from the discussions above, ascites is accessible and often therapeutically removed from patients; it is therefore a valuable source of tumour material, and consequently of information on the molecular perturbations of the tumour. The ability to isolate and analyse malignant ascites and its cellular components may thus facilitate the identification of therapeutic targets, as well as prognostic and predictive biomarkers. In addition, evidence to support clonal evolution, as well as molecular heterogeneity, throughout a patient's progress during treatment may be obtained. Various high-throughput techniques are now available to characterize the cellular and acellular components of ascites at the genetic, transcriptomic and proteomic levels. These include microarray-based gene expression profiling¹³³, proteomic methods¹³⁴ and next-generation sequencing technologies¹³⁵.

Translational research using cellular components in ascites

Do cancer cells in ascites harbour molecular changes that render them phenotypically distinct from those retained in the primary tumour? Indeed, it would seem that a degree of clonal evolution is necessary for the transcoelomic metastatic process and that a dynamic regulation of cell adhesion molecules, such as E-cadherin, throughout the process may also be required⁷. Nonetheless, the frequent presence of ascites at first presentation, and subsequent relapse in EOC, provides an accessible pool of tumour cells that, in most current

studies, are assumed to mirror some or most of the molecular characteristics of cells in the primary tumour or metastatic deposits. In order to study the cellular component of ascites, malignant cells from ascites can be preserved in cell blocks for immunohistochemical testing^{136,137} or can be frozen for further molecular analysis¹³⁷. Molecular and functional analysis of malignant ascitic cells has been greatly facilitated by the ability to isolate and culture cells derived from the ascitic fluid of patients with advanced EOC^{138,139}, and this presents further opportunities for the identification and validation of therapeutic targets and predictive biomarkers.

The potential utility of this approach was demonstrated by Mukhopadhyay *et al.*¹⁴⁰ where a functional assay for cells deficient in homologous recombination (HR)-mediated DNA repair was developed using primary cell cultures that were derived from malignant ascitic cells. This was used to identify patients who were most likely to benefit from poly(ADP-ribose) polymerase (PARP) inhibitors, a novel class of targeted therapy effective in tumour cells that are deficient in HR repair¹⁴¹. In another study by Elattar *et al.*¹⁴², primary ovarian cancer cultures were established from the ascitic fluid of 11 patients to assess the expression of androgen receptor (AR) mRNA and response to androgenic stimulation. The study revealed a strong correlation between increased nuclear AR expression by immunohistochemistry and increased S phase fraction changes in primary cultures, suggesting that intervention with anti-androgenic agents may be effective at an earlier stage of the disease¹⁴².

In an effort to elucidate the role of PI3K pathway activation in drug-resistant ovarian cancer, Carden *et al.*¹⁴³ immuno-magnetically separated ovarian cancer cells from ascites before quantifying the levels of phosphorylated AKT, p70S6K (also known as RPS6K β 1) and glycogen synthase kinase 3 β (GSK3 β) by enzyme-linked immunosorbent assay (ELISA) and sequencing *PIK3CA* and *AKT2* by PCR-amplified mass spectroscopy detection methods. In addition, *PIK3CA* and *AKT2* amplifications and *PTEN* deletions were analysed by fluorescence *in situ* hybridization (FISH). The study observed that mutations of *PIK3CA* and amplification of *PIK3CA* and *AKT2* or deletion of *PTEN* did not correlate with levels of phosphorylation of AKT, p70S6K and GSK3 β . However, significantly higher levels of phosphorylated p70S6K levels were present in ascitic tumour cells from patients who did not respond to subsequent chemotherapy compared with samples of patients who did respond ($P = 0.01$). This suggested that other as yet unidentified factors that are independent of PI3K and that influence p70S6K activation may be important for clinical drug resistance.

Ascitic cells from patients with EOC have also been used to study subpopulations of ovarian tumour cells with stem cell-like properties — the side population of cells that take up low levels of Hoechst 33342 dye in flow cytometry analyses^{139,144}. Side-population cells are characterized by prolonged anchorage-independent growth as spheroids, tumorigenicity when injected at low cell numbers into mice, inherent drug resistance leading to tumour recurrence following chemotherapy and ABC transporter-mediated efflux of the Hoechst 33342 dye^{144,145}. Side-population cells that are capable of forming tumours in xenogeneic mice have been isolated from ascites of patients with ovarian cancer¹³⁹, and a higher proportion of side-population cells has also been observed in ascites from patients who have relapsed following chemotherapy compared with chemo-naïve patients¹⁴⁴. Further

characterization of these cells has suggested that the histone methyltransferase EZH2, a key component of the Polycomb-repressive complex 2, may be crucial for maintaining the stem cell state, as small interfering RNA-mediated knockdown of EZH2 results in the loss of side-population cells in ovarian tumour models, reduced anchorage-independent growth and reduced tumour growth *in vivo*¹⁴⁴. Recent evidence suggests that interferon- α (IFN α) may have specific antitumour effects in side-population cells¹⁴⁵, as might Muellierian-inhibiting factor¹⁴⁶. The presence of these cells provides circumstantial evidence of the molecular heterogeneity in the population of malignant ascitic cells, and their clinical relevance is supported by a recent study that suggests that tumour cells isolated from ascites of chemoresistant patients had mRNA profiles that were characteristic of cancer stem cells¹⁴⁷. However, it remains unclear how much of this is reflected in the population of malignant cells in the primary tumour.

Translational research using ascitic fluid

As discussed above, ascitic fluid harbours factors such as VEGF, epidermal growth factor and LPA⁷, which provide a supportive microenvironment for malignant cells to overcome anoikis and to survive in the hypoxic, but otherwise rich, liquid milieu⁷. Identification of additional crucial signals that promote and support cellular survival in ascites should permit the identification of new treatment strategies to control ascites. Methods used to elucidate the molecular characteristics of malignant ascitic fluid have included proteomic studies¹³⁴ using mass spectroscopy (MS)-based techniques such as liquid chromatography (LC), surface enhanced laser desorption and ionization (SELDI), and matrix-assisted laser desorption and ionization (MALDI). More recently, high-throughput automated array-based proteomics techniques have been used such as reverse phase protein arrays (RPPAs) in which only very small amounts of tissue lysates are deposited onto slides covered with nitrocellulose, and proteins of interest are subsequently detected using specific antibodies¹⁴⁸.

In a study using RPPA analysis by Davidson *et al.*¹⁴⁸, malignant effusions (both ascitic and pleural effusions) from patients with EOC were found to have significantly higher expression levels of AKT, cAMP-responsive element binding protein (CREB) and JUN N-terminal kinase (JNK) than benign effusions (following RPPA analysis). Furthermore, high levels of p38, an increase in the ratio of phosphorylated EGFR and increased levels of phosphorylated JNK were associated with worse outcome in these patients¹⁴⁸. Given that upregulation of the AKT-PI3K pro-survival and invasion pathways has been demonstrated in ovarian cancer¹⁴⁹, it would seem that the proteomic profile of the ascitic milieu may reflect the protein expression profile of the underlying tumour. The results of this small study are an indication of the potential use of this technique for the discovery of diagnostic and prognostic biomarkers, but it will nonetheless require further validation in independent cohorts. Analysis of serial samples, such as pre- and post-chemotherapy levels, from each patient could also be compared to assess other molecular changes that may be predictive of treatment response and, in the case of clinical trials with targeted agents, indicative of target validation and inhibition^{134,148,150}. It might be anticipated that ascitic fluid would have a generally stimulatory effect on the invasion and growth of EOC cells, but a study by Puiffe *et al.*¹³³ demonstrated that the protein content of ascitic fluid can be either stimulatory or

inhibitory to EOC cells *in vitro*, suggesting that both positive and negative regulators of tumour progression may be present in ascitic fluid. A wide variability of cytokine expression profile between ascites from different patients was recently demonstrated by Matte *et al.*⁶⁸ using a multiplex cytokine array technique that simultaneously measured the level of 120 cytokines in ascites from patients with EOC, further illustrating the intrapatient heterogeneity of ascitic fluid content. Among the cytokines assayed, higher levels of osteoprotegerin (OPG), IL-10 and leptin in ascitic fluid were associated with worse outcome, and IL-10 was also found to mediate the survival-promoting activity of ascites against TRAIL-induced apoptosis⁶⁸.

Conclusions

Malignant ascites presents a substantial clinical challenge, but also provides a wealth of potential opportunities for translational research in ovarian cancer. The accessibility of ascites translates into a readily available source of tumour tissue during the course of a patient's treatment. In addition, it provides the opportunity for the identification of prognostic and predictive biomarkers, pharmacodynamic information, as well as options for molecular profiling and systems-biology-based analyses. In the current zeitgeist of personalized cancer medicine, the emerging picture from studies on malignant ascites seems to suggest that its molecular profile is subject to considerable inter-patient variability and that the ability to provide accurate molecular characterization will be crucial in designing the most appropriate intervention for patients in the era of targeted therapy.

Undeniably, although the best way to treat malignant ascites is to eradicate the underlying malignancy, the limits of our current therapeutic armamentarium are such that, in many cases of advanced ovarian cancer, tumours will inevitably become resistant to treatment, with the focus of care at that point being shifted to palliation of symptoms. In this context, given its significant associated morbidity, the ability to successfully manage the problem of recurrent ascites associated with treatment-resistant cancer through pharmacological or non-pharmacological methods with minimal risk, toxicity and discomfort, has the potential to vastly improve the level of palliative benefit and quality of life for patients. Therefore, it will be important to also include methods to assess ascites formation, such as time to paracentesis, as an end point in future clinical trials.

Inevitably, what we now know about malignant ascites has only served to highlight the considerable gap in our knowledge that still remains. Nonetheless, with the advances in our understanding of its pathophysiology, coupled with the development of new methods to delineate the molecular features of its various cellular and acellular components, it is anticipated that more rational, targeted and effective treatment strategies for malignant ascites and ovarian cancer will emerge in the future.

Acknowledgements

The Drug Development Unit, the Institute of Cancer Research and the Royal Marsden Hospital NHS Foundation Trust acknowledge support from Cancer Research UK, through core support and an Experimental Cancer Medicine Centre grant and from the National Institute of Health Research, as a Biomedical Research Centre. E.K. acknowledges support from the Wellcome Trust as a clinical research fellow.

Glossary

Omentum	A fold of peritoneum connecting or supporting abdominal structures, such as the stomach and liver.
Paracentesis	The procedure to remove fluid that has accumulated in the abdominal cavity (peritoneal fluid) by inserting a wide-bore needle through the abdominal wall.
Satiety	A condition of being full beyond capacity.
Starling's hypothesis of capillary haemodynamics	The direction and rate of fluid transfer between blood plasma in the capillary and fluid in the tissue spaces depend on the hydrostatic pressure on each side of the capillary wall, on the osmotic pressure of protein in plasma and in tissue fluid, and on the properties of the capillary walls as a filtering membrane.

References

- Runyon BA. Care of patients with ascites. *N. Engl. J. Med.* 1994; 330:337–342. [PubMed: 8277955]
- Parsons SL, Lang MW, Steele RJ. Malignant ascites: a 2-year review from a teaching hospital. *Eur. J. Surg. Oncol.* 1996; 22:237–239. [PubMed: 8654603]
- Ayantunde A, Parsons S. Pattern and prognostic factors in patients with malignant ascites: a retrospective study. *Ann. Oncol.* 2007; 18:945–949. [PubMed: 17298959]
- Garrison RN, Kaelin LD, Galloway RH, Heuser LS. Malignant ascites. Clinical and experimental observations. *Ann. Surg.* 1986; 203:644–651. [PubMed: 3718029]
- Howlader, N., et al. SEER Cancer Statistics Review, 1975–2009 (Vintage 2009 Populations). National Cancer Institute; 2012. [online], http://seer.cancer.gov/csr/1975_2009_pops09/
- Lopez RI, et al. Prognostic factor analysis, for patients with no evidence of disease after initial chemotherapy for advanced epithelial ovarian carcinoma. *Int. J. Gynecol. Cancer.* 1996; 6:8–14.
- Tan DS, Agarwal R, Kaye SB. Mechanisms of transcoelomic metastasis in ovarian cancer. *Lancet Oncol.* 2006; 7:925–934. [PubMed: 17081918]
- Bagnato A, Rosano L. Epithelial-mesenchymal transition in ovarian cancer progression: a crucial role for the endothelin axis. *Cells Tissues Organs.* 2007; 185:85–94. [PubMed: 17587812]
- Auersperg N, Wong AS, Choi KC, Kang SK, Leung PC. Ovarian surface epithelium: biology, endocrinology, and pathology. *Endocr. Rev.* 2001; 22:255–288. [PubMed: 11294827]
- Gardner MJ, Catterall JB, Jones LM, Turner GA. Human ovarian tumour cells can bind hyaluronic acid via membrane CD44: a possible step in peritoneal metastasis. *Clin. Exp. Metastasis.* 1996; 14:325–334. [PubMed: 8878406]
- Strobel T, Swanson L, Cannistra SA. *In vivo* inhibition of CD44 limits intra-abdominal spread of a human ovarian cancer xenograft in nude mice: a novel role for CD44 in the process of peritoneal implantation. *Cancer Res.* 1997; 57:1228–1232. [PubMed: 9102203]
- Wagner BJ, et al. Simvastatin reduces tumor cell adhesion to human peritoneal mesothelial cells by decreased expression of VCAM-1 and β 1 integrin. *Int. J. Oncol.* 2011; 39:1593–1600. [PubMed: 21874229]
- Rump A, et al. Binding of ovarian cancer antigen CA125/MUC16 to mesothelin mediates cell adhesion. *J. Biol. Chem.* 2004; 279:9190–9198. [PubMed: 14676194]
- Gubbels JA, et al. Mesothelin-MUC16 binding is a high affinity, N-glycan dependent interaction that facilitates peritoneal metastasis of ovarian tumors. *Mol. Cancer.* 2006; 5:566–580. [PubMed: 17067392]
- Renkin EM. Some consequences of capillary permeability to macromolecules: Starling's hypothesis reconsidered. *Am. J. Physiol.* 1986; 250:H706–710. [PubMed: 3706547]

16. Mutsaers SE. The mesothelial cell. *Int. J. Biochem. Cell Biol.* 2004; 36:9–16. [PubMed: 14592528]
17. Mutsaers SE. Mesothelial cells: their structure, function and role in serosal repair. *Respirology.* 2002; 7:171–191. [PubMed: 12153683]
18. von Recklinghausen FT. Zur fette sorption. *Arch. Pathol. Anat. Physiol.* 1863; 2666:172.
19. Sodek KL, Murphy KJ, Brown TJ, Ringuette MJ. Cell-cell and cell-matrix dynamics in intraperitoneal cancer metastasis. *Cancer Metastasis Rev.* 2012; 31:397–414. [PubMed: 22527451]
20. Holm-Nielsen P. Pathogenesis of ascites in peritoneal carcinomatosis. *Acta Pathol. Microbiol. Scand.* 1953; 33:10–21. [PubMed: 13113944]
21. Feldman GB, Knapp RC, Order SE, Hellman S. The role of lymphatic obstruction in the formation of ascites in a murine ovarian carcinoma. *Cancer Res.* 1972; 32:1663–1666. [PubMed: 5044130]
22. Nagy JA, Herzberg KT, Dvorak JM, Dvorak HF. Pathogenesis of malignant ascites formation: initiating events that lead to fluid accumulation. *Cancer Res.* 1993; 53:2631–2643. [PubMed: 8495427]
23. Garrison RN, Galloway RH, Heuser LS. Mechanisms of malignant ascites production. *J. Surg. Res.* 1987; 42:126–132. [PubMed: 2434730]
24. Hirabayashi K, Graham J. Genesis of ascites in ovarian cancer. *Am. J. Obstet. Gynecol.* 1970; 106:492–497. [PubMed: 5412843]
25. Senger DR, et al. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science.* 1983; 219:983–985. [PubMed: 6823562]
26. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z. Vascular endothelial growth factor (VEGF) and its receptors. *FASEB J.* 1999; 13:9–22. [PubMed: 9872925]
27. Geva E, Jaffe RB. Role of vascular endothelial growth factor in ovarian physiology and pathology. *Fertil. Steril.* 2000; 74:429–438. [PubMed: 10973633]
28. Zebrowski BK, et al. Markedly elevated levels of vascular endothelial growth factor in malignant ascites. *Ann. Surg. Oncol.* 1999; 6:373–378. [PubMed: 10379858]
29. Barton DP, et al. Angiogenic protein expression in advanced epithelial ovarian cancer. *Clin. Cancer Res.* 1997; 3:1579–1586. [PubMed: 9815846]
30. Kassim SK, et al. Vascular endothelial growth factor and interleukin-8 are associated with poor prognosis in epithelial ovarian cancer patients. *Clin. Biochem.* 2004; 37:363–369. [PubMed: 15087251]
31. Paley PJ, et al. Vascular endothelial growth factor expression in early stage ovarian carcinoma. *Cancer.* 1997; 80:98–106. [PubMed: 9210714]
32. Bamias A, et al. Correlation of NK T-like CD3+CD56+ cells and CD4+CD25+(hi) regulatory T cells with VEGF and TNF α in ascites from advanced ovarian cancer: association with platinum resistance and prognosis in patients receiving first-line, platinum-based chemotherapy. *Gynecol. Oncol.* 2008; 108:421–427. [PubMed: 18036640]
33. Santin AD, et al. Secretion of vascular endothelial growth factor in ovarian cancer. *Eur. J. Gynaecol. Oncol.* 1999; 20:177–181. [PubMed: 10410879]
34. Schumacher JJ, et al. Modulation of angiogenic phenotype alters tumorigenicity in rat ovarian epithelial cells. *Cancer Res.* 2007; 67:3683–3690. [PubMed: 17440080]
35. Byrne AT, et al. Vascular endothelial growth factor-trap decreases tumor burden, inhibits ascites, and causes dramatic vascular remodeling in an ovarian cancer model. *Clin. Cancer Res.* 2003; 9:5721–5728. [PubMed: 14654557]
36. Mesiano S, Ferrara N, Jaffe RB. Role of vascular endothelial growth factor in ovarian cancer: inhibition of ascites formation by immunoneutralization. *Am. J. Pathol.* 1998; 153:1249–1256. [PubMed: 9777956]
37. Yukita A, Asano M, Okamoto T, Mizutani S, Suzuki H. Suppression of ascites formation and re-accumulation associated with human ovarian cancer by an anti-VPF monoclonal antibody *in vivo*. *Anticancer Res.* 2000; 20:155–160. [PubMed: 10769648]
38. Herr D, et al. VEGF induces ascites in ovarian cancer patients via increasing peritoneal permeability by downregulation of Claudin 5. *Gynecol. Oncol.* 2012; 127:210–216. [PubMed: 22579791]

39. Rodewald M, et al. Regulation of tight junction proteins occludin and claudin 5 in the primate ovary during the ovulatory cycle and after inhibition of vascular endothelial growth factor. *Mol. Hum. Reprod.* 2007; 13:781–789. [PubMed: 18006538]
40. Saitou M, et al. Complex phenotype of mice lacking occludin, a component of tight junction strands. *Mol. Biol. Cell.* 2000; 11:4131–4142. [PubMed: 11102513]
41. Dejana E. Endothelial cell-cell junctions: happy together. *Nature Rev. Mol. Cell Biol.* 2004; 5:261–270. [PubMed: 15071551]
42. Takahashi A, Kondoh M, Kodaka M, Yagi K. Peptides as tight junction modulators. *Curr. Pharm. Des.* 2011; 17:2699–2703. [PubMed: 21728977]
43. Nitta T, et al. Size-selective loosening of the blood-brain barrier in claudin-5-deficient mice. *J. Cell Biol.* 2003; 161:653–660. [PubMed: 12743111]
44. Dvorak HF, Nagy JA, Feng D, Brown LF, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor and the significance of microvascular hyperpermeability in angiogenesis. *Curr. Top. Microbiol. Immunol.* 1999; 237:97–132. [PubMed: 9893348]
45. Esser S, Lampugnani MG, Corada M, Dejana E, Risau W. Vascular endothelial growth factor induces VE-cadherin tyrosine phosphorylation in endothelial cells. *J. Cell Sci.* 1998; 111:1853–1865. [PubMed: 9625748]
46. Horiuchi A, et al. Hypoxia-induced changes in the expression of VEGF, HIF-1 α and cell cycle-related molecules in ovarian cancer cells. *Anticancer Res.* 2002; 22:2697–2702. [PubMed: 12529984]
47. Hu YL, et al. Lysophosphatidic acid induction of vascular endothelial growth factor expression in human ovarian cancer cells. *J. Natl Cancer Inst.* 2001; 93:762–768. [PubMed: 11353786]
48. Kulbe H, et al. The inflammatory cytokine tumor necrosis factor- α generates an autocrine tumor-promoting network in epithelial ovarian cancer cells. *Cancer Res.* 2007; 67:585–592. [PubMed: 17234767]
49. Salani D, et al. Role of endothelin-1 in neovascularization of ovarian carcinoma. *Am. J. Pathol.* 2000; 157:1537–1547. [PubMed: 11073813]
50. Gupta RA, et al. Cyclooxygenase-1 is overexpressed and promotes angiogenic growth factor production in ovarian cancer. *Cancer Res.* 2003; 63:906–911. [PubMed: 12615701]
51. Stadlmann S, et al. Ovarian carcinoma cells and IL-1 β -activated human peritoneal mesothelial cells are possible sources of vascular endothelial growth factor in inflammatory and malignant peritoneal effusions. *Gynecol. Oncol.* 2005; 97:784–789. [PubMed: 15943987]
52. Belotti D, et al. Matrix metalloproteinases (MMP9 and MMP2) induce the release of vascular endothelial growth factor (VEGF) by ovarian carcinoma cells: implications for ascites formation. *Cancer Res.* 2003; 63:5224–5229. [PubMed: 14500349]
53. Chen Y, Gou X, Ke X, Cui H, Chen Z. Human tumor cells induce angiogenesis through positive feedback between CD147 and insulin-like growth factor-I. *PLoS ONE.* 2012; 766:e40965. [PubMed: 22844419]
54. Liu LZ, et al. Reactive oxygen species regulate epidermal growth factor-induced vascular endothelial growth factor and hypoxia-inducible factor-1 α expression through activation of AKT and P70S6K1 in human ovarian cancer cells. *Free Radic. Biol. Med.* 2006; 41:1521–1533. [PubMed: 17045920]
55. Matei D, et al. PDGF BB induces VEGF secretion in ovarian cancer. *Cancer Biol. Ther.* 2007; 6:1951–1959. [PubMed: 18075302]
56. Liao S, et al. TGF- β blockade controls ascites by preventing abnormalization of lymphatic vessels in orthotopic human ovarian carcinoma models. *Clin. Cancer Res.* 2011; 17:1415–1424. [PubMed: 21278244]
57. Mills GB, May C, McGill M, Roifman CM, Mellors AA. Putative new growth factor in ascitic fluid from ovarian cancer patients: identification, characterization, and mechanism of action. *Cancer Res.* 1988; 48:1066–1071. [PubMed: 3422589]
58. Fang X, et al. Mechanisms for lysophosphatidic acid-induced cytokine production in ovarian cancer cells. *J. Biol. Chem.* 2004; 279:9653–9661. [PubMed: 14670967]

59. Murph MM, et al. Lysophosphatidic acid-induced transcriptional profile represents serous epithelial ovarian carcinoma and worsened prognosis. *PLoS ONE*. 2009; 4:e5583. [PubMed: 19440550]
60. Jankowski M. Autotaxin: its role in biology of melanoma cells and as a pharmacological target. *Enzyme Res*. 2011; 19:48–57. [PubMed: 21423677]
61. Bast RC Jr, Hennessy B, Mills GB. The biology of ovarian cancer: new opportunities for translation. *Nature Rev. Cancer*. 2009; 9:415–428. [PubMed: 19461667]
62. Mills GB, Moolenaar WH. The emerging role of lysophosphatidic acid in cancer. *Nature Rev. Cancer*. 2003; 3:582–591. [PubMed: 12894246]
63. Abrahams VM, et al. Epithelial ovarian cancer cells secrete functional Fas ligand. *Cancer Res*. 2003; 63:5573–5581. [PubMed: 14500397]
64. Bjorge L, et al. Ascitic complement system in ovarian cancer. *Br. J. Cancer*. 2005; 92:895–905. [PubMed: 15726105]
65. Webb TJ, et al. Molecular identification of GD3 as a suppressor of the innate immune response in ovarian cancer. *Cancer Res*. 2012; 72:3744–3752. [PubMed: 22649190]
66. Milliken D, Scotton C, Raju S, Balkwill F, Wilson J. Analysis of chemokines and chemokine receptor expression in ovarian cancer ascites. *Clin. Cancer Res*. 2002; 8:1108–1114. [PubMed: 11948121]
67. Guo Y, et al. Effects of siltuximab on the IL-6-induced signaling pathway in ovarian cancer. *Clin. Cancer Res*. 2010; 16:5759–5769. [PubMed: 20699329]
68. Matte I, Lane D, Laplante C, Rancourt C, Piche A. Profiling of cytokines in human epithelial ovarian cancer ascites. *Am. J. Cancer Res*. 2012; 2:566–580. [PubMed: 22957308]
69. Penson RT, et al. Cytokines IL-1 β , IL-2, IL-6, IL-8, MCP-1, GM-CSF and TNF α in patients with epithelial ovarian cancer and their relationship to treatment with paclitaxel. *Int. J. Gynecol. Cancer*. 2000; 10:33–41. [PubMed: 11240649]
70. Kryczek I, Grybos M, Karabon L, Klimczak A, Lange A. IL-6 production in ovarian carcinoma is associated with histotype and biological characteristics of the tumour and influences local immunity. *Br. J. Cancer*. 2000; 82:621–628. [PubMed: 10682675]
71. Huang S, Robinson JB, DeGuzman A, Bucana CD, Fidler IJ. Blockade of nuclear factor- κ B signaling inhibits angiogenesis and tumorigenicity of human ovarian cancer cells by suppressing expression of vascular endothelial growth factor and interleukin 8. *Cancer Res*. 2000; 60:5334–5339. [PubMed: 11034066]
72. Yoneda J, et al. Expression of angiogenesis-related genes and progression of human ovarian carcinomas in nude mice. *J. Natl Cancer Institute*. 1998; 90:447–454. [PubMed: 9521169]
73. Obata N, Tamakoshi K, Shibata K, Kikkawa F, Tomoda Y. Effects of interleukin-6 on in vitro cell attachment, migration and invasion of human carcinoma. *Anticancer Res*. 1997; 17:337–342. [PubMed: 9066674]
74. Woolery KT, Kruk PA. Ovarian epithelial-stromal interactions: role of interleukins 1 and 6. *Obstet. Gynecol. Int*. 2011; 35:84–93. [PubMed: 21765834]
75. Nilsson MB, Langley RR, Fidler IJ. Interleukin-6, secreted by human ovarian carcinoma cells, is a potent proangiogenic cytokine. *Cancer Res*. 2005; 65:10794–10800. [PubMed: 16322225]
76. Alberti C, et al. Ligand-dependent EGFR activation induces the co-expression of IL-6 and PAI-1 via the NF κ B pathway in advanced-stage epithelial ovarian cancer. *Oncogene*. 2011; 31:4139–4149. [PubMed: 22158046]
77. Lane D, Matte I, Rancourt C, Piche A. Prognostic significance of IL-6 and IL-8 ascites levels in ovarian cancer patients. *BMC Cancer*. 2011; 1166:210. [PubMed: 21619709]
78. Naldini A, et al. Identification of thrombin-like activity in ovarian cancer associated ascites and modulation of multiple cytokine networks. *Thromb. Haemost*. 2011; 106:705–711. [PubMed: 21833453]
79. Gubbels JA, et al. MUC16 provides immune protection by inhibiting synapse formation between NK and ovarian tumor cells. *Mol. Cancer*. 2010; 966:11. [PubMed: 20089172]
80. Sica A, Sacconi A, Mantovani A. Tumor-associated macrophages: a molecular perspective. *Int. Immunopharmacol*. 2002; 2:1045–1054. [PubMed: 12349942]

81. Hagemann T, et al. Ovarian cancer cells polarize macrophages toward a tumor-associated phenotype. *J. Immunol.* 2006; 176:5023–5032. [PubMed: 16585599]
82. Balkwill F. Cancer and the chemokine network. *Nature Rev. Cancer.* 2004; 4:540–550. [PubMed: 15229479]
83. Wang E, et al. Peritoneal and subperitoneal stroma may facilitate regional spread of ovarian cancer. *Clin. Cancer Res.* 2005; 11:113–122. [PubMed: 15671535]
84. Lin YG, et al. EphA2 overexpression is associated with angiogenesis in ovarian cancer. *Cancer.* 2007; 109:332–340. [PubMed: 17154180]
85. Niu G, et al. Constitutive Stat3 activity up-regulates VEGF expression and tumor angiogenesis. *Oncogene.* 2002; 21:2000–2008. [PubMed: 11960372]
86. Rosen DG, et al. The role of constitutively active signal transducer and activator of transcription 3 in ovarian tumorigenesis and prognosis. *Cancer.* 2006; 107:2730–2740. [PubMed: 17063503]
87. Lane D, Robert V, Grondin R, Rancourt C, Piche A. Malignant ascites protect against TRAIL-induced apoptosis by activating the PI3K/Akt pathway in human ovarian carcinoma cells. *Int. J. Cancer.* 2007; 121:1227–1237. [PubMed: 17534891]
88. Lane D, Goncharenko-Khaider N, Rancourt C, Piche A. Ovarian cancer ascites protects from TRAIL-induced cell death through $\alpha\beta 5$ integrin-mediated focal adhesion kinase and Akt activation. *Oncogene.* 2010; 29:3519–3531. [PubMed: 20400979]
89. Goncharenko-Khaider N, Matte I, Lane D, Rancourt C, Piche A. Ovarian cancer ascites increase Mcl-1 expression in tumor cells through ERK1/2-Elk-1 signaling to attenuate TRAIL-induced apoptosis. *Mol. Cancer.* 2012; 1166:84. [PubMed: 23158473]
90. Peart TM, Correa RJ, Valdes YR, Dimattia GE, Shepherd TG. BMP signalling controls the malignant potential of ascites-derived human epithelial ovarian cancer spheroids via AKT kinase activation. *Clin. Exp. Metastasis.* 2012; 29:293–313. [PubMed: 22249415]
91. Bookman MA, et al. Evaluation of new platinum-based treatment regimens in advanced-stage ovarian cancer: a Phase III Trial of the Gynecologic Cancer Intergroup. *J. Clin. Oncol.* 2009; 27:1419–1425. [PubMed: 19224846]
92. De Placido S, et al. Topotecan compared with no therapy after response to surgery and carboplatin/paclitaxel in patients with ovarian cancer: Multicenter Italian Trials in Ovarian Cancer (MITO-1) randomized study. *J. Clin. Oncol.* 2004; 22:2635–2642. [PubMed: 15226331]
93. du Bois A, et al. Addition of epirubicin as a third drug to carboplatin-paclitaxel in first-line treatment of advanced ovarian cancer: a prospectively randomized gynecologic cancer intergroup trial by the Arbeitsgemeinschaft Gynaekologische Onkologie Ovarian Cancer Study Group and the Groupe d'Investigateurs Nationaux pour l'Etude des Cancers Ovariens. *J. Clin. Oncol.* 2006; 24:1127–1135. [PubMed: 16505432]
94. Perren TJ, et al. A phase 3 trial of bevacizumab in ovarian cancer. *New Engl. J. Med.* 2011; 365:2484–2496. [PubMed: 22204725]
95. Burger RA, et al. Incorporation of bevacizumab in the primary treatment of ovarian cancer. *New Engl. J. Med.* 2011; 365:2473–2483. [PubMed: 22204724]
96. Kristensen GP, et al. Result of interim analysis of overall survival in the GCIG ICON7 phase III randomized trial of bevacizumab in women with newly diagnosed ovarian cancer. *J. Clin. Oncol. Abstr.* 2011; 29:LBA5006.
97. Aghajanian C, et al. OCEANS: a randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer. *J. Clin. Oncol.* 2012; 30:2039–2045. [PubMed: 22529265]
98. Pujade-Lauraine E, et al. AURELIA: a randomized phase III trial evaluating bevacizumab (BEV) plus chemotherapy (CT) for platinum (PT)-resistant recurrent ovarian cancer (OC). *J. Clin. Oncol. Abstr.* 2012; 30:LBA5002.
99. Sennino B, McDonald DM. Controlling escape from angiogenesis inhibitors. *Nature Rev. Cancer.* 2012; 12:699–709. [PubMed: 23001349]
100. Runyon BA. Management of adult patients with ascites due to cirrhosis: an update. *Hepatology.* 2009; 49:2087–2107. [PubMed: 19475696]

101. Lee CW, Bociek G, Faught W. A survey of practice in management of malignant ascites. *J. Pain Symptom Manage.* 1998; 16:96–101. [PubMed: 9737100]
102. Becker G, Galandi D, Blum HE. Malignant ascites: systematic review and guideline for treatment. *Eur. J. Cancer.* 2006; 42:589–597. [PubMed: 16434188]
103. Pockros PJ, Esrason KT, Nguyen C, Duque J, Woods S. Mobilization of malignant ascites with diuretics is dependent on ascitic fluid characteristics. *Gastroenterology.* 1992; 103:1302–1306. [PubMed: 1397889]
104. Cavazzoni E, Bugiantella W, Graziosi L, Franceschini MS, Donini A. Malignant ascites: pathophysiology and treatment. *Int. J. Clin. Oncol.* Mar 31.2012 (doi:10.1007/s10147-012-0396-6).
105. Kalambokis G, et al. Renal effects of treatment with diuretics, octreotide or both, in non-azotemic cirrhotic patients with ascites. *Nephrol. Dial. Transplant.* 2005; 20:1623–1629. [PubMed: 15886218]
106. Jatoi A, et al. A pilot study of long-acting octreotide for symptomatic malignant ascites. *Oncology.* 2012; 82:315–320. [PubMed: 22572824]
107. Dedrick RL, Myers CE, Bungay PM, DeVita VT Jr. Pharmacokinetic rationale for peritoneal drug administration in the treatment of ovarian cancer. *Cancer Treat. Rep.* 1978; 62:1–11. [PubMed: 626987]
108. Armstrong DK, et al. Intraperitoneal cisplatin and paclitaxel in ovarian cancer. *N. Engl. J. Med.* 2006; 354:34–43. [PubMed: 16394300]
109. Ostrowski MJ. An assessment of the long-term results of controlling the reaccumulation of malignant effusions using intracavity bleomycin. *Cancer.* 1986; 57:721–727. [PubMed: 2417681]
110. Maiche AG. Management of peritoneal effusions with intracavitary mitoxantrone or bleomycin. *Anticancer Drugs.* 1994; 5:305–308. [PubMed: 7522646]
111. Link KH, et al. Intraperitoneal chemotherapy with mitoxantrone in malignant ascites. *Surg. Oncol. Clin. N. Am.* 2003; 12:865–872. [PubMed: 14567037]
112. Schilsky RL, et al. Phase I clinical and pharmacologic study of intraperitoneal cisplatin and fluorouracil in patients with advanced intraabdominal cancer. *J. Clin. Oncol.* 1990; 8:2054–2061. [PubMed: 2230897]
113. Mackey JR, Wood L, Nabholz J, Jensen J, Venner P. A phase II trial of triamcinolone hexacetanide for symptomatic recurrent malignant ascites. *J. Pain Symptom Manage.* 2000; 19:193–199. [PubMed: 10760624]
114. Stuart GC, Nation JG, Snider DD, Thunberg P. Intraperitoneal interferon in the management of malignant ascites. *Cancer.* 1993; 71:2027–2030. [PubMed: 7680276]
115. Rath U, et al. Effect of intraperitoneal recombinant human tumour necrosis factor α on malignant ascites. *Eur. J. Cancer.* 1991; 27:121–125. [PubMed: 1827272]
116. Heiss MM, et al. The trifunctional antibody catumaxomab for the treatment of malignant ascites due to epithelial cancer: results of a prospective randomized phase II/III trial. *Int. J. Cancer.* 2010; 127:2209–2221. [PubMed: 20473913]
117. Ott MG, et al. Humoral response to catumaxomab correlates with clinical outcome: results of the pivotal phase II/III study in patients with malignant ascites. *Int. J. Cancer.* 2012; 130:2195–2203. [PubMed: 21702044]
118. Beattie GJ, Smyth JF. Phase I study of intraperitoneal metalloproteinase inhibitor BB94 in patients with malignant ascites. *Clin. Cancer Res.* 1998; 4:1899–1902. [PubMed: 9717817]
119. Numnum TM, Rocconi RP, Whitworth J, Barnes MN. The use of bevacizumab to palliate symptomatic ascites in patients with refractory ovarian carcinoma. *Gynecol. Oncol.* 2006; 102:425–428. [PubMed: 16797681]
120. El-Shami K, Elsaid A, El-Kerm Y. Open-label safety and efficacy pilot trial of intraperitoneal bevacizumab as palliative treatment in refractory malignant ascites. *J. Clin. Oncol.* 2007; 25:9043.
121. Hamilton CA, et al. Intraperitoneal bevacizumab for the palliation of malignant ascites in refractory ovarian cancer. *Gynecol. Oncol.* 2008; 111:530–532. [PubMed: 18561992]

122. Bellati F, et al. Complete remission of ovarian cancer induced intractable malignant ascites with intraperitoneal bevacizumab. Immunological observations and a literature review. *Invest. New Drugs*. 2010; 28:887–894. [PubMed: 19936983]
123. Holash J, et al. VEGF-Trap: a VEGF blocker with potent antitumor effects. *Proc. Natl Acad. Sci*. 2002; 99:11393–11398. [PubMed: 12177445]
124. Colombo N, et al. A phase II study of aflibercept in patients with advanced epithelial ovarian cancer and symptomatic malignant ascites. *Gynecol. Oncol*. 2012; 125:42–47. [PubMed: 22112608]
125. Gotlieb WH, et al. Intravenous aflibercept for treatment of recurrent symptomatic malignant ascites in patients with advanced ovarian cancer: a phase 2, randomised, double-blind, placebo-controlled study. *Lancet Oncol*. 2012; 13:154–162. [PubMed: 22192729]
126. LeVeen HH, et al. Peritoneo-venous shunting for ascites. *Ann. Surg*. 1974; 180:580–591. [PubMed: 4415019]
127. Mamada Y, et al. Peritoneovenous shunts for palliation of malignant ascites. *J. Nippon Med. Sch*. 2007; 74:355–358. [PubMed: 17965529]
128. White MA, Agle SC, Padia RK, Zervos EE. Denver peritoneovenous shunts for the management of malignant ascites: a review of the literature in the post LeVeen Era. *Am. Surg*. 2011; 77:1070–1075. [PubMed: 21944526]
129. Saiz-Mendiguren R, et al. Permanent tunneled drainage for malignant ascites: initial experience with the PleurX[®] catheter. *Radiologia*. 2010; 52:541–545. [PubMed: 20863540]
130. Fleming ND, Alvarez-Secord A, Von Gruenigen V, Miller MJ, Abernethy AP. Indwelling catheters for the management of refractory malignant ascites: a systematic literature overview and retrospective chart review. *J. Pain Symptom Manage*. 2009; 38:341–349. [PubMed: 19328648]
131. Tapping CR, Ling L, Razack A. PleurX drain use in the management of malignant ascites: safety, complications, long-term patency and factors predictive of success. *Br. J. Radiol*. 2012; 85:623–628. [PubMed: 21427184]
132. Kmietowicz Z. Cancer patients should have access to device to treat fluid retention at home, says NICE. *BMJ*. 2012; 344:e2272. [PubMed: 22442355]
133. Puiffe M-L, et al. Characterization of ovarian cancer ascites on cell. Invasion, proliferation, spheroid formation, and gene expression in an *in vitro* model of epithelial ovarian cancer. *Neoplasia*. 2007; 9:820–829. [PubMed: 17971902]
134. Lee, J. m.; Kohn, EC. Proteomics as a guiding tool for more effective personalized therapy. *Ann. Oncol*. 2010; 21:205–210. [PubMed: 20943616]
135. Morozova O, Marra MA. Applications of next-generation sequencing technologies in functional genomics. *Genomics*. 2008; 92:255–264. [PubMed: 18703132]
136. Hetland TE, et al. Class III β -tubulin expression in advanced-stage serous ovarian carcinoma effusions is associated with poor survival and primary chemoresistance. *Hum. Pathol*. 2011; 42:1019–1026. [PubMed: 21315408]
137. Gillet J-P, et al. Clinical relevance of multidrug resistance gene expression in ovarian serous carcinoma effusions. *Mol. Pharm*. 2011; 8:2080–2088. [PubMed: 21761824]
138. Shepherd TG, Thériault BL, Campbell EJ, Nachtigal MW. Primary culture of ovarian surface epithelial cells and ascites-derived ovarian cancer cells from patients. *Nature Protoc*. 2007; 1:2643–2649. [PubMed: 17406520]
139. Hu L, McArthur C, Jaffe RB. Ovarian cancer stem-like side-population cells are tumorigenic and chemoresistant. *Br. J. Cancer*. 2010; 102:1276–1283. [PubMed: 20354527]
140. Mukhopadhyay A, et al. Development of a functional assay for homologous recombination status in primary cultures of epithelial ovarian tumor and correlation with sensitivity to poly(ADP-ribose) polymerase inhibitors. *Clin. Cancer Res*. 2010; 16:2344–2351. [PubMed: 20371688]
141. Fong PC, et al. Poly(ADP-ribose) polymerase inhibition: frequent durable responses in BRCA carrier ovarian cancer correlating with platinum-free interval. *J. Clin. Oncol*. 2010; 28:2512–2519. [PubMed: 20406929]

142. Elattar A, et al. Androgen receptor expression is a biological marker for androgen sensitivity in high grade serous epithelial ovarian cancer. *Gynecol. Oncol.* 2012; 124:142–147. [PubMed: 22001143]
143. Carden CP, et al. The association of PI3 kinase signaling and chemoresistance in advanced ovarian cancer. *Mol. Cancer Ther.* 2012; 11:1609–1617. [PubMed: 22556379]
144. Rizzo S, et al. Ovarian cancer stem cell-like side populations are enriched following chemotherapy and overexpress EZH2. *Mol. Cancer Ther.* 2011; 10:325–335. [PubMed: 21216927]
145. Moserle L, et al. The side population of ovarian cancer cells is a primary target of IFN- α antitumor effects. *Cancer Res.* 2008; 68:5658–5668. [PubMed: 18632618]
146. Meirelles K, et al. Human ovarian cancer stem/progenitor cells are stimulated by doxorubicin but inhibited by Mullerian inhibiting substance. *Proc. Natl Acad. Sci. USA.* 2012; 109:2358–2363. [PubMed: 22308459]
147. Latifi A, et al. Isolation and characterization of tumor cells from the ascites of ovarian cancer patients: molecular phenotype of chemoresistant ovarian tumors. *PLoS ONE.* 2012; 766:e46858. [PubMed: 23056490]
148. Davidson B. Proteomic analysis of malignant ovarian cancer effusions as a tool for biologic and prognostic profiling. *Clin. Cancer Res.* 2006; 12:791–799. [PubMed: 16467090]
149. Yap TA, Carden CP, Kaye SB. Beyond chemotherapy: targeted therapies in ovarian cancer. *Nature Rev. Cancer.* 2009; 9:167–181. [PubMed: 19238149]
150. Lee J-M, Han JJ, Altwerger G, Kohn EC. Proteomics and biomarkers in clinical trials for drug development. *J. Proteomics.* 2011; 74:2632–2641. [PubMed: 21570499]
151. Runyon BA, et al. The serum-ascites albumin gradient is superior to the exudate-transudate concept in the differential diagnosis of ascites. *Ann. Intern. Med.* 1992; 117:215–220. [PubMed: 1616215]
152. Sheid B. Angiogenic effects of macrophages isolated from ascitic fluid aspirated from women with advanced ovarian cancer. *Cancer Lett.* 1992; 62:153–158. [PubMed: 1371714]
153. Starling EH. On the absorption of fluids from the connective tissue spaces. *J. Physiol.* 1896; 19:312–326.
154. Cattau EL Jr, Benjamin SB, Knuff TE, Castell DO. The accuracy of the physical examination in the diagnosis of suspected ascites. *JAMA.* 1982; 247:1164–1166. [PubMed: 7057606]
155. Inadomi J, Cello JP, Koch J. Ultrasonographic determination of ascitic volume. *Hepatology.* 1996; 24:549–551. [PubMed: 8781322]
156. Akriviadis EA. Hemoperitoneum in patients with ascites. *Am. J. Gastroenterol.* 1997; 92:567–575. [PubMed: 9128301]
157. von Riedenauer WB, Janjua SA, Kwon DS, Zhang Z, Velanovich V. Immunohistochemical identification of primary peritoneal serous cystadenocarcinoma mimicking advanced colorectal carcinoma: a case report. *J. Med. Case Rep.* 2007; 166:150. [PubMed: 18036260]

Box 1**The composition of ascites**

Ascites fluid contains variable proportions of suspended cells and debris depending on the pathogenesis.

Fluid composition

The protein content of ascites can be useful in determining the cause of ascites. Ascites that is secondary to portal hypertension (most commonly secondary to liver failure) can be readily differentiated from ascites that is due to other causes by the serum–ascites albumin gradient (SAAG = [albumin] serum – [albumin] ascites). If the SAAG is greater than or equal to 1.1 g per dl (or 11 g per L), ascites is ascribed to portal hypertension with an approximate 97% accuracy¹⁵¹. A lower SAAG strongly suggests an alternative diagnosis, such as malignant ascites or tuberculosis. Total glucose and lactic dehydrogenase levels also vary, typically measuring <3.33 mmol per L and >200 international units (IU) per L, respectively, in ascites secondary to malignancy and tuberculosis. With pancreatic ascites, the levels of ascitic fluid amylase may exceed 2,000 IU per L.

Cellular composition

In non-malignant ascites, the cell count and white cell differential is useful in determining an infective or inflammatory cause. With spontaneous bacterial peritonitis, the upper limit of the neutrophil count of 250 cells per μ L is exceeded, and represents >50% of the total white cell count in the ascitic fluid. In malignant ascites, the cellular population consists of differing proportions of tumour cells, mesothelial cells, fibroblasts, macrophages, white blood cells and red blood cells¹⁵². Analysis of ascites samples from patients with ovarian cancer has shown considerable variation in the cell populations⁶⁶, with a higher proportion of red blood cells characteristically seen when ascites has rapidly developed. A typical distribution of the cellular components comprising the ascitic fluid of women with ovarian cancer is 37% lymphocytes, 29% mesothelial cells, 32% macrophages and <0.1% adenocarcinoma cells¹⁵².

"with the advances in our understanding of its pathophysiology ... it is anticipated that more rational, targeted and effective treatment strategies for malignant ascites and ovarian cancer will emerge"

Figure 1. The peritoneum, peritoneal membrane and ascites

a | The peritoneal membrane covers the visceral organs, as well as the abdominal and pelvic cavities, and consists of five layers. The first layer is made up of endothelial cells that line the intravascular space of capillaries. These cells have an extracellular glycocalyx and fixed anionic charge that makes it difficult for large blood plasma proteins, such as albumin, to pass through. Intracellular pores provide transport through this layer. The endothelial cell basement membrane provides the second layer. The interstitial space is the third layer and contains fibroblasts, collagen and hyaluronic acid, and it can block the diffusion of macromolecules before the submesothelial basement membrane (the fourth layer). The final layer consists of mesothelial cells. Bound by tight junctions and secreting surface glycosaminoglycans (hyaluronan) into the abdominal space, mesothelial cells provide an effective anti-adhesive surface and a protective barrier against physical damage. In physiological conditions, the difference in oncotic pressure across the peritoneal membrane (high at the endothelial layer and low at the mesothelial layer) limits capillary fluid filtration and prevents oedema that is due to water reabsorption into the capillaries from the interstitial space. **b** | In patients with tumours in the abdominal cavity, the cross-sectional area of microvessels lining the peritoneal cavity is increased, and this results in an increased filtration surface for fluid. In addition, malignant ascites has a high protein concentration that is secondary to increased capillary permeability. Inflammatory cytokines and chemokines, as well as reduced lymphatic flow, all contribute to alterations of the peritoneal

membrane. These changes decrease the plasma to peritoneal oncotic pressure difference, so the direction of flow of fluid is into the peritoneal cavity, obeying Starling's hypothesis of capillary haemodynamics¹⁵³. This leads to the build up of pathological volumes of fluid in the peritoneal cavity. **c** | A computed tomography image of a patient with ovarian cancer showing the accumulation of ascites in the abdomen, leading to the compression of organs and tissues in the abdominal cavity. Image in part **c** courtesy of N. Tunariu, Royal Marsden Hospital, UK.

Table 1
Malignant ascites: clinical features and investigations

Investigations and indications	Signs (% of patients) ^{2,3}
Clinical examination	Dullness to percussion, a shifting dullness and fluid wave. This requires >1,500 mls of ascites to be present for detection. This means of diagnosis has a 50% to 94% sensitivity ¹⁵⁴
Patient symptoms	Anorexia (36%), nausea (37%), abdominal swelling (55%), abdominal pain (53%), early satiety (6%), weight change (5%), dyspnoea (11%), vomiting (25%) and fatigue (17%)
Imaging	Ultrasound is used to assess and to mark the area most suitable for drainage. Using this method 100 mls of ascitic fluid can be detected ¹⁵⁵ , whereas >20 mls can be detected using computer-aided tomography or magnetic resonance imaging. Disease status can also be assessed. It is not possible to distinguish between malignant and benign ascites on the basis of examination or radiological imaging alone
Cytological characteristics	This is a standard technique for diagnosing malignant ascites. It is essential for differentiating between malignant, infective and inflammatory causes of ascites (97% sensitivity). Cellular content of ascitic fluid represents shedding of tumour cells from the tumour into the peritoneal fluid. The yield of cytology is greater with primary peritoneal tumours ¹⁵⁶
Immunohistochemical staining	Immunohistochemical staining of the ascites is important in determining the primary site of the tumour. CA125 is a glycoprotein secreted by and overexpressed on the cell surface of ovarian cancer cells. Other markers include oestrogen receptor, cytokeratin 7, Wilm's tumor suppressor gene, cytokeratin 20, progesterone receptor, calretinin, carcinoembryonic antigen, gross cystic disease fluid protein, and thyroid transcription factor 1 (REF. 157)