Chapter 8
Body Cavity Fluids

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8.1 Introduction

Body cavity fluid specimens come from the mesothelium-lined pleural, peritoneal, and pericardial cavities and have much in common with one another. These specimens can pose unique challenges for the cytologist. Although the specimens are relatively simple to obtain, diagnosis is often rendered difficult by the very abundance of material provided for analysis. Isolating and identifying small numbers of diagnostically critical cells in large volumes of fluid can prove difficult. The frequent presence of abundant inflammation or blood in these fluids compounds the problem. Furthermore, the properties of the cells that normally line serous cavities, the mesothelium, cause many interpretive dilemmas that extend beyond morphology into the realm of immunocytochemistry and other ancillary techniques. Most malignancies in these specimens are adenocarcinomas, but mesothelioma, melanoma, and lymphoid malignancies also occur. When malignancy can be confidently identified, determining the precise source of origin can be very difficult because of the tendency for all epithelioid processes to take on similar appearances when the cells are suspended in fluid. Immunocytochemistry can be extremely useful when determining the origin of the malignancy, but in many cases clinical history and radiological findings render ancillary testing unnecessary. The high clinical stakes involved in diagnoses of malignancy in these fluids also adds to the challenge. Positive body cavity fluids often signal end-stage disease progression and preclude some forms of treatment with curative intent. This fact makes cytologists particularly cautious in their handling of difficult cases to avoid false positive or false negative interpretations that may have a harmful impact on the patient.

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Although some cases remain challenging in everyday practice, common sources of error are well-known and can usually be avoided. Most cytologists see enough body cavity fluid specimens to become comfortable with the range of normal findings, wide though it is, and provide confident diagnoses in the majority of cases. This chapter will outline the methods used by cytologists to arrive at their diagnoses and explain the potential pitfalls and dilemmas that may create problems for pathologists and, consequently, other care-givers.

8.2 Preparation

8.2.1 Gross Appearance

Prior to any processing, the first task with any pathology specimen, including fluids, is gross examination. The volume, color, and transparency of the fluid are routinely noted and generally appear on final cytology reports. If the fluid is viscous or contains flecks or chunks this information should also be recorded. Odor may also contain some clues to the underlying process. The observations of the laboratory generally match those of the physicians responsible for collecting the specimen. Significant discrepancies between the reported description and the appearance at the time of removal from the patient should raise concern about a specimen misidentification. In general, fluids are very well preserved by refrigeration alone. Sterile collection containers, anticoagulant, and fixatives are not necessary. Indeed, fixatives may interfere with processing, staining, and interpretation of the findings.

The most common "abnormal" finding in body cavity fluid specimens is the presence of blood. Since the traumatic introduction of blood at the time of the extraction procedure is commonplace, a small amount of blood is probably best considered a normal finding. Large amounts of blood, however, may accompany malignant processes, though gross blood by itself has low sensitivity and specificity. The presence of macroscopically detectable pieces of tissue or debris is another gross finding that has been associated with malignancy. Cholesterol crystals may also give a similar gross appearance. A shimmering effect noted upon agitation of the sample may serve as a clue to the presence of crystals.

High viscosity may be seen in cases of pseudomyxoma peritonei. Indeed, the viscosity may be so high as to preclude effective sampling, or effective processing in the cytology laboratory. Inadvertent sampling from a mucinous tumor, such as an ovarian cystic neoplasm, rather than the peritoneum itself may give similar findings. Increased viscosity, though to a lesser degree, may be manifest in some mesothelioma cases in which large amounts of hyaluronic acid are produced by the tumor. Such effusions are often described as having the consistency of honey.

As a general rule, grossly purulent effusions are infectious in origin until proven otherwise. Malignancy may however occur in this setting. Brown-tinged fluid most often results from the presence of hemosiderin-laden macrophages and is

indicative of prior bleeding into the space with partial digestion of the heme. Rarely, melanoma within body cavity fluids may produce enough pigment to be grossly visible. Chylous effusions often show a layering effect, with a creamy lipid layer forming at the surface if given time to settle.

8.2.2 Preparatory Techniques

Body cavity fluid specimens are very well preserved by refrigeration at 4 °C, and therefore they can sit overnight or over the weekend with essentially no loss of quality for diagnostic purposes [1]. The fundamental problem in processing body cavity fluids is the separation of the cells of interest from the large volumes of fluid in which they are dispersed. Most laboratories concentrate the cells by use of a machine that spins the sample. One common method uses a cytocentrifuge designed to simultaneously concentrate the cells and distribute them onto a slide. Alternatively, wire-loop cell collection from a cell pellet, followed by smearing on a slide, may be employed. For markedly hypocellular specimens the fluid may be spun through a filter that is stained and directly analyzed. Over the last 15 years the use of liquid-based thin-layer Papanicolaou-stained preparations, for body cavity fluids has gone from experimental to mainstream. Thin-layer preparations yield comparable or higher sensitivity than conventional processing while being simultaneously easier to examine [2–5]. Large-scale interlaboratory comparisons of diagnostic performance on thin-layer body fluid specimens indicate that they perform at least as well as other preparation types [6].

Body cavity fluids typically make good cell blocks, especially in cases where malignancy is suspected, because the specimens are highly cellular with abundant residual material. Cell blocks are useful either to look for additional examples of rare atypical cells or to facilitate immunocytochemical testing. Due to the high cellularity, flow cytometry can often be performed easily on these specimens as well.

8.3 Normal Body Cavity Fluids

8.3.1 Anatomy and Physiology

There are three major serous cavities: pleural, pericardial, and peritoneal. An outpouching of the peritoneal cavity also forms the tunica vaginalis in males, but this structure is of minimal importance in cytology. All of these cavities are lined by mesothelial cells whose primary function is to secrete lubricating serous fluids that allow the organs within each cavity to glide smoothly over the cavity walls or, in the case of the peritoneum, each other. Under normal circumstances these cavities contain only minimal fluid and few cells.

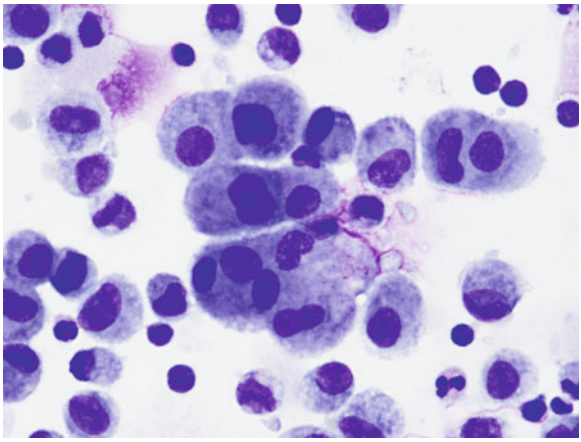

Fig. 8.1 Benign mesothelial cells. The nuclei are centrally located and surrounded by vacuolated cytoplasm. Note the presence of windows between cells and skirts on the outer edges of the cells. There is also binucleation, nuclear enlargement, and nuclear contour irregularity present in an inflammatory background, showing some of the reactive changes often seen in these specimens. (DiffQuik stain, 600× magnification.)

Clinically, increased volumes of fluids are much more readily apparent than hypercellularity in disease states. The important distinction between transudative effusions and exudative effusions can be made on the basis of chemical tests. Indeed, this testing is often the primary driver of fluid collection for analysis. Many times fluid is also removed for symptom relief. In such situations cytology is often just an "add on" test ordered for the sake of completeness. Transudative effusions are usually hypocellular. Exudative effusions commonly contain inflammatory cells but malignancy is only occasionally found in the setting of low clinical suspicion. Generally speaking, these scenarios account for most body cavity fluid specimens in cytology laboratories and lead to few diagnostic problems.

8.3.2 Benign Cells

8.3.2.1 Mesothelial Cells

The most important normal cells present in body cavity fluids are, of course, mesothelial cells (Fig. 8.1). Mesothelium is a specialized type of epithelium derived from mesoderm that exists only along the linings of the serous cavities. It is similar in many ways to other epithelial types, not only in terms of appearance under the microscope but also at the ultrastructural and molecular levels. It is important to keep in mind the fundamental kinship of these cells to understand why they so frequently cause problems for pathologists.

Mesothelial cells differ from typical epithelial cells in that they have numerous very long microvilli. This feature creates diagnostically helpful findings, in the form of gaps between cells ("windows") or attenuated cytoplasm at the edges of cells ("skirts") that can be used by cytologists to confidently identify mesothelial cells in many instances.

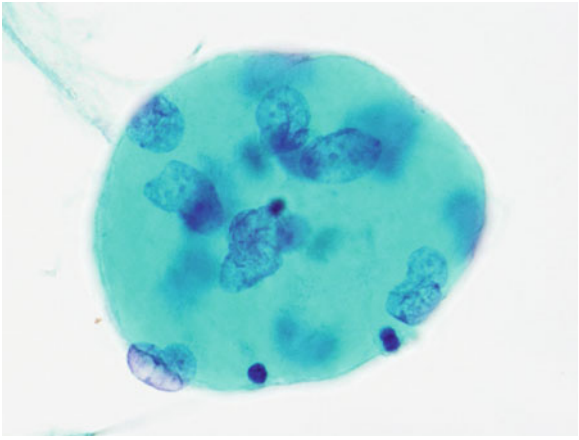

Fig. 8.2 Collagen ball. The cells surround a homogeneous round ball of collagen, with the nuclei seen only on the edge of the structure when examined on high power. The surrounding mesothelial cells are bland with small nuclei that lack atypia or prominent nucleoli. Comparison with Fig. 8.5 shows why these structures might cause confusion with adenocarcinoma at low power. (Papanicolaou stain, 600× magnification.)

Mesothelial cells have additional cellular features that are useful in identifying them. The nuclei are generally centrally placed within the cell, round, and contain noticeable nucleoli. The cytoplasm takes on the appearance of "ground glass" or may be foamy due to many small vacuoles. Individual cell contours tend to be round in tapped fluids. Cells in washings often have a more scale-like squamoid appearance.

At low power, mesothelial cells form up into a number of different patterns. Spontaneously shed cells in effusion specimens are usually present as a mixture of rounded cell clusters of various sizes, chains, and individual cells. This spectrum is reassuring, especially if the nuclei are uniform and bland. The groups are said to resemble florets, due to the tendency for cells on the edge to bulge from the surface like flowers in a bouquet.

In washings, honeycomb sheets are often identified in addition to or instead of spheroid clusters. This reflects the physics of cells in fluids. Cells of any type will tend to round up over time because this is the least energetic conformation due to the minimization of the surface area exposed to charged water molecules. Cell groups in washing specimens often do not have sufficient time to undergo this transformation.

In some instances mesothelial cells may be found in association with a central core of homogenous material, forming a three-dimensional structure known as a collagen ball (Fig. 8.2). These represent fragments of mesothelium still associated with underlying stroma, perhaps due to forceful removal by washing or the detachment of a small papillary tuft with an intact core. Their primary importance lies in the fact that they may mimic malignancy by creating large structures that stand out from the background. The presence of small bland cells around the outside of the ball, in combination with an acellular center, usually dispels concern.

Mesothelial "atypia" is an extremely common problem. Generally the report is worded in such a way as to avoid the loaded term "atypia", often with the word

"reactive" used in its place, but the changes are truly atypical in the sense that they may raise concern for malignancy. Whole cell and nuclear enlargement, multinucleation, vacuolization, frequent mitoses, and cell-in-cell configurations are not uncommon in benign mesothelial processes, especially in response to inflammation. These often appear against a backdrop of high cellularity that may compound concern. Such findings may induce suspicion of a more serious process, especially if considering the criteria used in other body sites where mesothelium is not present. Mitoses, in particular, can be seen more frequently in benign mesothelium than in many malignancies. The finding of one cell "hugging" another, in other words completely wrapping its cytoplasm around its neighbor, is also very worrisome in most contexts but has little import in these specimens. Marked vacuolization, creating a single "signet ring"-like central vacuole with a peripherally placed nucleus, may also raise concern for a single-cell adenocarcinoma pattern. This dilemma can often be resolved by comparing the other cytologic features of the "signet ring" cell with adjacent mesothelium. Furthermore, the nucleus is rarely as atypical, or as deeply or irregularly indented by the vacuole, as what is seen in adenocarcinoma.

When analyzing body cavity fluid specimens, the wide range of "normality" in mesothelial cells must be kept in mind. Even in surgical pathology specimens markedly reactive mesothelium cannot be reliably separated from malignancy in the absence of frank invasion [7]. Only with experience can cytologists develop some sense for the full range of "reactive" mesothelial changes that may accompany inflammation or radiation exposure [8].

8.3.2.2 Blood Elements

Blood and blood elements are almost always present in effusion specimens received by laboratories, either due to trauma from the acquisition procedure or the true presence of those cells in the cavity. Red cells are easily recognized as benign but may cause diagnostic difficulty by obscuring other more significant cell types. For this reason, laboratories routinely use techniques designed to lyse red cells so as to remove them from the visual field.

Nucleated cells of hematopoietic origin are always present in body cavity fluid specimens, and are usually of little or no clinical significance, but occasionally may be a key to diagnosis, especially if they are numerous. Some of the most important clinical associations for the different cell types have been listed in Table 8.1.

Lymphocytes are frequently identified in body cavity fluid specimens, but usually have little or no diagnostic importance. Small numbers of such lymphocytes may be physiologic and are usually not mentioned in cytology reports. Differentiating normal B cells from normal T cells is difficult or impossible in these specimens without ancillary studies. For the most part, reactive lymphocytes in effusions are of predominantly T cell lineage, but this is rarely confirmed in clinical practice unless lymphoma is suspected. The term "chronic inflammation"

Table 8.1 The potential implications of the presence of increased numbers of hematopoietic cells in body cavity fluid specimens

Cell type	Major associations
Lymphocytes	Long-standing effusions
	Lymphoma
	Response to solid malignancies
	Tuberculosis
Neutrophils	Bacterial infection
	Infarction of intracavity organs
	Malignancy
	Collagen vascular disease
Eosinophils	Idiopathic
	Air in the body cavity
	Hypersensitivity
	Parasites
	Pulmonary infarct or pneumonia
	Malignancy

may be used in cytology reports for body cavity fluids. Generally, this merely means that significant numbers of benign-appearing lymphocytes are present with no further clinical implications. Cytologists worried about a specific infection or a malignant process should use more definitive terminology.

Neutrophils are also frequently identified in effusion specimens. A true increase in neutrophils in body cavity fluids is indicative of an acute inflammatory process. Cases with abundant neutrophils generally correspond to grossly evident pus and often the diagnosis is already known or suspected clinically. However, the presence of only occasional neutrophils is more difficult to interpret because it may be an artifact of blood contamination. If the red cells have been lysed away, a frequent occurrence, differentiating true inflammation from an incidental finding can be difficult because a comparison of the ratio of neutrophils to red cells is not possible. Many cytologists prefer to report the presence of neutrophils whenever present because it may provide a clue to the presence of spontaneous bacterial peritonitis or some other treatable acute infectious process that might otherwise go undetected. The use of the term "acute inflammation" on a cytology report should be taken to mean that significant numbers of neutrophils are present in the specimen and that the treating physician should be alert to the possibility of bacterial infection of the space. A special type of neutrophil seen in the setting of systemic lupus erythematosus is the so-called "LE cell", which contains homogenized partially degraded nuclear material within a large cytoplasmic vacuole, presumably reflecting phagocytosis of the material (Fig. 8.3).

Eosinophils are not commonly seen in substantial numbers in body cavity fluids, and are outright rare in the peritoneum and pericardium. The cells are easily recognized, when present, by their bilobed nuclei and prominent eosinophilic granules. Trauma/bleeding is the most common cause of eosinophilic effusions. Eosinophilic effusions only occasionally correspond to hypersensitivity or parasitic infections. In many cases no identifiable cause is found. Occasionally, there may

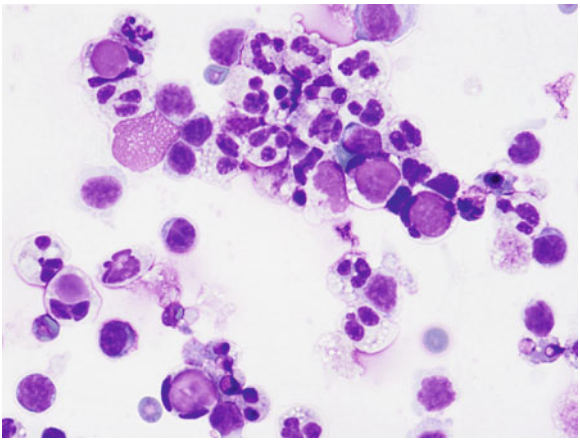

Fig. 8.3 LE cells. Here several "LE cells" can be seen, neutrophils can be seen that contain homogenized engulfed nuclear material within vacuoles. There is an inflammatory background consistent with the history of systemic lupus erythematosus. (DiffQuik stain, 600× magnification.)

be an underlying malignancy [9]. Basophils and mast cells may also be found in effusion specimens but usually make up a minor population and have no diagnostic significance.

Histiocytes, also known as macrophages, often appear in effusion specimens. These cells typically have abundant foamy or lacy cytoplasm and so-called "bean shaped" off-center nuclei. They are not always morphologically distinctive and confusion with benign mesothelial cells or malignancy can occur. The presence of large clear vacuoles or engulfed cells may suggest the possibility of adenocarcinoma. Their presence is not typically reported unless they are very numerous. The finding of histiocytes, in and of itself, has little diagnostic specificity unless they take on elongated and giant forms and are associated with necrotic debris, in which case they are strongly suggestive or even diagnostic of rheumatoid arthritis.

8.3.2.3 Psammoma Bodies

Psammoma bodies are lamellated round calcifications formed from papillary cellular structures. Most psammoma bodies seen in body cavity fluids are found in the context of serous carcinoma. Typically, the malignancy is obvious and psammomatous calcifications are noted as an interesting background phenomenon, often with a ring of obviously malignant viable cells surrounding them. Isolated psammoma bodies, however, create a diagnostic dilemma. In all likelihood, rare psammoma bodies not associated with malignant cells derive from benign processes, but in the context of high clinical suspicion complete dismissal of the finding may not be possible. Reporting of the presence of psammoma bodies without an outright diagnosis of malignancy, therefore, represents an equivocal diagnosis.

8.4 Malignancy in Body Cavity Fluids

8.4.1 Mesothelioma

Mesothelioma attracts interest out of proportion to its frequency due to its unusual risk associations and the socio-legal implications that follow this diagnosis. The link between this disease and prior asbestos exposure, particularly to amphibole subtypes, has made this one of the most feared occupational exposure-related diseases. The grim prognosis of the disease compounds the anxiety of those potentially exposed to asbestos fibers and contributes to the large legal settlements that victims and their families often lay claim to following diagnosis. Asbestos is a mineral that occurs in nature and is, in some areas, a not uncommon component of the rock. The use of asbestos by various industries led to a spike in mesothelioma cases some decades after its introduction, but many countries have now banned it for commercial use since its attendant risks are widely known. Unfortunately, history-taking almost always reveals some possible asbestos exposure, limiting the usefulness of that information. Analysis of body cavity fluids for asbestos bodies has a very low yield.

The diagnosis of mesothelioma by cytology is a daunting challenge. As has been noted, the range of reactive changes in benign mesothelial cells extends very broadly. This means that most "atypia" seen in mesothelial cells or clusters will not represent malignancy. Furthermore, mesothelioma may be extremely bland cytologically, making diagnosis difficult even in extensive surgical pathology specimens. As a general rule, mesothelioma recapitulates the appearance of normal mesothelium with central round nuclei, vacuolated cytoplasm, the presence of windows and skirts, and clusters with a knobby contour. Some features are suggestive of malignant mesothelium in cytology preparations: cellularity and cell size. It has been stated that mesothelioma is best separated from reactive mesothelial cells in body cavity fluids by the presence of "more and bigger cells in more and bigger clusters" (Fig. 8.4). This rule of thumb certainly applies, but suggests the vagaries involved in reaching this diagnosis. Attempts to create objective standards for cell cluster size have not proven successful, since sufficiently stringency to exclude benign changes reduces the sensitivity to a very low level. Cell size evaluation is also subjective. Uniform marked enlargement of mesothelial cells with concomitant nuclear enlargement, chromatin clumping, and prominent nucleoli is suggestive of malignancy. However, cells with these features are not uncommon in benign cases, and drawing a sharp line between positive and negative cannot be reliably done.

Ancillary studies are of no value in distinguishing benign from malignant mesothelium. Electron microscopy shows the same characteristic long cilia that are seen in normal mesothelium. Immunocytochemistry also shows the same patterns of staining in both mesothelioma and benign mesothelium. Molecular studies may in the future find characteristic markers that could distinguish malignant or premalignant processes.

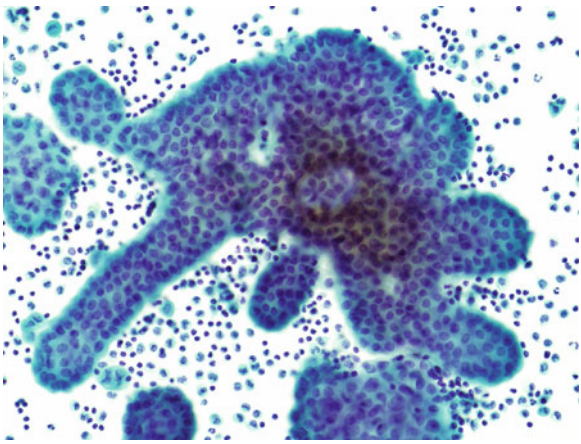

Fig. 8.4 Mesothelioma. There is an enormous and complex cell cluster with large cells as demonstrated by comparison with background inflammatory cells. Individual atypical mesothelial cells can also be seen nearby. Nucleoli and clumpy chromatin can be discerned in many of the cells, even at this power. (Papanicolaou stain, 200× magnification.)

Many cytologists believe that a primary diagnosis of mesothelioma should not be made in body cavity fluid specimens. It should be noted that there are those who do not accept this position [10, 11]. However, the Mesothelioma Reference Panel group has recently recommended that mesothelioma should not be diagnosed unless unequivocal invasive malignancy can be demonstrated on surgical pathology material [7]. As a result, cases where mesothelioma is highly suspected in body cavity fluid cytology are frequently interpreted as "atypical mesothelial proliferation", often with an accompanying comment suggesting additional correlation or follow-up. In patients with an established diagnosis of mesothelioma on surgical pathology material, most cytologists would feel comfortable rendering an outright diagnosis if the findings were compatible.

Even when a diagnosis of mesothelioma has been established, it may not be possible to diagnose persistent or recurrent disease from fluid alone. Many mesotheliomas are predominantly or entirely composed of spindled cells, resembling sarcoma rather than adenocarcinoma. Such tumors virtually never shed diagnostic cells into body cavity fluids.

8.4.2 Other Malignancies

Most effusions containing malignant cells do not derive from the relatively rare mesotheliomas that are primary to the body cavities. Rather, positive body cavity fluids almost always represent involvement of body cavities by malignancy derived from other types of epithelium with secondary spread. Occasionally, non-epithelial malignancies may also appear in fluids: lymphoma/leukemia, melanoma, and sarcomas. Differentiating these various types of malignant process from benign fluids, and from each other, constitutes the primary challenge of cytological body cavity fluid interpretation. Knowledge of patterns of spread can aid in the

diagnosis of malignancy in any area of pathology. The different compartments of the thorax have unique patterns of involvement by tumor type. Gender and age are also key considerations.

Malignant effusions are rare in pediatric populations. Body cavity fluid cytology specimens are correspondingly unusual. Most malignancies that are found will be hematologic, especially leukemias [12]. Pediatric solid tumors, including neuroblastoma, Wilms tumor, and others may rarely appear in peritoneal fluid. The diagnosis of such tumors would be a once-in-a-lifetime event for most cytologists.

The frequency of malignancy involving effusions rises through adulthood in accord with the increasing incidence of carcinomas, which make up the predominant causes of positive body cavity fluid specimens in both genders. Among younger adults, melanoma, breast cancer, and gastric cancer are relatively more likely due to the epidemiology of those malignancies.

Older adults, who are the source of most of the positive specimens, have well-defined risks by site (Table 8.2). Peritoneal fluids much more commonly harbor malignancies in women, among whom serous ovarian/primary peritoneal carcinomas and breast carcinomas predominate. Gastrointestinal primaries also involve peritoneal fluid not uncommonly in both genders and are the most important consideration in men. Gastric carcinomas have an especially marked tendency to cause malignant effusions, whereas colon cancer only rarely involves peritoneal fluid. Despite the fact that most hepatocellular carcinoma arises in the setting of cirrhosis, and is therefore associated with ascites, the tumor virtually never enters into the fluid. Attempts to diagnose hepatocellular carcinoma in ascites fluid have a very low yield [13].

In pleural fluid, lung cancer predominates in both genders. Adenocarcinomas make up the vast majority of these, due to their tendency to arise peripherally in the lung and their proclivity to grow through and along the parietal pleura. Squamous and small cell lung carcinomas involve pleural fluid more rarely. Among women, metastatic breast cancer is the most common malignancy in pleural fluid after lung.

Pericardial fluid malignancies are essentially always from distant sites. Lung adenocarcinoma, due to its high frequency and proximity, is the most frequently discovered type. In women, breast carcinoma predominates. Melanoma also has a proclivity to appear in this location.

Of course, more detailed clinical and radiological history provided with the request for examination, or obtained through a careful review of the history at the time of analysis, often provides more precise information that can be of the utmost usefulness. In most instances, malignancy can be suspected prior to submission of the specimen to cytology [14, 15]. History is also extremely useful when dealing with a malignant process that is not "following the rules" such as breast cancer in a male or lung adenocarcinoma in a 25-year old. In such instances, a lack of clinical correlation can lead to serious mistakes. It is the responsibility of the requestors of the test, as well as the pathologists, to make sure that patient history is known in sufficient detail before a final interpretation is rendered. In many instances cytologists struggle to try to interpret a difficult case, perhaps with the

Table 8.2 The most frequently seen malignancies, by gender, in each of the body cavities

Cavity	Females	Males
Peritoneal	Gynecologic tract	Gastrointestinal tract
	Breast	Lung
	Gastrointestinal tract	Lymphoma
	Lung	Melanoma
	Lymphoma	Renal
	Melanoma	Urothelial
	Renal	
Pleural	Lung	Lung
	Breast	Gastrointestinal tract
	Gynecologic tract	Lymphoma
	Gastrointestinal tract	Melanoma
	Lymphoma	
	Melanoma	
Pericardial	Breast	Lung
	Lung	Melanoma
	Melanoma	

aid of expensive and delay-causing tests, only to find out later that the primary care-givers already knew something that would have instantly resolved the difficulty. Electronic medical records and universal access to radiological images via computer have tremendously reduced this problem, but the key remains good communication between pathologists and the rest of the patient care team.

8.4.2.1 Adenocarcinoma

The most frequent malignancies identified in body cavity fluids are adenocarcinomas (Fig. 8.5). They arise from many different body sites, but, insofar as they are all malignant epithelial tumors with glandular differentiation, they have a great deal in common. Indeed, they often resemble one another so much as to make distinguishing them impossible by morphology alone. Adenocarcinomas stand out from background mesothelial cells found in body cavity fluids in two ways: changes related to malignancy and changes related to origin from glandular epithelium. Neither of these is entirely reliable, but when both are present a diagnosis of adenocarcinoma can usually be made with confidence, especially with corroborating history and radiology. A fundamental rule of body cavity cytology is to look for a "two-cell population". This maxim refers to the dimorphic character of body cavity fluid specimens with the most common manifestation of adenocarcinoma: large clusters of obviously malignant cells, with distinctive cytologic features, that stand out from the adjacent normal mesothelial cells.

Adenocarcinomas show a number of cytomorphologic patterns. The nuclei show enlargement with correspondingly large nucleoli. The chromatin becomes clumpy and the nuclear membranes develop grooves and other contour

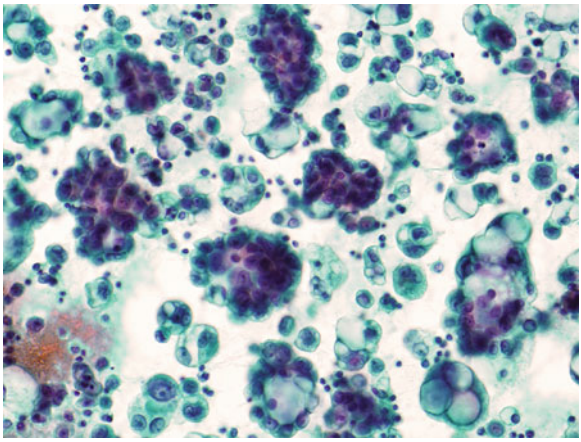

Fig. 8.5 Lung adenocarcinoma. This is a relatively straightforward example of adenocarcinoma that is predominantly composed of obviously malignant cells containing prominent mucin vacuoles and forming large gland-like structures. Comparison with Fig. 8.4 shows some of the similarities between adenocarcinoma and mesothelioma that may cause a diagnostic dilemma: high cellularity, large irregular clusters, atypical nuclei, and prominent nucleoli. (Papanicolaou stain, 400× magnification)

irregularities; however, these features are relatively nonspecific, being common in other malignancies also. This makes sense, because all of these features result from rapid and disordered cell growth and division, common events in all malignant processes. Individual cells typically produce mucin in adenocarcinomas. This may manifest as either a foamy cytoplasm with many small vacuoles or a so-called "signet ring" appearance caused by the presence of a single large vacuole that pushes the nucleus off to one side (Fig. 8.6). Unfortunately, mesothelial cells, whether benign or malignant, also frequently have foamy cytoplasm and may form signet ring-like structures with a large central vacuole. The irregularity and sharp edges of the nuclei in true signet rings of adenocarcinoma may help in distinguishing them from mesothelial imitators, but this may be difficult if only a few such cells are present. In adenocarcinomas, nuclei tend to be off-center even in cells without a dominant vacuole, which also may help in differentiating such cells from mesothelium, which tends to maintain a centrally located nucleus.

Another feature of glands often recapitulated in body fluids is the formation of acinar structures. In fluid cytology, this manifests as a tendency for cells to cling to one another in a tight spherical cluster with a sharp outer border. The presence of many such groups is often the first indication of malignancy in body cavity fluid specimens. In distinguishing adenocarcinoma from mesothelioma, in particular, the presence of smooth outer borders of clusters, as opposed to the "florets" typical of mesothelioma, can be a vital clue. However, as with essentially every other morphologic feature, the finding is not entirely reliable.

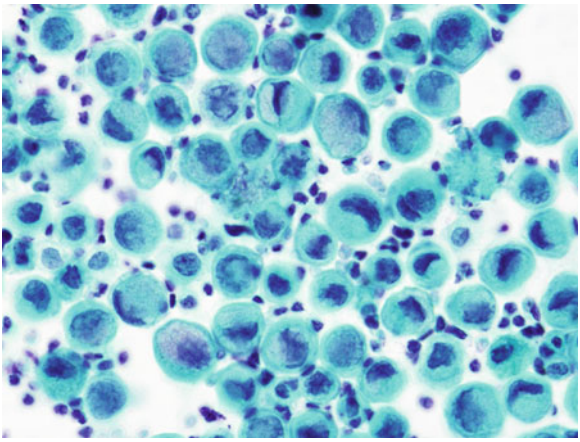

Fig. 8.6 Breast adenocarcinoma with signet ring morphology. This adenocarcinoma is composed of individual malignant cells rather than clusters. The cells show a signet ring configuration with the nucleus displaced to one side and indented by mucin vacuoles. This specimen also has prominent nuclear pleomorphism and atypia. (Papanicolaou stain, 600× magnification.)

Most adenocarcinomas have overlapping features by cytology. Certain findings discussed below may increase suspicion for one particular type over another, but pathognomonic morphologic findings are rare. Generally, the distinction is not important. The primary tumor is often already known or suspected on the basis of radiological findings or previous biopsy or excision.

Adenocarcinoma of the lung is by far the most common malignancy in pleural fluid and among the most frequent in pericardial and peritoneal fluids as well. Because of the nonspecific cytologic appearance of lung adenocarcinoma, differentiation from reactive mesothelial cells or mesothelioma often requires ancillary techniques. The most common peritoneal adenocarcinoma, serous carcinoma, can also be difficult to differentiate from mesothelioma due to the similarity of its papillary structures to mesothelial proliferations. Differentiating primary peritoneal carcinomas from ovarian or uterine primaries cannot be done by cytology alone. Breast carcinoma, whether manifesting as a signet ring morphology or as more typical clusters of cells, may also pose diagnostic difficulties. One scenario of special concern is in women harboring BRCA mutations, who are prone to both serous and breast carcinomas.

Adenocarcinomas presenting in fluids as single cells, whether showing clear signet ring morphology or not, pose special problems and deserve special attention [16]. These malignancies are as much a pitfall in body cavity fluid cytology as in fine needle aspiration or surgical pathology. The cells tend to be uniform, bland, and infrequent. Even when they are very numerous, their similarity to normal mesothelial cells leads to an absence of the “two-cell population” appearance typical of most adenocarcinomas. Indeed, many such cases appear benign or even normal at low power. Only careful investigation of the cells, often prompted by history, will lead to the correct diagnosis. The danger of false negative body fluid cytology results is high for these specimens, especially when suspicion is low.

8.4.2.2 Other Epithelioid Malignancies

Lung carcinomas other than adenocarcinoma occasionally appear in pleural and, even more rarely, peritoneal or pericardial fluid specimens. Small cell carcinoma, when it does manifest in body cavity fluids, has a distinctive appearance. As the name suggests, the cells are relatively small, composed almost entirely of nucleus, with neuroendocrine features including coarse "salt and pepper" chromatin and inconspicuous or absent nucleoli. The cells are often noted first because they form into characteristic cell rows with nuclear molding, referred to variously as "vertebral columns" or "stacks of coins". When such structures are absent, however, small cell carcinoma may be extremely difficult to detect, especially if carcinoma cells are few or similarly appearing lymphocytes are numerous. Neither scenario is uncommon. Other neuroendocrine carcinomas, including carcinoid tumors, atypical carcinoids, and large cell neuroendocrine tumors may also rarely appear in fluid specimens.

Squamous cell carcinoma also has a distinctive appearance that usually allows for relatively easy identification. The cells typically have a "hard" cytoplasm caused by the accumulation of dense keratins in accordance with the differentiation of the tumor. The cytoplasm takes on a distinctive orange coloration in Papanicolaou stains that can be readily recognized by cytologists, most of whom spend a great deal of time looking for similar cells in cervicovaginal specimens. The presence of "tadpoles" and other bizarre forms caused by keratinization is as diagnostic in body cavity fluids as elsewhere. Unfortunately, not all squamous cell carcinomas announce themselves so clearly. Poorly differentiated examples may be difficult to distinguish from adenocarcinoma, reactive or malignant mesothelium, or other malignancies.

Both squamous cell and neuroendocrine carcinomas may arise in other sites, though even less commonly. This fact needs to be kept in mind when such tumors appear in the absence of a detectable lung mass. Continued searching generally yields a different primary site without too much difficulty. Urothelial carcinomas, in particular, should be kept in mind when squamous or squamous-like cells appear in peritoneal fluid.

Renal cell carcinomas are another unusual tumor type in fluids with a potentially distinctive morphology. Conventional renal cell carcinoma often has a clear cell appearance that can be identified in body cavity fluids. Such cells contain numerous small cytoplasmic vacuoles and often have unusually prominent red nucleoli.

Although not of epithelial derivation, melanoma must always be kept in the differential for epithelioid tumors. Known for their mimicry, melanoma cells can take on a number of appearances. Although formation of large cohesive clusters is very unusual for melanoma, it can imitate just about any other manifestation of epithelial malignancies in body cavity fluids. Occasionally, distinctive features may be present that aid diagnosis. The presence of melanin pigment is

pathognomonic but quite unusual. The presence of "double mirror-image nuclei" may also help distinguish melanoma when they are present. Such cells contain paired, prominent nuclei with large red nucleoli, which often bulge the outer nuclear membrane. Prior knowledge of a patient history of melanoma may increase the likelihood of noticing these features, but melanoma is also known for arising in obscure or long-forgotten sites. For these reasons pathologists must constantly remind themselves of the possibility of melanoma.

8.4.3 Immunocytochemistry for Epithelial Malignancies

8.4.3.1 Immunocytochemistry in Body Cavity Fluid Specimens

For the most part, immunocytochemistry for body cavity fluid specimens is similar in principle and practice to what is done for other cytology specimen types. Most laboratories attempt to make a cell block from residual fluid for staining. Body cavity fluids are generally very cellular, especially in malignant cases, facilitating the use of this technique. However, there are some cases with low cellularity or limited material that may require other methods. Many laboratories that have a large volume of cytology specimens are proficient in immunostaining thin-layer liquid preparations such as ThinPrep and/or direct smears. Older techniques such as electron microscopy have been almost entirely replaced by immunocytochemistry. Special stains not based on antibody binding continue to have a limited role. In particular, mucicarmine stain is often used to detect mucin production in adenocarcinomas as a part of a broader work-up.

8.4.3.2 Mesothelioma Versus Lung Adenocarcinoma

The predominant differential diagnostic consideration in malignant pleural fluids is the separation of adenocarcinoma of the lung from mesothelioma. This differential is especially difficult because the two tumors resemble one another not only morphologically but also have similar clinical history and radiological findings.

There is consensus that multiple markers for mesothelioma and for adenocarcinoma should always be used because every marker has significant false negative rates and many are also prone to false positives. Expert panels on the diagnosis of mesothelioma have pointedly declined to recommend any particular panel [17]. This is because different laboratories have different degrees of experience and success with the various markers. The belief is that the best results are obtained when pathologists have the freedom to choose a panel tailored to their own laboratories. A great many different markers have been reported to be useful in differentiating mesothelial cells from adenocarcinoma (Table 8.3) [18].

Table 8.3 Immunocytochemical markers used for differentiating mesothelioma from adenocarcinoma, with the most commonly used markers listed nearer to the top

Mesothelioma	Adenocarcinoma
D2-40	MOC-31
Calretinin	Ber-EP4
WT-1	B72.3
Cytokeratin 5/6	CEA
Podoplanin	Leu-M1 (CD 15)
Mesothelin	EMA
HBME-1	E-cadherin
Thrombomodulin	BG-8 (Lewis y)
HMW Cytokeratin	CA 19-9
N-cadherin	
Vimentin	

8.4.3.3 Immunocytochemical Markers for Other Carcinomas Frequently Encountered in Body Cavity Fluid Specimens

For the most part, carcinomas of other types can be more easily differentiated from mesothelioma on clinical and radiological grounds. In some instances a distinctive cytological appearance may also make mesothelioma unlikely. Immunocytochemistry is still useful, however, for those occasional cases where mesothelioma remains in the differential and, more commonly, when carcinoma can be confidently diagnosed but the site of origin remains ambiguous.

For the differentiation of serous carcinoma from mesothelioma, many of the same markers used in the panel for lung adenocarcinoma can be used with similar effectiveness. Important alterations include the replacement of the lung marker TTF-1 with the gynecologic marker PAX-8 and the exclusion of WT-1, which is typically positive in both entities.

Many other tumor types have characteristic markers that can be tested for by immunocytochemistry (Table 8.4). Adenocarcinomas arising from the breast and pancreas pose special problems for immunocytochemistry. Breast prognostic markers can be performed in cytology specimens but have poor sensitivity and specificity for breast origin. Although specific breast markers are available, they are frequently negative and therefore only have value when they stain the cells of interest. No good markers exist for pancreatic adenocarcinomas. Therefore this diagnosis is frequently impossible to make in fluid cytology, though it may be suggested by a lack of staining with specific markers for other frequent carcinomas with a similar appearance.

Squamous cell carcinomas have a less pronounced tendency to involve body cavity fluids than adenocarcinomas. When keratinization can be detected cytologically, the diagnosis poses few problems. However, squamous cell carcinomas involving fluids often are poorly differentiated and may be difficult to recognize by morphology alone. Differentiating squamous cell carcinomas arising from different sites of origin is an essentially impossible task using immunomarkers.

Table 8.4 Commonly used immunocytochemical markers with specificity for particular carcinoma types

Immunomarker	Target Tumor Type(s)	Caveat
BRST-2	Breast	Poor sensitivity
CA-125	Gynecologic	Poor specificity
CDX-2	Gastrointestinal	Poor sensitivity except for colon
Cytokeratin 5/6	Squamous	Also positive in mesothelioma
Estrogen receptor	Breast	Poor sensitivity and specificity
Mammaglobin	Breast	Poor sensitivity
p63	Squamous	Breast carcinomas may be positive
PAX-8	Gynecologic, renal, thyroid	A new marker with limited availability
TTF-1	Lung, thyroid	Negative in squamous lung carcinomas
WT-1	Gynecologic	Also positive in mesothelioma

As a general rule, a good clinical history and knowledge of radiological findings is often much more useful than a panel of immunocytochemical markers for differentiating these different types of adenocarcinoma. Many expensive, time-consuming, and nonconclusive work-ups are performed by pathologists needlessly because of poor communication between patent care-givers. It is the responsibility of all parties to try to avoid this outcome. In most cases, a cytological diagnosis of adenocarcinoma, without any further specification, is entirely sufficient for the purposes of patient care.

8.4.3.4 Immunocytochemistry for Melanoma

Melanoma is frequently considered in the differential diagnosis for malignant epithelioid cells, especially if they are poorly differentiated, dyscohesive, and no site of origin is readily identified. Immunocytochemistry can be extremely useful for ruling out melanoma in body cavity fluids because there are several markers that will almost never stain anything else seen in these specimens: S-100, melan-A (MART-1), HMB-45, and tyrosinase. The less-than-perfect sensitivity of these markers may require the use of several of them, but, in general, they can be used to confidently identify melanoma in most instances.

8.4.3.5 Hematopoietic Malignancies

In hematopoietic malignancies, positive fluid cytology usually represents a late finding. Typically, the cytologist only needs to confirm or refute the presence of malignancy that has already been diagnosed previously. As with carcinoma, however, this may be a more difficult task that it would seem. Lymphomas often cause serous effusions even when malignant cells are not present in the fluid or are present only in minute amounts [19]. Fortunately, flow cytometry provides a very useful tool that enables the confident separation of benign from malignant

processes. Body cavity fluid specimens generally work well for flow cytometry and in most cases there is abundant material left for further analysis following the preparation of cytology slides. Flow cytometry is somewhat similar in principle to immunocytochemistry in that it uses antibody binding to determine expression patterns for panels of markers of differential diagnostic usefulness. The small size and dyscohesive nature of lymphocytes, however, makes possible the more sophisticated techniques of flow cytometric analysis that enable much larger panels to be performed on relatively small volumes of material.

8.4.3.6 Small Cell Lymphomas

Lymphomas composed entirely or predominantly of small cells are especially difficult to diagnose in body cavity fluids. The reactive T-lymphocytes characteristically seen in all effusions have nuclear features very similar to those of many lymphomas. Indeed, in the majority of cases of small cell lymphomas the distinction cannot be made with absolute confidence, even if a history of lymphoma is known. One distinctive feature that may offer a clue in cases of small lymphocytic lymphoma is distinct clumping of the chromatin referred to as "cellules grumelees" or "clotted cells". If this finding is present in numerous, monomorphic lymphocytic nuclei, the diagnosis can be strongly suspected. Flow cytometric confirmation would nonetheless be employed in most instances, however. In general, flow cytometry is a useful adjunct whenever a small cell lymphoma is suspected because it considerably improves the sensitivity for diagnosis in these specimens [20].

8.4.3.7 Intermediate and Large Cell Lymphomas

Lymphomas with larger cells are typically more obviously malignant and stand out more distinctly from the background of reactive lymphocytes. The nuclei often show marked pleomorphism, clumpy chromatin, and prominent nucleoli, findings rarely observed in reactive lymphocytes in substantial numbers. The presence of numerous mitoses within enlarged lymphocytes may also serve as a useful clue to malignancy. Lymphocyte apoptosis may manifest as "mercury drop karyorrhexis," the breakdown of nuclear material into multiple very dense and degenerated blobs. This finding is particularly common in cells from processes such as Burkitt lymphoma or diffuse large B cell lymphoma that also show frequent apoptosis in tissue.

As with small cell lymphomas, confirmation of clonality by flow cytometry is of great utility. At times, very ugly large cell lymphomas may raise carcinoma or melanoma as a differential diagnostic possibility. An immunocytochemical panel of CD45 for lymphoma, pan-cytokeratin for carcinoma, and S-100 for melanoma can resolve this problem.

One rare type of lymphoma deserves special attention because of its unique proclivity to manifest in body cavity fluids at presentation: primary effusion lymphoma. This relatively recently described entity is similar in morphology to

other large cell lymphomas and is distinguished by its clinical presentation. It essentially always occurs in immune-suppressed individuals, especially AIDS patients. It has been linked to the virus HHV-8, the same herpesvirus responsible for causing Kaposi sarcoma [21]. If the diagnosis is suspected, immunocytochemistry can be performed for HHV-8 as a confirmatory step [22].

8.4.3.8 Other Hematopoietic Malignancies

Hodgkin lymphoma, despite being relatively common, very seldom can be diagnosed in body cavity fluids. Reed-Sternberg cells are only rarely seen in fluid cytology specimens. Effusions associated with Hodgkin lymphoma generally only contain nonspecific reactive inflammatory cells.

Plasma cell myeloma is also very rare in effusion specimens. Very infrequently, however, body cavity fluids can be massively involved by malignant plasma cells.

Leukemias may also involve body cavity fluids, though the disease is virtually always diagnosed by other means prior to this presentation. The presence of large numbers of blasts in these specimens is very distinctive and generally does not cause diagnostic problems. Leukemias compose a relatively large share of malignancies in childhood effusions.

Chronic idiopathic myelofibrosis may cause a very interesting finding in body cavity fluid specimens: cells derived from extramedullary hematopoiesis. The presence of mixed blood precursors in effusion specimens, including nucleated red cells, cells of various stages of the myeloid series, and megakaryocytes, is strongly suggestive of this diagnosis.

8.4.4 Sarcomas

Sarcomas are quite rare in body cavity fluid specimens. Bone and soft tissue tumors manifesting in effusions are generally easily recognized as malignant [23]. Distinctive morphologies may be recognizable in some cases, but such findings are of more intellectual than clinical interest. Usually, the underlying tumor has already been diagnosed by other means as involvement of fluids is a late manifestation.

8.5 Intraoperative Body Cavity Washing Specimens

8.5.1 Introduction

Pelvic washings are routinely performed in conjunction with hysterectomy and salpingo-oophorectomy for malignant disease, as well as at the time of second-

look procedures. For ovarian primary tumors, the presence of positive cytology will cause an upstage of the disease to FIGO Stage IC or IIC depending on other parameters. Cytology has, up until recently, also been a part of the staging for endometrial carcinomas. In other settings, cytology may not change the official staging but is used to direct treatment decisions. As such, the cytology laboratory plays a key role in the determination of the ultimate treatment course for many of these patients.

Washings are easy and inexpensive to obtain, facilitating studies regarding their utility in surgical settings. So far, body cavity washing cytology has not become a standard procedure outside of gynecologic surgery, but this may change in the future as more studies are performed and more data is gathered regarding the clinical utility of the information such specimens can provide.

8.5.2 *Differences between Washing and Effusion Cytology*

As has been discussed above, the primary difference between intraoperative washing specimens and effusion specimens is the degree of rounding of the cell clusters. Mesothelial cells usually take on a flat sheet-like configuration in washings, and often form into larger groups than are seen with spontaneously exfoliated mesothelium due to the abrading action of the fluid. Direction of washings toward areas of the peritoneal lining with a gross appearance suspicious for tumor implants often facilitates diagnosis as well. Most positive washings are "loaded" with tumor cells, making their identification straightforward, even for low-grade processes [24]. The opportunity to compare the washing cytology with the appearance of the tumor in simultaneously resected specimens for surgical pathology also facilitates diagnosis.

Directed washings may occasionally, however, cause problems if endometriosis or endosalpingiosis are targeted. The resulting presence of a large number of endometrial or tubal cells, especially, if they have reactive atypia or psammoma bodies, or if the primary tumor is bland and low grade, may lead to a diagnostic dilemma. Immunocytochemistry is of little use in such situations. Comparison with simultaneous surgical pathology specimens can be very helpful.

8.5.3 *Controversies in the Use of Cytology for Staging of Endometrial Adenocarcinoma*

There has been an ongoing disagreement in the literature about the true prognostic significance of positive cytology alone in the setting of hysterectomy for endometrial adenocarcinoma. Some authors report no difference in recurrence rate or survival in women upstaged by cytology alone from Stage I to Stage III [25, 26].

Others argue that the finding is significant and the practice of intraoperative washings should continue [27–31].

The introduction of less invasive techniques in gynecologic surgery has created additional controversy regarding the value of the pelvic washing specimens from those procedures. Hysteroscopy prior to surgery has been suggested as a cause of positive peritoneal washings [32], but later studies have allayed concern [33]. It has also been argued that more intensive uterine manipulation in laparoscopic procedures may cause tumor spillage from the uterus [34, 35], though not all studies agree [36]. Furthermore, the clinical significance of rare false positives caused by artifactual spillage is far from clear. It may still be better to over-treat a few women rather than under-treat many others who are true positives.

8.5.4 Body Cavity Washings for Non-Gynecologic Tumors

There has been an upsurge in interest in recent years of applying intraoperative washing cytology more broadly outside the pelvis. Numerous studies have been conducted regarding the value of washings following pulmonary [37–43] and gastrointestinal [44–48] surgeries. As a general rule, these studies show that post-operative positive cytology does correspond to a worse prognosis for the patient. However, this finding is often fairly weak or even nonexistent when other staging parameters are controlled for, raising the question of the practical utility of performing washings on a routine basis in clinical practice. Furthermore, lung and gastrointestinal tumors have a fundamentally different biology from gynecologic tumors. They are less likely to spread via implants on serosal surfaces and more likely to recur in the form of distant metastasis. All of these factors mean that the significance of a positive washing cytology for any particular patient is relatively difficult to deduce, precluding broader acceptance of these procedures for clinical practice.

8.6 Fine Needle Aspiration Cytology of the Omentum and Peritoneum

For the most part, directed aspiration cytology of masses occurring in the omentum, or elsewhere in the peritoneum, yield findings similar to effusion or washing cytology. If anything, in these specimens interpretation is made easier by the fact that a defined tumor mass is being heavily sampled with relatively few cells in the background derived from benign processes. These specimens are virtually always taken from gynecologic malignancies and the cytologist only needs to confirm the suspected diagnosis.

8.7 Molecular Pathology of Body Cavity Fluid Specimens

As with every other area of pathology, ancillary molecular techniques are becoming more important in body cavity fluid specimens. A few genetic tests are already being used clinically. The use of body cavity fluid specimens as substrates for EGFR mutation analysis has been demonstrated in multiple studies [49–51]. Similar testing for other cancers, such as colorectal carcinoma, as well as other genes, such as K-RAS, should also be possible. Perhaps more exciting is the prospect of using molecular techniques to aid in diagnosis of problematic cases. Because body cavity fluid specimens are typically highly cellular and leave abundant residual material after routine cytologic processing, they would seem like ideal candidates for research in this area. A number of studies have been published [52, 53], especially, with regard to methylation of DNA as an ancillary marker [54–56]. Although this work shows promise, the findings so far have not been revolutionary. Nevertheless, it seems inevitable that body cavity fluid cytology, like all areas of pathology, will be gradually transformed by molecular insights and techniques.

8.8 Conclusions

Body cavity fluid cytology is a well-established field whose pitfalls and diagnostic dilemmas are widely known and extensively studied. False positive, false negative, and ambiguous results can occur because of the inherent challenges posed by these specimens. The importance of providing pathologists with every possible advantage when making their interpretations, in particular complete patient histories and information about radiological findings, cannot be overstated.

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