Chapter 9

Ascites in Advanced Ovarian Cancer

Katarina Cerne and Borut Kobal

Additional information is available at the end of the chapter

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Abstract

The presence of ascites is one of the general ovarian cancer (OC) symptoms detected at initial diagnosis and can be present at an early stage but is most often seen in advanced disease. In newly diagnosed OC patients, ascites is treated by the standard treatment for the underlying disease. However, once the chemoresistant and recurrent features of the disease develop, management of a large volume of ascites can be a major problem. By increasing abdominal pressure, ascites can cause severe symptoms; thus, palliation of symptomatic patients is the main goal. The elimination of fluid accumulation in OC patients with these symptoms will certainly improve their quality of life and may even prolong survival. Unfortunately, no standard treatment for OC-associated ascites exists. There are several traditional therapies for ascites, with limited effectiveness and significant adverse effects. Catumaxomab is the only medicine approved for intraperitoneal treatment of malignant ascites in patients with EpCAM-positive carcinomas. Advances in our understanding of malignant ascites aetiology and more effective treatment strategies for ascites and OC will help reduce the symptoms associated with ascites.

Keywords: advanced ovarian cancer, malignant ascites, aetiology, treatment, diagnosis

1. Introduction

Ascites is an abnormal accumulation of serous fluid (>50 mL) in the peritoneal cavity between the membrane lining the abdominal wall and the membrane covering the abdominal organs. Although ascites is most commonly observed in patients with cirrhosis, 7–10% of patients with ascites develop it secondary to malignancy. The commonest primary tumour associated with the development of ascites is ovarian cancer (OC) [1]. Large amounts of ascites in a patient with OC usually indicate the presence of peritoneal metastasis; therefore, ascites is found in the majority of patients (89%) with advanced disease (FIGO stages III and IV). However, the absence of ascites may not exclude malignant disease, since ascites is rarely

(17%) observed in the early disease (FIGO stages I and II) and is absent in nearly half of borderline tumours. Unlike in primary OC, recurrent disease is not strongly associated with ascites, which was found in 38% of patients with recurrent OC [2].

Throughout history, ascites has always been regarded as a poor prognostic sign. In the 1700s, Sir Thomas Spencer Wells wrote "surgeons stood and trembled on the brink of ovarian waters" [3]. Studies addressing the prognostic significance of ascites in patients with stage III or IV have shown a significantly poorer survival [4]. Ascites is also associated with pharmacoresistance [4]. Patch et al. showed that matched primary ascites (tumour cells isolated from ascites) share most genomic changes of acquired resistance with primary tumour samples across the whole genome [5].

In newly diagnosed ovarian cancer patients, ascites is treated by using the standard treatment for the underlying disease. However, once the chemoresistant and recurrent features of the disease develop, management of a large volume of ascites can be a major problem. Palliation of symptomatic patients is therefore the foremost goal, and elimination of fluid accumulation in patients with these symptoms will certainly improve their quality of life and may even prolong survival [6, 7]. An understanding of malignant ascites aetiology is of utmost importance if more effective treatment strategies for ascites and OC are to emerge in the future.

This chapter considers the aetiology and pathophysiology of malignant ascites in OC as well as current diagnostic modalities and explores the best form of management.

2. Mechanisms of malignant ascites formation

The word ascites originates from the ancient Greek askos, meaning a sac or bag. Celsus (c.30 BC–c.50 AD) postulated a link between ascites and renal disease, and he coined the term [8]. The peritoneal cavity, located between the parietal and visceral peritoneum, contains approximately 100 mL of serous fluids. Free fluid in the peritoneal cavity acts as a lubricant of the serosal surfaces and originates from the transduction of plasma through capillary membranes of the peritoneal serosa. Healthy women may normally have as much as 20 mL of free peritoneal fluid, depending on the phase of the menstrual cycle [9]. Under physiological conditions, transudation is balanced by efflux of the peritoneal fluid via lymphatic vessels. Tumour growth eventually disrupts the normal regulation of intraperitoneal fluid flow and the maintenance of a steady state in the peritoneal cavity by simultaneously causing a greater fluid inflow and a reduced outflow. Four major factors that contribute to the formation of ascites: two cause increased influx due to tumour-related factors and two cause decreased efflux due to lymphatic obstruction and mechanical obstruction by accumulation of tumour cells at the peritoneal surface (**Figure 1**) [1, 10]. The percentage of cases with a greater ascites volume increased as the stage of ovarian malignancy progressed [1].

2.1. Efflux from the peritoneal cavity into the blood

The peritoneal lymphatic system collects excess fluid, proteins, other macromolecules (>16 kDa) and cells and returns them to systemic circulation [11]. Decreased efflux from the peritoneal cavity due to lymphatic obstruction by tumour cells was first proposed as a hypothesis for ascites

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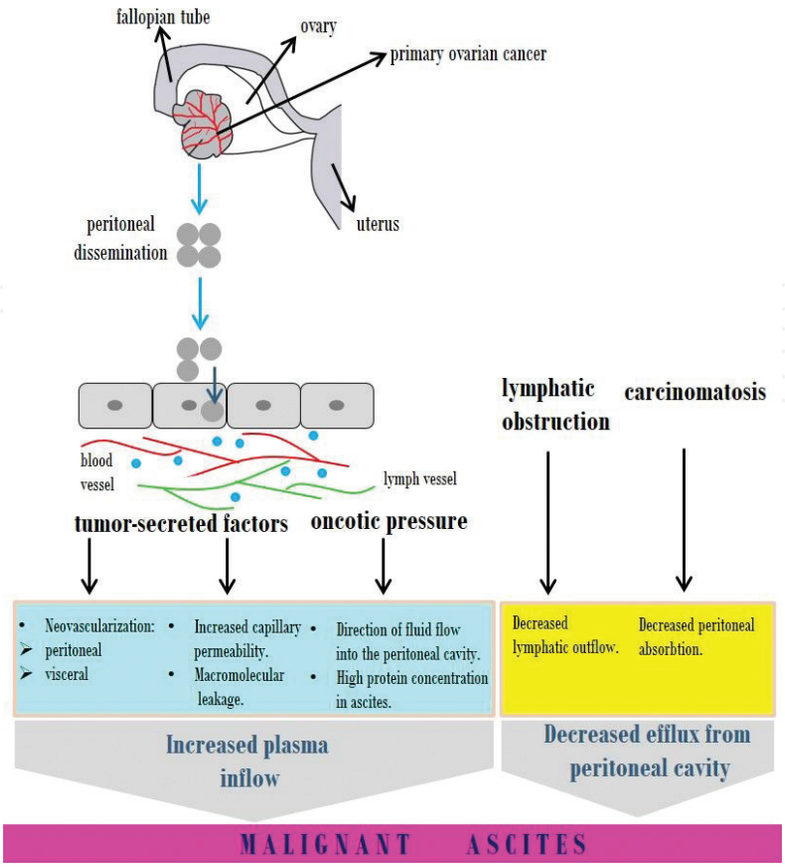

Figure 1. Aetiology of malignant ascites in ovarian cancer.

formation by Holm-Nielsen more than 60 years ago [12]. Published data using lymphoscintigraphy showed that patients with malignant ascites had no activity above the diaphragm after intraperitoneal injection of the isotope, in contrast to control patients with no ascites or cirrhotic ascites. Bronskill et al. [13] showed that OC patients with persistent, intractable ascites, who were approaching their terminal illness, had low peritoneal drainage rates (below 50 ml/h). This result generally indicates obstruction of the diaphragmatic plexus [13]. Initial events that lead to fluid accumulation were studied by Nagy et al. [14] who showed that in mice efflux the peritoneal cavity of ^{125}I-labelled human serum albumin and ^{51}Cr-labelled red blood cells is markedly reduced (fivefold) within 1 day of *i.p.* ovarian tumour cell line injection. A significant reduction preceding a detectable increase in tumour cell number was not attributable to the blockage of peritoneal lymphatics by tumour cells and by itself did not provoke peritoneal fluid accumulation. These results suggest a prominent role for nonobstructive mechanisms, including contraction of lymph vessels induced by secretion of tumour cell product(s). At later periods, the absorption of fluid from the peritoneal cavity might also be affected due to carcinomatosis [14].

2.2. Influx into the peritoneal cavity

Nagy et al. studied the influx of fluid into the peritoneal cavity of mice [14]. They found that after *i.p.* ovarian tumour cell line injection, influx of ^{125}I-labelled human serum albumin rose between days 5 and 7 to values 13- to 25-fold higher than control values, when the tumour cell number had increased >500-fold. By day 10, influx had increased sufficiently to exceed efflux,

resulting in net accumulation of fluid [14]. An increase in influx is a result of various factors: (1) increased capillary permeability, (2) angiogenesis, (3) increased area for filtration and (4) decreased oncotic pressure difference. In malignant ascites, various factors secreted by tumour cells are present, which increase vascular permeability and induce angiogenesis. An early step leading to angiogenesis is partial proteolysis of vascular basal lamina, resulting in hyperpermeability. Vascular endothelial growth factor (VEGF) is the most potent and specific angiogenic factor, secreted by a large variety of tumours, peritoneal mesothelial cells, monocyte/macrophages in malignant ascites and even tumour-infiltrating T cells [7]. Additionally, VEGF increases the permeability of vessels to plasma proteins, including albumin and fibrinogen, with a potency 10,000 times higher than histamine [15, 16]. Other factors that may also induce angiogenesis have been identified in malignant ascites and include basic fibroblast growth factor (bFGF), angiogenin, transforming growth factor alpha and beta (TGF-alpha, TGF-beta), interleukin-8, placental growth factor (PIGF) and platelet-derived endothelial cell growth factor (PD-EGF) [11]. Influx into the peritoneal cavity after *i.p.* ovarian tumour cell line injection rose significantly when the surface area for filtration also increased; the size and number of vessels lining the peritoneal cavity increased as much as 15-fold [16]. The protein content of malignant ascites is greater than in peritoneal fluid of healthy women [11]. The oncotic pressure difference between plasma and ascites therefore decreases, and as a consequence, reabsorption decreases and interstitial fluid accumulation results [11]. Liver metastasis causing hepatic vein obstruction may be an important aetiology factor in some cases of malignant ascites [1].

3. Diagnosis

The absence of symptoms or the presence of symptoms that mimic other conditions often results in diagnostic delay with OC, and this worsens prognosis. Evaluation consists of physical examination, imaging [ultrasonography, computerized tomography (CT), magnetic resonance image (MRI)], serum tumour markers analysis and ascitic fluid analysis (visual inspection, biochemical analysis, cytology and tumour markers). Diagnostic laparoscopy is an additional investigation and may be useful in patients with whom simple investigations have failed to determine the cause of ascites (**Figure 2**) [6, 17–21].

3.1. Symptoms

The most common complaint in the presentation of OC is abdominal swelling or bloating [17]. These symptoms are commonly associated with the physical and surgical finding of ascites. As the amount of fluid increases, ascites can cause significant symptoms referable to the gastrointestinal and genitourinary tracts. Malignant ascites is associated with abdominal and pelvic pain, while liver disease tends to be relatively painless [6, 17].

3.2. Imaging

Transabdominal and transvaginal ultrasonographies are the most sensitive techniques for the detection of ascitic fluid (**Figure 3**) [19]. Uncomplicated ascites appears as ahomogeneous, freely mobile, anechoic collection in the peritoneal cavity that demonstrates deep acoustic enhancement. Generally, free ascites do not displace organs situated between them

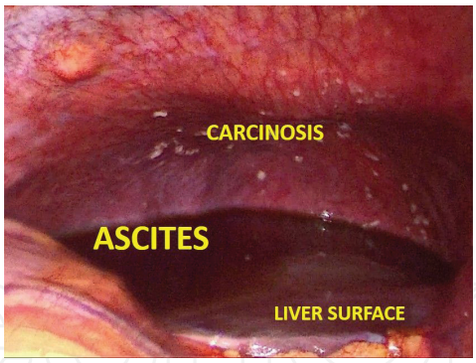

Figure 2. Diagnostic laparoscopy showing ascites and peritoneal carcinosis.

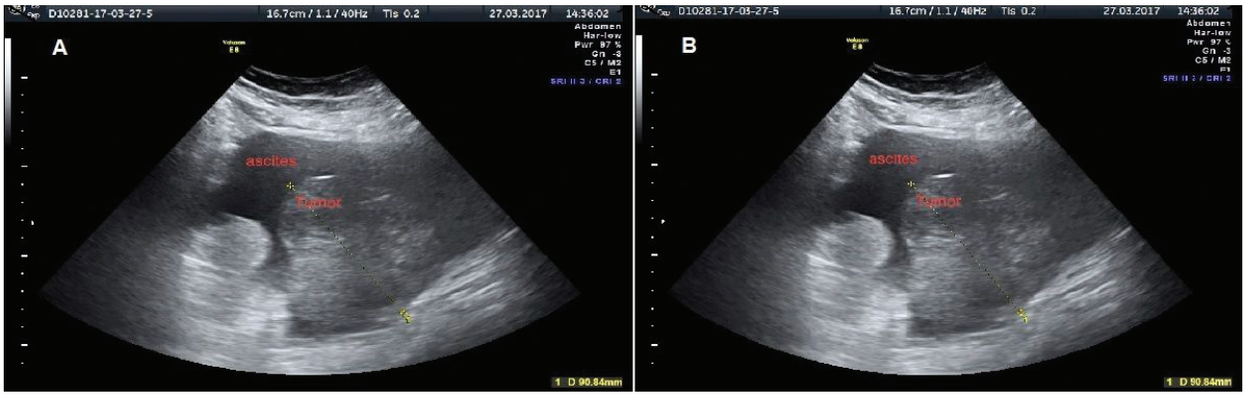

Figure 3. Ultrasound images of ascites. (A) Transabdominal ultrasound image demonstrates ascites and ovarian tumour. (B) Transvaginal ultrasound image demonstrates ascites and intestinal carcinosis.

(**Figure 3A**) [20]. Sometimes, bowel loops do not float freely but may be tethered along the posterior abdominal wall, plastered to organs or surrounded by loculated fluid collections (**Figure 3B**) [21]. When small amounts of ascitic fluid localise in the Morison pouch and the pouch of Douglas, CT scan demonstrated the best sensitivity [21, 22].

3.3. Ascitic fluid analysis

In patients with new-onset ascites of unknown origin, peritoneal fluid analysis may provide some information regarding the origin of the disease. However, it remains difficult to differentiate malignant ascites from other types [23].

On inspection, most ascitic fluids are transparent and tinged yellow. In the case of malignancy, it could also appear pink or red (when at least 10,000 red blood cells/μL are present). Any inflammatory condition can cause an elevated white blood cell count. In case of malignant ascites, lymphocytes usually predominate [24].

Conventional cytological examination shows high specificity, but its sensitivity is low (58–75%) [23]. The cellular components of malignant ascites contain a complex mixture of cell populations, including tumour cells and stromal cells [25]. Immunohistochemistry (ICH) staining and cytological diagnosis by using cell block (CB) sections prepared with the ascites cytological specimen are useful in delineation of the primary origin of the tumour cells. Since

multiple sections can be obtained by the CB method, this technique is particularly valuable when the ICH staining is required for a battery of markers. Typically, primary ovarian epithelial cancers are positive for ER/PR, PAX8, CK7 and negative for CK20 and CDX2. The reverse is true for gastrointestinal cancers. By using a combination of cytological conventional smears and CB methods, the primary site could be detected with 81% accuracy [26, 27].

A number of soluble factors are present in abundance in malignant ascites, but few have been validated for their biomarker potential [28].

4. Treatment

Many factors influence the optimal therapeutic interventions. The aim is palliation in a significant number of patients; only in a selected subgroup is it to improve survival [29].

4.1. Non-pharmacological treatment of ascites

Surgical treatment of malignant ascites involves a variety of different options, each with a certain degree of efficacy but not without risks [30]. Very few studies concern the benefits and harm of differing surgical interventions for intra-peritoneal fluid drainage. Numerous questions, such as how long should the drain stay in place, whether the volume of fluid drained should be replaced intravenously, whether the drain should be clamped to regulate the drainage of fluid and whether any particular vital observations should be regularly recorded, remain partially unanswered [31]. The most common surgical option for ascites drainage is abdominal paracentesis followed, in recent years, by the insertion of permanent tunnelled catheters (PleurX®) and peritoneal-venous shunts.

4.1.1. Paracentesis

Paracentesis (needle drainage of fluid) is an effective and widely used procedure for the management of treatment-resistant, recurrent malignant ascites [32]. It can provide good short-term symptomatic relief in up to 90% of hospital cases, although it may also be offered as a day-case procedure [33].

The procedure involves the placement of a fine tube into the peritoneal cavity to drain ascitic fluid. The procedure can be done all at once but, especially for large volume paracentesis, the catheter can remain in place for several hours and sometimes for days [31]. The volume of drained fluid can vary according to the patient's general conditions, from a few litres up to a maximum of 20 l [30]. Complications of the procedure may include peritonitis, sepsis, visceral injuries, bleeding and fluid leak. Moreover, especially for large volume drainage or repeated procedures, paracentesis may be associated with significantly higher incidence of hypotension and renal impairment [32, 34].

In general, intravenous fluid replacement is not routinely required for paracentesis with less than 5 l removed, but it depends on the patient's clinical condition [32]. However, some reports suggest the use of 5% dextrose infusion during the procedure to avoid severe hypotensive episodes [30, 34]. There is no evidence albumin infusion is of benefit during paracentesis for

malignant ascites, even though many studies focusing on cirrhosis related ascites have demonstrated great benefits of albumin infusion (6–8 g per litre of ascites removed) to maintain intravascular volume [30].

4.1.2. Peritoneal-venous shunting

Common peritoneal-venous shunts drain ascites from the peritoneal cavity into the superior vena cava and have a one-way valve that prevents reflux of blood [32, 34]. They are rarely used due to the high rate of complications such as occlusion, infection, coagulopathy and the widespread dissemination of malignant cells [35]. The only advantage compared to other techniques is related to saving electrolytes and proteins, preserving the body fluid balance [30, 32]. Two shunts are commonly used: the older LeVeen and the most recent Denver shunt, which require different pressures to open the valves [32, 36].

Contraindications of shunt positioning are as follows: congestive heart disease or renal failure due to the significant hemodilution and blood volume overload produced by the shunt, portal hypertension, and severe pleural effusion and clotting disorders [35].

A novel type of technique, automated low-flow ascites pump, drains ascites from the peritoneal cavity to the bladder. This novel device seems effective (even though tested only on liver disease patients) for symptom relief, although data about safety (especially linked to catheter dislodgement and infections) are only preliminary [37].

4.1.3. Catheter drainage

In cases of recurrent or refractory malignant ascites, when frequent paracentesis is required, patients may benefit from an indwelling catheter [32]. This device allows easy and self-drainage, eliminating the need for hospitalisation and frequent paracentesis. The most common permanent catheters are the tunnelled PleurX®, Tenckhoff, Port-a-Cath and cope-type loop catheters [30, 38]. Most authors prefer tunnelled catheters due to greater stability (higher long-term patency rate and success rate) and lower infection rate [30, 39]. Recent trials have suggested that untunnelled catheters have a 21–34% risk of developing peritonitis compared to 4.4% with tunnelled Tenckhoff and 2.5% for tunnelled PleurX® [30].

Catheter placement can be performed with ultrasound guidance or with CT guidance in cases of particular anatomical conditions or widespread carcinomatosis [30]. Antibiotic prophylaxis is recommended for catheter placement [39]. Patients should be instructed to drain the fluid frequently enough to avoid the development of tense ascites, usually once or twice per week. Intravenous fluid replacement and/or albumin supplementation is indicated according to clinical conditions and ascites volume [30, 39].

The safety and cost-effective profiles of tunnelled catheters for the management of recurrent malignant ascites have been demonstrated by several observational studies [38, 40].

4.2. Pharmacological treatment of ascites

If the patient's malignant disease is sensitive to chemotherapy, reduction of ascites production and relief of symptoms may be achieved. However, most patients with ascites have already been treated with several lines of treatment, and their disease has become refractory

to chemotherapy, and carcinomatosis may not be amenable to surgery. For such patients, no pharmacological therapy has been approved except catumaxomab in the EU. The effectiveness of other drugs to treat ascites has been explored in a few studies, with the majority of treatments having been studied in a small series.

4.2.1. Catumaxomab

Catumaxomab (Removab®; Fresenius Biotech) was approved in 2009 by the European Medicine Agency (EMA) for the intraperitoneal treatment of malignant ascites in adults with EpCAM-positive carcinomas, where standard therapy is not available or no longer feasible [41]. Catumoxomab is a trifunctional rat-mouse hybrid monoclonal antibody that is specifically directed against the epithelial cell adhesion molecule (EpCAM) and CD3 antigen (**Figure 4**). EpCAM (CD326) antigen is overexpressed in epithelial ovarian cancer of serous (68%), endometrioid (82%), clear cell (92%) and mucinous (49%) histological subtypes. EpCAM correlates with lower overall survival [42]. Over 80% of ovarian cancer patients have EpCAM over-expressed in tumour cells present in ascites [43]. EpCAM has been reported to initiate cell proliferation by upregulating the oncogene c-myc and to dampen antitumour immunity by blocking antigen presentation in dendritic cells [44, 45]. Mesothelial cells do not express EpCAM on their surface, so catumaxomab applied to the peritoneal cavity specifically targets epithelial tumour cells but not the normal tissue. CD3, as a second antigen, is expressed in mature T-cells as a component of the T-cell receptor. A third functional binding site in the Fc-region of catumaxomab enables the interaction with accessory immune cells (macrophages, dendritic cells and NK cells) via Fcγ receptors. Due to catumaxomab's binding properties, tumour cells and immune effector cells come in close proximity, and complex "crosstalk"

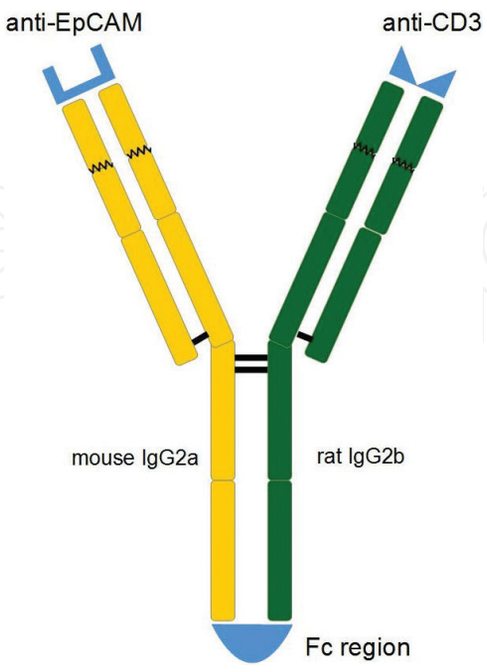

Figure 4. Schematic structure of catumaxomab.

between the T cell and accessory cell can occur, which includes cytokines and co-stimulatory signalling necessary for T-cell activation cascade, resulting in the killing of tumour cells [41].

The clinical efficacy of catumaxomab in the treatment of malignant ascites has been demonstrated in two clinical studies: a phase I/II study (STP-REM-01) and a pivotal phase II/III study (IP-REM-AC-01) [41]. In the first study treatment resulted in a significant reduction of the ascites flow rate from a median of 105 mL/h at baseline to 23 mL/h 1 day after the fourth infusion. Twenty-two of 23 patients did not require paracentesis between the last infusion and the end of the study. Tumour cell-count monitoring revealed a mean reduction up to 99.9% of EpCAM-positive malignant cells in ascites. In a pivotal study, 129 ovarian cancer patients with recurrent symptomatic malignant ascites were randomised to treatment with catumaxomab (as four 6-h *i.p.* infusions on days 0, 3, 7, and 10 at doses 10, 20, 50, and 150 μg, respectively) plus paracentesis or paracentesis alone (the control group). The median time to the next paracentesis was significantly longer for catumaxomab plus paracentesis than paracentesis alone: 77 versus 13 days ($P < 0.0001$) [41].

The safety profile of catumaxomab was established from five completed studies (STP-REM-01, IP-REM-AC-01, IP-REM-PC-01-DE, AGO-Ovar-2.10 and IP-REM-PK-01-EU) [41]. A total of 258 patients were treated with *i.p.* administration of catumaxomab and 207 (80%) patients completed treatment, underlining the good tolerability for the drug. Catumaxomab may cause symptoms related to local and systemic cytokine release: pyrexia, nausea and vomiting. In 48% of patients, abdominal pain was reported, which is considered in part a consequence of the *i.p.* route of administration. All mentioned adverse drug reactions (ADRs) are fully reversible. One hundred and twenty-seven (49%) patients had at least one ADR of grade 3/4, according to the Terminology Criteria for Adverse Events (CTCAE). Abdominal pain, pyrexia and vomiting were the most common symptomatic grade 3 ADRs. Grade 4 ADRs were isolated cases (1%), mostly related to the progression of the underlying malignant disease, such as ileus. In 1% of patients, symptoms of systemic inflammatory response syndrome (SIRS) were observed within 24 h after catumaxomab infusion, such as tachycardia, fever and dyspnea. These reactions resolve under symptomatic treatment. Conditions such as hypovolemia, hypoproteinaemia, hypotension, circulatory decompensation and acute renal impairment must be resolved before each infusion. Since patients with severe hepatic or renal impairment have not been investigated, treatment of these patients should only be considered after a thorough evaluation of benefit/risk. Catumaxomab is potentially immunogenic when administered to humans. In clinical studies, almost all patients (94%) developed human anti-mouse antibodies (HAMAs) or human anti-rat antibodies (HARAs) 1 month after the last infusion; however, patients who developed HAMAs 8 days after the fourth infusion showed a better clinical outcome as measured by puncture-free survival, compared with HAMA-negative patients, suggesting that HAMA development may be a biomarker for catumaxomab response. No hypersensitivity reactions were observed [41].

4.2.2. Other immunological approaches

Evidence to suggest that an immunological approach to the treatment of malignant ascites in OC may be effective and has been observed in small studies of intraperitoneal administration of triamcinolone (long acting corticosteroid), interferons and TNFα [10, 46].

Interferon alpha-2b (IFNα-2b), administered *i.p.* inserted with a 9-French catheter, was evaluated in a study by Sartory et al. Twelve of 41 patients had OC. A complete response (no fluid recurrence) within 30 days of treatment (six courses with an interval of 4 days with six or nine million units depending on a body weight) occurred in 65% of OC patients. The fluid reaccumulated after 11.4 days before and 70.5 days after the treatment. Adverse effects were flu-like symptoms, vomiting and infection with staphylococcus (two patients). If there is no response after the first three courses, the treatment should be stopped [47].

TNFα, installed inside the abdomen of advanced OC patients for 24–48 h (the procedure was repeated on day 8 at a dose of 0.08–0.014 mg/m^2), was evaluated in a study by Kaufmann et al. Production of ascites was supressed or reduced to a minimum for at least 4 weeks in 87% of patients. The treatment was not effective in patients with malignant ascites due to mucinous OC. Patients often suffer from flu-like symptoms, which can be reduced by taking indomethacin or paracetamol before the infusion [47].

4.2.3. Bevacizumab

Bevacizumab (Avastin®, Genentech, Inc., a member of the Roche Group) was approved as an *i.v.* infusion in 2014 by the Food and Drug Administration (FDA) and by EMA in combination with paclitaxel, topotecan, or pegylated liposomal doxorubicin, for the treatment of adult patients with recurrent epithelial ovarian cancer that is resistant to platinum-containing chemotherapy. Bevacizumab is a humanised monoclonal antibody directed against vascular endothelial growth factor (VEGF). Bevacizumab binds to VEGF and thereby prevents the binding of VEGF to its receptors, Flt-1 (VEGFR-1) and KDR (VEGFR-2), on the surface of endothelial cells. Neutralising the biological activity of VEGF regresses the vascularisation of tumours and inhibits the formation of a new tumour vasculature and thereby inhibits tumour growth. Interestingly, the delay of tumour growth induced by anti-VEGF antibody was mainly attributed to the blockage of ascites development and vascular permeability and to a lesser degree to the inhibition of VEGF-induced angiogenesis [7].

Approval of bevacizumab in the USA and EU was based on results of a phase III AURELIA study that involved 361 women with recurrent, platinum-resistant OC, who received either chemotherapy or bevacizumab in combination with chemotherapy. In the subgroup of patients with ascites at baseline, the absence of paracentesis after the first bevacizumab dose suggests that adding bevacizumab to chemotherapy improved the control of ascites [48]. Bevacizumab has been associated with serious (but rare) side effects, and the use of bevacizumab remains significantly more expensive than cytotoxic therapies. The identification of predictive clinical and biological factors that could be utilised to select patients with a greater likelihood of clinical benefit therefore remains a high priority. Using data from Phase III trial GOG218 (Gynaecologic Oncology Group), ascites as a prognostic factor and as a predictor of efficacy for bevacizumab in advanced OC was investigated. In multivariate survival analysis, ascites was prognostic of poor overall survival (OS) but not progression-free survival (PFS). In predictive analysis, patients without ascites treated with bevacizumab had no significant improvement in either PFS or OS, whereas patients with ascites treated with bevacizumab had significantly improved PFS ($p < 0.001$) and OS ($p = 0.014$). These findings support the

plausible biologic rational that patients with malignant ascites have cancer with a phenotype representative of the initiation phase of angiogenesis and are therefore more likely to respond to anti-VEGF therapy. If these findings could be validated through a similar analysis of data from one or more independent randomised phase III trials, the clinical determination of malignant ascites could be a simple and cost-effective way of selecting patients with the greatest probability of benefit from bevacizumab. However, it is possible that volume of ascites could be a more robust predictor of the degree of benefit from VEGF-targeted therapy [49].

Intraperitoneal administration of bevacizumab has also been explored, although only very few OC patients with malignant ascites have received this route of administration. In all patients, ascites resolved after a single *i.p.* dose (5 mg/kg) without re-accumulation or repeat paracentesis over a median observation period >2 months. Moreover, no grade 2–5 adverse events were observed [50]. To evaluate the great potential that preclinical data and clinical case reports have suggested for *i.p.* administration of bevacizumab, clinical trials should be undertaken regarding the safety of treatment, specifically for the palliation of ascites. Bevacizumab may have the potential advantage so that it could be used in patients with reduced performance [47].

4.2.4. Aflibercept (VEGF-TRAP)

Aflibercept (Zaltrap®, Sanofi-Aventis group) was approved for the treatment of adult metastatic colorectal cancer that is resistant or has progressed after an oxalipatin-containing regimen. Aflibercept, also known as VEGF trap (it binds to VEGF trapping it and inhibiting it) in the scientific literature, is a fusion protein, comprising a portion of human VEGF receptor Fit-1 (VEGFR-1) + KDR (VEGFR-2) extracellular domains fused to the Fc-portion of human IgG. Aflibercept binds to VEGF-A, VEGF-B and placental growth factor (PIGF). By acting as a ligand trap, aflibercept prevents binding of endogenous ligands to their cognate receptors and thereby blocks receptor-mediated signalling. VEGF-A acts via VEGFR-1 and VEGFR-2 present on the surface of endothelial cells. PIGF and VEGF-B bind only to VEGFR-1, which is also present on the surface of leucocytes. Excessive activation of receptors by VEGF-A can result in pathological neovascularisation and excessive vascular permeability. PIGF is also linked to pathological neovascularisation and recruitment of inflammatory cells into tumours [51]. In addition to the approved indication, aflibercept has demonstrated the ability to reduce the formation of ascites in patients with advanced epithelial OC [10, 52, 53]. In pre-clinical xenograft models, aflibercept inhibited tumour growth, angiogenesis, reduced blood vessel density and inhibited metastases [10]. The safety and efficacy of aflibercept, administrated *i.v.* at a dosage of 4 mg/kg every 2 weeks, was tested in two-phase II clinical trials in chemoresistant advanced OC patients with recurrent symptomatic ascites [52, 53]. In a randomised, double blind, placebo-controlled, parallel trial, 29 patients were treated. The mean time to paracentesis was significantly ($p = 0.0019$) longer in the aflibercept arm (55.1 days) than in the placebo arm (23.3 days). Two patients receiving aflibercept did not need paracentesis for a period of 6 months. The most common grade 3 or 4 adverse effects were dyspnea, fatigue or asthenia, and dehydration. The frequency of fatal gastrointestinal perforation was higher with aflibercept (three-bowel perforation) than with the placebo. In spite of the effectiveness of aflibercept in the reduction of malignantascites, the authors acknowledge that the limitation of this

treatment is the risk of significant morbidity associated with bowel perforation in patients with very advanced OC. Thus, the advantages of aflibercept over bevacizumab are unclear [53].

4.2.5. Matrix metalloproteinase inhibitors (MMPIs)

MMPs, mainly MMP9, play a role in the release of biologically active VEGF and, consequently, play a role in the formation of ascites. Batistamat, a potent reversible inhibitor of a broad spectrum of MMP, has been developed and has been shown to resolve ascites when given *i.p.* to mice ascites secondary to an ovarian carcinoma xenograft; treatment was accompanied by a 6.5-fold increase in survival [54]. Sixteen patients with OC (out of 23 patients) were included in a Phase I study of *i.p.* administration of batistamat after drainage of ascites. Patients acquired a predicted survival of 1 month or more. Of the 23 patients in the study, 16 did not require redrainage within 28 days of the initial treatment. Five of the 23 patients neither reaccumulated ascites nor died up to 112 days after dosing. Seven patients died without reaccumulating ascites. Adverse effects considered at least possibly related to the treatment occurred in 16 patients, the most common of which were fatigue, fever, vomiting and abdominal pain [46]. MMP inhibitors may warrant further study.

4.2.6. Intraperitoneal chemotherapy

Intraperitoneal chemotherapy is an effective way to palliate malignant ascites. By destroying the surface cancer, it induces a progressive fibrotic process, which will prevent the formation of fluid. If the sclerotic process is not complete, it may produce fluid loculation, which will interfere with uniform drug distribution, may cause obstructions and makes subsequent paracentesis difficult and risky [29]. Intraperitoneal therapy with cisplatin has been evaluated for the first-line treatment of optimal debulked OC patients with FIGO stage III. Despite a 16-month survival advantage, the catheter-related complications rate was 34%, and only 42% of women in the trial completed six cycles of chemotherapy [46].

The procedure called intraperitoneal hyperthermic chemotherapy (HIPEC) is an attempt to increase the cytotoxicity of selected cytotoxic drugs by a hyperthermic medium (40.5–43°C), thereby improving tissue penetration and reducing drug resistance. The primary objective is an increase of PFS and OS, not the control of ascites itself [47]. Finally, aggressive cytoreductive surgery combined with laparoscopic installation of HIPEC is reserved for selected patients with malignant ascites. In well-selected patients, results are encouraging, and this procedure not only controls ascites, but prolongation of OS is possible [29].

Laparoscopic installation of HIPEC has been recently reported as an option to treat resistant malignant ascites not suitable for surgery. The biggest series published, which also included patients with OC, was by Valle et al. [55], who achieved complete remission of ascites in 94% of 52 patients after 1 month of follow-up. There were no complications of the procedure, demonstrating the feasibility and safety of this technique [55].

4.2.7. Diuretics

Some patients with liver metastasis and malignant ascites have raised plasma renin concentrations, and these patients showed a good response to aldosterone competitive antagonist

spironolactone, which decreases reabsorption of water and sodium in the renal collecting duct. Packros et al. [56] found that 13 of 15 patients treated with increasing doses of spironolactone had a good response, with eight remaining free of ascites until death. Renin levels were raised in all of these patients [56].

5. Role of ascites in translational science

Ascites is often therapeutically removed from patients and is therefore an available source of valuable tumour material. Representing the local tumour environment, ascites is composed of cellular and acellular components. In addition to tumour cells present, either as single cells or as spheroids, the cellular component of ascites is composed of stromal cells, including fibroblasts, mesothelial cells, endothelial cells, adipocytes and inflammatory cells. Cells in ascites communicate with each other through acellular components, including cytokines, proteins, metabolites and exosomes. All these components work in coordination to create a tumour-friendly microenvironment. Better knowledge of the tumour microenvironment represented by ascites would thus certainly help to overcome the limitations of current anticancer treatments [23].

Targeting ascites components that cause immunosuppression of T-cells is an interesting future therapeutic option. T-cells present in ovarian tumour ascites do not respond properly to stimulation via the T-cell receptor. Since these T-cells were assayed in the absence of ascites, they gained their normal function, but when ascites was added to T-cells, this effect was rapidly reversed. This might explain why human tumours grow despite the presence of T-cells and other cells of immunological response [10].

In the study by Latifi et al. [57], it was demonstrated that cells in malignant ascites belong to two types of tumour cells, adherent cells (expressed mesenchymal features) and non-adherent cells with an epithelial phenotype, as expressed by EpCAM and cytokeratin 7. Patients with chemoresistant tumours had more tumorogenic, non-adherent cells in the ascites than non-tumorogenic adherent cells. Non-adherent cells featured increased mRNA expression of cancer stem cell-associated genes [10]. Since catumaxomab selectively kills epithelial tumour cells belonging to the non-adherent cell type, this might explain why it is beneficial for patients with OC.

Ascites is highly attractive as a source for biomarker discovery study. The concentrations of cancer-associated soluble factors are usually much higher in ascites than in serum [47, 58]. Moreover, investigation of the relationship between biomarker concentrations in ascites and serum in OC patients may help elucidate whether concentration changes in the local environment can be detected with a blood test [58].

6. Conclusions

The development of malignant ascites is probably dependent on a combination of factors, which disrupts the normal regulation of intraperitoneal fluid flow and the maintenance of a steady state in the peritoneal cavity. Each factor plays a greater or lesser role in each individual patient, so the results of available treatment alternatives are inconsistent. In advanced OC,

palliation of symptomatic patients is the foremost goal, and elimination of fluid accumulation in a patient with these symptoms will certainly improve the patient's quality of life and may even prolong survival. However, effective palliation of malignant ascites remains a difficult management issue. Present treatments have been developed, particularly for malignant ascites, with the primary aim of prolonging the time until a need for subsequent paracentesis. Further clinical trials are therefore necessary in order to investigate the influence on ascites-triggered intervention not only for symptomatic relief but also for the prolongation of both PFS and OS. For the use of targeted therapeutics in malignant ascites (catumaxomab, bevacizumab, aflibercept), it is mandatory to select patients carefully and to identify their risk factors so that the incidence of adverse effects can be minimised. The identification of predictive clinical and biological factors that could be utilised to select patients with a greater likelihood of clinical benefit remains a high priority. With advances in our understanding of malignant ascites pathophysiology, more effective treatment strategies for malignant ascites and ovarian cancer will emerge in the future.

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Author details

Katarina Cerne[1]* and Borut Kobal[2,3]

*Address all correspondence to: katarina.cerne@mf.uni-lj.si

1 Department of Pharmacology and Experimental Toxicology, Faculty of Medicine, University Ljubljana, Ljubljana, Slovenia

2 Department of Gynaecology, Division of Gynaecology and Obstetrics, University Medical Centre Ljubljana, Ljubljana, Slovenia

3 Department of Gynaecology and Obstetrics, Faculty of Medicine, University Ljubljana, Ljubljana, Slovenia

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