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REVIEW ARTICLE

Cytopathologic interpretation of ascites due to malignancy

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Summary

The diagnosis of metastatic cancer in peritoneal fluid is of great importance for the patient and the attending physician. A cytopathologist's responsibility is twofold: (1) to accurately identify malignant cells; (2) to interpret tumor type and if possible the site of its origin even in the absence of complete clinical history of other clues. The difficulty in the diagnosis of metastatic neoplasms in peritoneal fluid is due to 2 factors: (1) abnormal mesothelial cells or macrophages may simulate cancer cells, or may conceal tumor cells; and (2) peritoneal fluid constitutes a natural and hitherto inadequately explored medium of cell culture, in which neoplastic cells may proliferate free of the boundaries imposed upon them by the framework of organs and tissues.

Immunocytochemistry (ICC) and molecular techniques are essential to establish an accurate diagnosis. From a great many points of view malignant peritoneal fluid is suitable for continuous study of cancer cells, thus providing knowledge about biologic aspects of human solid tumors.

Key words: immunocytochemistry, malignant ascites, molecular biology, peritoneal cytology

Introduction

The word ascites is of Greek origin (askos), meaning bag or sac, and is defined as the pathological accumulation of excessive fluid within the peritoneal cavity. The most common cancers associated with malignant ascites are adenocarcinomas.

It is usually a manifestation of end-stage events in a variety of cancers and is associated with significant morbidity.

Peritoneal cytology has been used in the evaluation of various malignancies since the early 1930s and modern cytology using ICC is considered to be helpful in achieving correct diagnosis.

This review summarizes the current knowledge in this field and the contribution of modern cytology in the accurate interpretation of the findings of malignant ascites.

Etiology, pathophysiology and clinical manifestations

Malignant ascites, the subject of this review, signifies disease progression and is associated with worse prognosis regardless of the tumor's origin. Malignant ascites accounts for about 10% of all cases of ascites and is usually caused by ovarian, endometrial, breast, gastric, colorectal, pancreatic, hepatobiliary carcinomas and malignant mesotheliomas (MM).

Sometimes ascites is the sole manifestation of internal malignancies [1-4].

Lymphatic obstruction seems to be the major pathophysiologic mechanism behind the formation of ascites. Recent evidence suggests that immunomodulators, vascular permeability factors and metalloproteinases contribute significantly to the process. The most acceptable theory for ascites formation is peripheral arterial vasodilatation, leading to underfilling of circulatory volume.

The usual clinical presentation is a protuberant abdomen with discomfort, difficulty in breathing, and pain. It is known that in about 50% of patients with malignant ascites, this condition represents the first manifestation of their cancer [5,6].

The onset and progression of malignant ascites is associated with deterioration in quality of life (QoL).

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According to the International Ascites Club, severity is classified as grade I (mild; not clinically evident, diagnosed on ultrasound), grade II (moderate, proportionate sensible abdominal distension) and grade III (severe, noticeable tense distension of the abdomen) [7-9].

Based on associated complications like spontaneous bacterial pneumonitis (SBP) or hepatorenal syndrome (HRS), and according to the therapeutic response, ascites can also be classified as uncomplicated, complicated, and refractory.

Pitfalls in the cytologic evaluation of ascites

The diagnosis of malignant ascites is cytologic but with difficulties because isolated malignant cells often go undetected among mesothelial cells and macrophage populations. In addition, mesothelial cells react to a wide variety of stimuli and injuries that break their continuity by proliferation and cellular changes, as in cirrhosis, including marked nuclear and cytoplasmic alterations that can mimic the morphology of malignant cells [10-14].

Differential diagnosis should include reactive mesothelial cells, but endosalpingiosis and endometriosis frequently contribute to diagnostic difficulties. Reactive mesothelial cells usually present as clusters of epithelioid cells with occasional cell ball or papillary cluster formation. Occasionally the cells may be vacuolated or contain prominent nucleoli. The presence of cellular "windows" may help identify the cells as mesothelial [15]. Cells from the fallopian tubes in pelvic washings may also lead to false positive diagnosis.

Endosalpingiosis could be nearly impossible to distinguish from well differentiated serous neoplasms such as serous borderline tumor and low grade serous carcinoma.

Cases of endosalpingiosis display organized, tight clusters with occasional nonbranching papillary formation. It is important to remember that psammoma bodies might be present in cases of endosalpingiosis [16-19]. The distinction of endosalpingiosis from serous papillary tumors is based on the presence of large papillary clusters with architectural disorientation in the latter. These findings of nuclear molding and nucleoli in papillary clusters also suggest a neoplastic process.

Endometriosis is another potential pitfall. It is characterized by the presence of round to oval cells arranged in 3-dimensional clusters, tubular structures and sheets. The nuclei are round or bean-shaped with fine chromatin and rare nucleoli. The cytoplasm is scant and vacuolated. The most sensitive finding in endometriosis is the presence of hemosiderin-laden macrophages.

The report format used in peritoneal washings from patients with serous borderline tumors can be problematic.

If the patient has a frozen section diagnosis of a serous borderline tumor and the peritoneal fluid shows the presence of atypical cells, the pathologist may elect to release the report describing the presence of neoplastic cells along with the differential diagnosis, which includes serous borderline tumor and carcinoma. Alternatively, the pathologist could wait for evaluation of the surgical pathology specimen before releasing the final diagnosis.

The cytologic investigation of ascitic fluid in patients with liver insufficiency is often of crucial clinical diagnostic importance. The clinical differential diagnosis usually comprises cirrhosis of the liver and various forms of primary or metastatic cancer. In cases of active cirrhosis with liver necrosis and jaundice, very atypical mesothelial cells in papillary or rosette-like arrangement, accompanied by nuclear hyperchromasia, nuclear enlargement and strikingly abnormal single cells may be noted.

Malignant ascites and cytology

Peritoneal washing cytology has been used in the evaluation of malignancies since the early 1950s.

Metastasis from ovarian, endometrial and breast adenocarcinoma is the most common etiology for the presence of malignant cells in peritoneal effusions in female patients, whereas cancers of gastrointestinal tract and MM account for a relatively large number of cases in both sexes. In two-thirds of ovarian carcinoma and malignant MM tumor cells have disseminated to the peritoneal cavity at diagnosis [20,21].

In contrast to the early appearance of peritoneal effusions in ovarian cancer and MM, the mean interval between primary tumor diagnosis and the appearance of malignant ascites was 41.5 months in one series of breast cancer patients [22]. Irrespective of their time of appearance, the finding of malignant cells in serous effusions is associated with significant therapeutic and prognostic implications, because it signifies the spread of disease beyond the organ of origin, hence, tumor progression.

As referred above, the presence of atypical mesothelial cells in malignant effusions is a big diagnostic problem for the correct cytologic interpretation of patients with malignant ascites. These reactive changes of mesothelial cells may be more pronounced after radiation or chemotherapy, common adjuncts to surgery in the treatment of various malignancies [1,2].

In view of the clinical implications related to the presence of cancer cells in effusions, accurate diagnosis of this condition is of paramount significance. Therefore

it has been in our practice to supplement morphological examination of cytological specimens with ancillary studies.

Ancillary studies

Most ancillary studies in our laboratory are based on ICC studies although there is a limited number of studies using flow cytometry and molecular studies. ICC studies can be performed in cytospins, Thin-Prep preparations, and cell blocks [23]. There is some controversy over what kind of preparation works best. It has been suggested that cell blocks provided the best morphologic interpretation and ICC study. However, another report obtained superior results with smear preparations [24,25].

At Memorial Sloan-Kettering Cancer Center (New York), Thin-Prep slides are used for ICC studies in most fluid specimens requiring it. Several panels of antibodies have been proposed in the literature to distinguish cells of mesothelial origin from adenocarcinoma. The antibodies include markers of mesothelial and epithelial origin. Mesothelial markers previously evaluated include D2-40, calretinin, mesothelin, cytokeratin 5/6, WT-1, and HBME-1.

D2-40, a monoclonal antibody directed against the oncofetal antigen M2A present on cell membranes, has been described as a useful marker of mesothelial cells. It has been shown to have a good sensitivity and specificity for distinguishing epithelioid MM and reactive mesothelial cells from adenocarcinoma [26-32].

Calretinin, a calcium-binding protein widely expressed throughout the central and peripheral nervous systems, is also expressed in mesothelial cells in the cytoplasm and frequently in the nucleus. In our laboratory is the best available marker to identify mesothelial cells. Calretinin expression has also been demonstrated in a small number of adenocarcinomas [33-47].

WT-1 is a DNA-binding protein predominantly located in the nucleus that plays a critical role in the development of the genitourinary tract. In adult tissues, it is expressed by mesangial cells of the kidney, Sertoli cells of the testis, ovarian stromal cells and surface epithelium, mesothelial cells, and some other stromal cells in the female genital tract. WT-1 is also expressed in MM and in tumors derived from the ovarian surface epithelium. Although some studies have confirmed the specificity of WT-1 for MM, cytokeratin 5 is expressed in normal mesothelium, squamous, transitional epithelia and myoepithelial cells but unfortunately some breast carcinomas express cytokeratin 5 [48].

Mesothelin is a surface protein that may be involved in cell-cell adhesion. Its expression is seen in mesothelial cells, surface ovarian epithelial cells and pancreatic ductal carcinoma [49,50].

HMBE-1 is a mouse monoclonal antibody prepared from a human MM but its specificity is not very high for mesothelial cells [51-55].

In our laboratory we use more often calretinin, WT-1, D2-40 and CK5/6. These markers are useful to characterize mesothelial cells-reactive or neoplastic-when the cells are abundant in a specimen, but when the cells are scare, very often these markers are not expressed. For this reason we prefer to use a combination of mesothelial and epithelial markers, which are more sensitive to identify epithelial cells.

The panel of epithelial markers for the detection of epithelial cells include carcinoembryonic antigen (CEA), CA125, MOC31, BerEp4, CA19-9, and B72.3. CEA was one of the first antibodies used to distinguish epithelial from mesothelial cells. Mesothelial cells are generally negative for CEA staining, as are most carcinomas derived from gynecologic sites [56-60]. In our laboratory we use CEA, and we believe that is a useful marker for carcinomas of the gastrointestinal tract.

Several investigators have also reported that CEA stains macrophages and other inflammatory cells owing to nonspecific cross reactivity and that it has been associated with a 5-15% false positive rate [61,62].

In our opinion, CEA sometimes stains mesothelial cells in about 5% of our cases.

CA125 is well established as a tumor marker for ovarian carcinoma. We use this marker very often in combination with WT-1, especially for serous ovarian carcinoma, but it can also be expressed in pancreatic and lung carcinomas [63].

The MOC-31 antibody recognizes an epithelial-associated transmembrane glycoprotein of unknown function in the GLS-1 small cell lung carcinoma cell line and in epithelial tumors. The BerEp4 antibody is generated from mice immunized with cells from the McF-7 breast carcinoma cell line and 2 noncovalently bound glycopeptides. Most studies [64,65] have documented that MOC-31 and BerEp4 are highly effective in distinguishing adenocarcinoma from reactive mesothelial cells, although few authors have reported possible expression of these markers in mesothelial cells. In our laboratory we use daily BerEp4 antibody and we believe that is the most sensitive epithelial marker in distinguishing epithelial cells from reactive mesothelial cells.

CA19-9 is usually expressed in ovarian and gastrointestinal adenocarcinomas and in our opinion it is more sensitive in cancers of the gastrointestinal tract, and is expressed in 89% of serous adenocarcinomas. B72.3 monoclonal antibody recognizes the glycoprotein TAG-72 expressed in 69% of adenocarcinomas.

Molecular diagnosis

The detection of cancer cells in peritoneal effusions is a straightforward issue when a large number of overtly atypical cells are present, but difficulty may arise when these cells are few and less conspicuous. A variety of molecular methods, therefore, have been applied in order to improve the diagnostic sensitivity and specificity in this setting. Because ICC was not performed in many of these studies, it is difficult to establish the superiority of these methods [66]. In our laboratory we use molecular methods such as *in situ* hybridization only for research studies in combination with ICC.

Molecular methods

The value of DNA analysis, using flow cytometry or image analysis, in the diagnosis of malignant cells in effusions (both epithelial and mesothelial) has been evaluated in several studies, generally with sensitivity and specificity that were not superior to current ICC panels [67-72]. Therefore, this method is in routine use in only a few laboratories worldwide.

The fluorescent *in situ* hybridization (FISH) and enzyme-linked immunosorbent assay (ELISA) have been used in breast, pancreatic and lung adenocarcinoma [73,74].

Recent studies using reverse transcription-polymerase chain reaction (RT-PCR) suggested a role for this method in the detection of cancer cells in effusions [75].

Two additional methods that may be of interest in the diagnostic setting are studies of telomerase expression and comparative genomic hybridization (CGH) [76,77]. Studies of peritoneal washings and effusions using PCR or the telomeric repeat amplification protocol (TRAP) assay to detect telomerase activity have generally shown high sensitivity and specificity [78-83].

Conclusions

Malignant peritoneal effusions may represent major interpretive challenges to the cytopathologists and their status has important clinical implications. ICC and molecular methods are useful to identify cancer cells in malignant ascites. We hope that the continuous work over this topic will finally provide a significant body of data regarding cancer-cell diagnosis and biology in effusions, data that will complement current and future knowledge from studies of solid tumors.

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