Chapter 6
Esophagus, Stomach, and Pancreas

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Cytologic examination using fine needle aspiration (FNA), exfoliative brushings, and needle core biopsy are essential tests in the diagnosis of esophageal, gastric and pancreatic lesions, staging of malignancy, and follow-up of patients. The sensitivity and specificity of cytology is increased by the concurrent use of radiologic imaging (endoscopic ultrasound (EUS), ultrasound, computed tomography (CT) and magnetic resonance imaging (MRI), immuno-cytochemistry, and molecular studies.

6.1 Utility of Cytology in Esophageal, Gastric and Pancreatic Lesions

6.1.1 Diagnostic Methodology

The following techniques can be used to obtain samples from the esophagus, stomach and pancreas, and their surrounding tissues or lymph nodes for cytologic examination of neoplastic, infectious, or inflammatory lesions.

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6.1.1.1 Esophageal, Common Bile Duct and Pancreatic Duct Brushing Cytology

Brush cytology is used for superficial mucosal lesions or lesions involving the mucosa [1–4]. Brushing cytology is more routinely used in the setting of pancreaticobiliary malignancy. In this setting, common bile duct and pancreatic duct brushings can be obtained during ERCP [3].

6.1.1.2 Ultrasound, CT or MRI-Guided Percutaneous FNA or Needle Core Biopsy

These techniques are used for gastric and pancreatic lesions, lesions surrounding the stomach and pancreas for diagnosis, and mediastinal, peri-gastric, and peri-pancreatic lymph nodes for staging.

6.1.1.3 EUS-Guided Fine Needle Aspiration

In conjunction with FNA, EUS has become a very effective tool for tissue acquisition for many malignant and benign processes both within and outside the gastrointestinal tract (e.g., lymph nodes) [5]. While ultrasound and CT-guided FNA biopsies are also alternative techniques for tissue acquisition, EUS-FNA has become the gold standard and offers greater efficacy with less complications.

6.1.1.4 Abrasive Balloons for Gastroesophageal Lesions

Although this less costly cancer surveillance method for gastroesophageal diseases is not used in USA, it is used in other countries [6, 7]. The sensitivity for detecting high-grade dysplasia or carcinoma in high incidence areas is 80 %, while the sensitivity for detecting low-grade dysplasia is much lower, at 25 % [6].

6.1.2 Diagnostic Application

Optimal tissue acquisition techniques as described above are essential to procure good cytologic samples for establishing a diagnosis of malignancy, infectious and inflammatory processes, as well as in the assessment for dysplasia in non-neoplastic entities (e.g., Barrett's esophagus, primary sclerosing cholangitis).

6.1.3 Staging Application

Utilization of cytologic samples is an important aspect of cancer staging. In many clinical scenarios, EUS-FNA and CT/ultrasound-FNA are utilized to improve cancer staging in the esophagus, stomach, and pancreaticobiliary trees. EUS-FNA allows for sampling of local and metastatic lymph nodes and ascitic fluid to stage gastrointestinal malignancies. CT/ultrasound-FNA allows sampling of many sites further away from the gastrointestinal tract to assess for metastatic disease. Both of these diagnostic modalities have become a mainstay in locoregional and distant cancer staging.

6.2 Esophagus

6.2.1 Non-Neoplastic Conditions: Esophagitis

The most common non-neoplastic disease of the esophagus is esophagitis. Esophagitis can be caused by gastroesophageal reflux disease (GERD), infections (e.g., bacteria, cytomegalovirus (CMV) [8], Herpes simplex virus (HSV) [8, 9], Candida spp. [9], and human papilloma virus (HPV) [10], irritants (e.g., alcohol, smoking, and hot drinks), trauma, radiation/chemotherapy, and autoimmune diseases (e.g., Crohn's disease, sarcoidosis, and eosinophilic esophagitis). Infectious esophagitis is commonly encountered in immunocompromised patients.

6.2.1.1 Diagnostic Challenges and Techniques

There are many challenges in diagnosis of esophagitis. The most difficult area for diagnosis occurs with infectious etiologies. While in some circumstances, there be numerous organisms present (Candida spp.), in other situations we may struggle to find more than a single organism or virus-infected cell. While endoscopic biopsy has largely replaced cytology in esophagitis, a combination of modalities may in fact provide the greatest yield [9]. Special and immunohistochemical (IHC) stains can be performed on smears, cytospin slides, cell blocks, or biopsies. Gram stain is for bacteria, GMS and PAS stains for fungi, and acid fast or FITE stains for mycobacteria. IHC stains for CMV, HSV and *Helicobacter pylori* are available for confirmation. Cultures are also useful for identification of infectious agents and are used to detect sensitivity/resistance to medications.

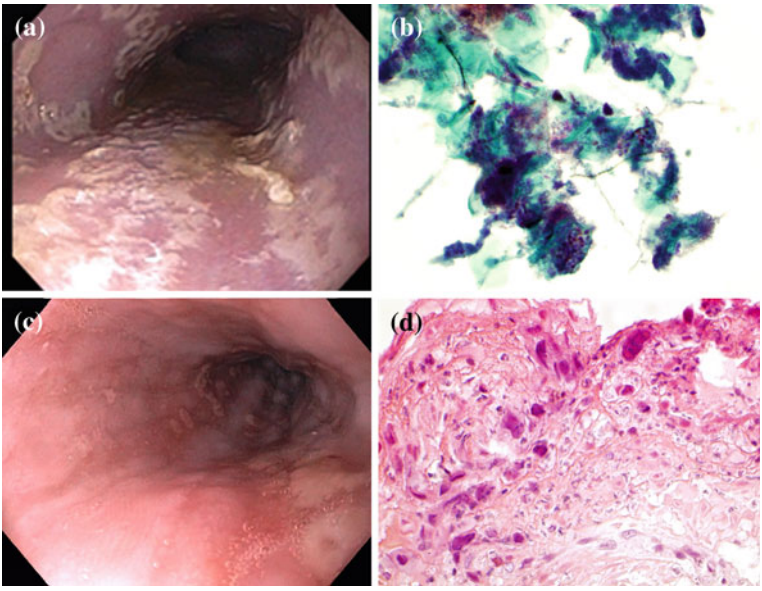

Fig. 6.1 **a** Endoscopy image of esophageal candidiasis. **b** Brushing cytology of esophageal candidiasis, ThinPrep, Pap stain, 600×. **c** Endoscopy image of esophageal HSV infection. **d** Biopsy of esophageal HSV infection, H and E stain, 600×

6.2.1.2 Application of Endoscopy and Cytology in Esophagitis

Esophageal candidiasis is the most common cause of infectious esophagitis and is often seen in immunosuppressed or antibiotic-treated patients. Endoscopy reveals a white plaque on an erythematous mucosa, and possibly erosion or ulceration (Fig. 6.1a). Brushing cytology is more sensitive than biopsy for diagnosing Candida esophagitis (sensitivity of 100 and 67 %, respectively), and shows typical budding yeasts and pseudohyphae (Fig. 6.1b) [9]. The clinician needs to distinguish disease from oral contamination. Inflammatory cells (predominantly neutrophils), necrotic debris, and reactive/reparative cells can be seen. GMS stain on smeared slides or cellblock and culture can be ordered for any suspicious cases, but is usually not necessary.

Herpes virus infection is more often seen with immunosuppression. Endoscopy may reveal vesicles and ulcers (Fig. 6.1c). Biopsy is similar or more sensitive than brushing cytology for Herpes esophagitis [8, 9]. Virus-infected cells are found at the edge of ulcers versus the base. Infected cells show intranuclear inclusions with multinucleation, nuclear molding, chromatin margination, and ground glass appearance (Fig. 6.1d). Reactive and reparative changes, inflammatory cells, necrotic debris, and granulation tissue may be seen. IHC staining should be ordered for suspicious cases, but culture is not helpful for diagnosis [8].

CMV esophagitis is generally associated with immunodeficiency. Endoscopic biopsy is the best method for diagnosis, while brushing cytology and culture add little to diagnose CMV (sensitivity, 0 and 27 %, respectively) [8]. Endoscopy generally reveals ulcers; contrary to Herpes infection, CMV-infected cells are found in the base of the ulcer. It is therefore imperative that the center and edges of all ulcers are sampled endoscopically to ensure adequate diagnosis. CMV infects glandular cells and endothelial cells. The infected cells are enlarged with large intranuclear or cytoplasmic inclusions with halos (Fig. 6.1). IHC stain for CMV can be used if there is suspicion for CMV infection.

HPV infection causes esophagitis [10], squamous papillomas [11], flat condylomas [12], and squamous cell carcinoma [13]. However, some studies showed no association between HPV infection and esophageal squamous cell carcinoma [14]. Cytologic features of HPV infection are koilocytes similar to those seen in cervical Pap tests. IHC p16 stain is helpful for confirming HPV infection and grading dysplasia.

6.2.2 Barrett's Esophagus

Barrett's esophagus is defined as "a change in the esophageal epithelium of any length that can be recognized at endoscopy and is confirmed to have intestinal metaplasia by biopsy" [11]. GERD is the most common type of esophagitis in the USA and may result in Barrett's esophagus, dysplasia, and adenocarcinoma with an odds ratio of 7 in chronic reflux and 43.5 in longstanding and severe reflux [15–17]. Long-term surveillance of patients with Barrett's esophagus is recommended to detect the occurrence of dysplasia and adenocarcinoma.

6.2.2.1 Diagnostic Challenges and Techniques

Tissue acquisition in Barrett's esophagus has numerous limitations. The endoscopist should utilize methods of sampling that allow for maximal surface area coverage. Despite the fact that endoscopic biopsy has largely replaced brushing cytology in dysplasia surveillance, it still remains suboptimal. Endoscopic sampling error accounts for much difficulties in dysplasia assessment in Barrett's esophagus (Fig. 6.2a). The majority of dysplasia in Barrett's esophagus may be unrecognizable from the surrounding intestinal metaplasia endoscopically. While enhanced endoscopic imaging modalities (e.g., narrow band imaging and confocal endomicroscopy) have been promising, their role is still unclear [18]. It is therefore essential to follow a methodical technique in tissue sampling (e.g., Seattle protocol) to minimize sampling error [19].

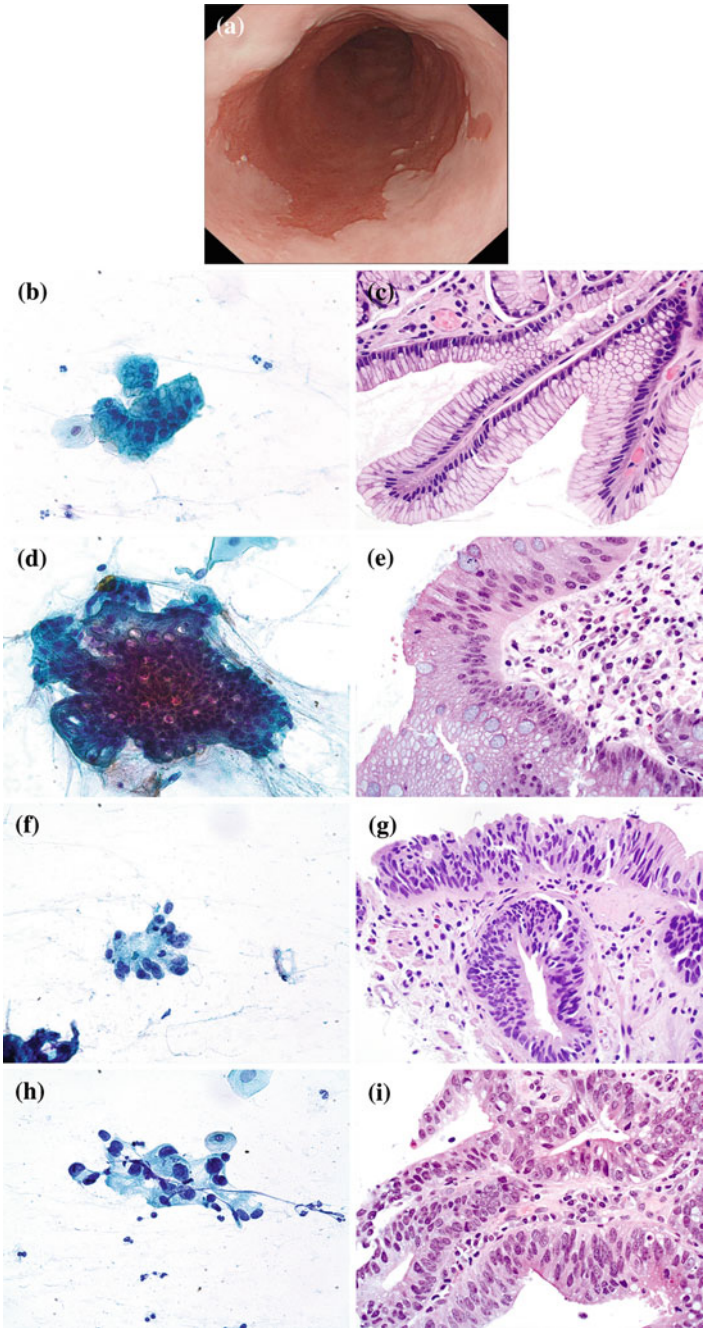

Fig. 6.2 **a** Endoscopic image of Barrett's esophagus. **b** Brushing cytology of normal mucosa, Pap stain, 600×. **c** Biopsy of normal mucosa, H and E stain, 400×. **d** Brushing cytology of Barrett's esophagus, Pap stain, 600×. **e** Biopsy of Barrett's esophagus, H and E stain, 400×. **f** Brushing cytology of low-grade dysplasia, Pap stain, 600×. **g** Biopsy of low-grade dysplasia, H and E stain, 400×. **h** Brushing cytology of high-grade dysplasia, Pap stain, 600×. **i** Biopsy of high-grade dysplasia, H and E stain, 400x

6.2.2.2 Application of Cytology in Barrett's Esophagus

Nowadays, most institutes in the USA utilize endoscopic biopsies to diagnose Barrett's esophagus and follow up the patients, as it is more sensitive and specific than brushing cytology to diagnose Barrett's esophagus (sensitivity, 92 vs. 60 %) [9]. Esophageal brushing cytology combined with detection of a broad panel of loss of heterozygosity (LOH) targeting tumor suppressor genes is more sensitive and accurate to diagnose Barrett's esophagus, low-, high-grade dysplasia and adenocarcinoma than biopsy histomorphology, brushing cytology, and combination of biopsy histomorphology with detection of the same panel of LOH [1]. As compared with brushing cytology, FISH targeting oncogenes and tumor suppressor genes increases sensitivity to detect low-grade dysplasia (from 5 to 50 %), high-grade dysplasia (from 32 to 82 %) and carcinoma (from 45 to 100 %) [20, 21]. The sensitivity of DNA ploidy analysis for these lesions is similar to that of cytology [20].

Barrett's Esophagus

The features of Barrett's esophagus detected by endoscopic biopsy are the presence of intestinal metaplasia including goblet cells (Fig. 6.2e). Proliferation of basal and parabasal cells, extension of papillae of connective tissue close to the mucosal surface (GERD), and an acute and/or chronic inflammation in the mucosa (reflux esophagitis) may be also seen. The features of Barrett's esophagus in brushing cytology (smears, cytospin, or ThinPrep) are the presence of goblet cells (large cytoplasmic vacuoles compressing the nuclei to one side) embedded in cuboidal or columnar intestinal metaplastic cells arranged in small nests or acini (Fig. 6.2d) [9, 22, 23]. Reactive/reparative epithelial cells, inflammatory cells (neutrophils, lymphocytes, and possibly eosinophils), and gastric epithelial cells may be seen. Alcian blue stain may be helpful to confirm the presence of goblet cells in intestinal metaplasia.

Low-Grade Dysplasia

Endoscopic biopsy histomorphology is much more sensitive for diagnosing low-grade dysplasia than brushing cytology (sensitivity, 97 vs. 20 %) [9]. The features of LGD on biopsy are the presence of columnar cells with crowded, enlarged, elongated, pseudostratified (confined to the lower half of the glandular epithelium), and hyperchromatic nuclei with mild pleomorphism, increased nuclear to cytoplasmic ratio, decreased cytoplasmic mucin, and minimal architectural changes (Fig. 6.2g) [17, 22]. The features of LGD on brushing cytology are the presence of small clusters or acini of columnar cells with crowded, enlarged, elongated and hyperchromatic nuclei, and increased nuclear to cytoplasm ratio (Fig. 6.2f) [22,

23] Differential diagnosis includes benign gastroesophageal junctional mucosa, Barrett's esophagus, and high-grade dysplasia.

High-Grade Dysplasia

Endoscopic biopsy histomorphology shows marked architectural aberrations including cribriform glands, marked pleomorphism, nuclear stratification extending to the upper part of the cells and glands, and decrease in or loss of mucin secretion, and frequent mitosis (Fig. 6.2i) [17, 22]. On brushing cytology, HGD shows two- to three-dimensional small clusters or acini of cuboidal to columnar cells with crowded, pleomorphic, enlarged and hyperchromatic nuclei with irregular nuclear membranes and prominent nucleoli, and increased nuclear to cytoplasm ratio (Fig. 6.2h) [22, 23]. Few single atypical glandular cells and rare mitoses may be seen. Tumor diathesis is absent. The differential diagnosis of HGD on cytology includes reactive and reparative atypia, LGD, and invasive adenocarcinoma.

6.2.2.3 Therapeutic Impact

Adequate tissue sampling in Barrett's esophagus has been a limitation. Utilization of jumbo biopsy forceps, addition of cytology to biopsy, enhanced imaging techniques (e.g., narrow band imaging), and emphasis to all endoscopists to follow some sampling protocol may ultimately improve our diagnostic yield. In an era of improved endoscopic therapies for Barrett's esophagus with dysplasia (e.g., radiofrequency ablation), it is more essential to improve our diagnostic technique. Improving our abilities to identify dysplasia early will allow for endoscopic therapies to be more effective in reducing the rise of esophageal adenocarcinoma.

6.2.3 Neoplastic Conditions

6.2.3.1 Esophageal Adenocarcinoma

The incidence of esophageal adenocarcinoma is estimated to be between 1 and 4 per 100,000 per year in the USA and approaches or exceeds that of squamous cell esophageal cancer [17]. There is a distinct predominance in white males (male: female ratio 7:1). Barrett's esophagus is the single most important precursor lesion and risk factor for distal esophageal adenocarcinoma [17]. Other etiologies include tobacco, obesity, alcohol, and *H. pylori* [17].

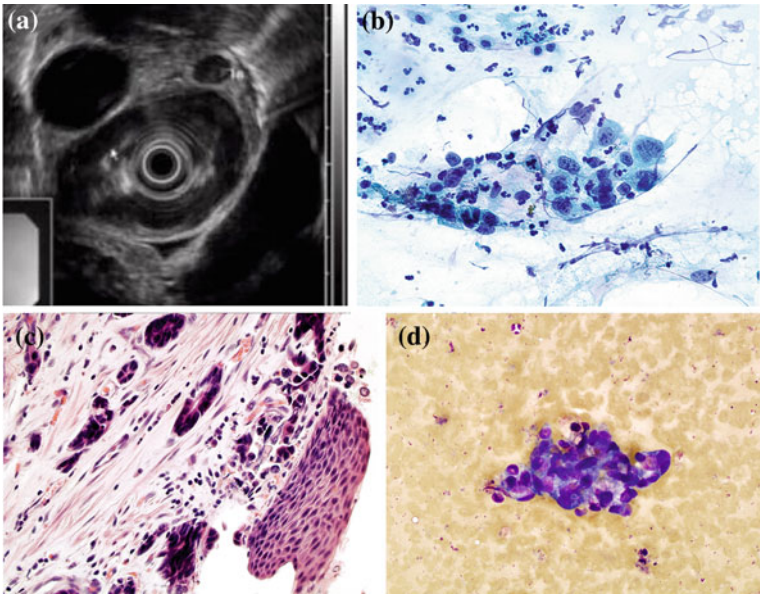

Fig. 6.3 Gastroesophageal junctional adenocarcinoma. **a** Endoscopy image. **b** Brushing cytology, Pap stain, 600×. **c** Biopsy, H and E stain, 400×. **d** EUS-FNA smear of metastatic gastroesophageal junctional adenocarcinoma in an enlarged 1.1-cm peri-esophageal lymph node, Diff Quik stain, 400×

Role of EUS-FNA in Staging Esophagus Adenocarcinoma

EUS has become an integral component of initial esophageal cancer staging (Fig. 6.3a). The accuracy of EUS for T-staging is 89 % [24]. The sensitivity and specificity for regional lymph node metastasis is 80 and 70 %, respectively and for celiac lymph node metastasis it is 85/96 % [25]. While EUS-FNA provides the most accurate locoregional staging, CT and PET are essential to assess for metastatic disease. Additional studies have demonstrated EUS-FNA to be cost-effective for preoperative staging of esophageal cancer [26]. Of note, EUS-FNA is not very effective in staging after neoadjuvant therapy.

Application of Cytology in Diagnosis of Esophageal Adenocarcinoma

Brushing cytology has similar sensitivity as biopsy for diagnosis of adenocarcinoma (96 vs. 91 %) [9]. The diagnostic accuracy of touch smear in esophageal malignancy was significantly higher (94.12 %) than brushing and crush smears (89.71 % each), and endoscopic biopsy had diagnostic accuracy of 88.24 % [27]. The diagnostic accuracy of combined brushing and biopsy was 100 %; it was 97.06 % for touch smears combined with biopsy [27].

Brushing and FNA cytology of adenocarcinoma is characterized by the presence of pleomorphic cuboidal, columnar or polygonal cells present singly or arranged in loosely cohesive and crowded three-dimensional clusters, acini or papillae (Fig. 6.3b) [23]. The nuclei are enlarged and hyperchromatic, with abnormal chromatin and one or more prominent nucleoli [23]. The cytoplasm is delicate, granular or vacuolar, and may contain mucin. More single pleomorphic cells and tumor diathesis are seen. Signet ring cell adenocarcinoma is characterized by the presence of single or loosely cohesive tumor cells with cytoplasmic mucin pushing hyperchromatic dysplastic nuclei to one side. Differential diagnosis includes HGD, reactive/reparative atypia, metastatic adenocarcinoma, and non-keratinizing squamous cell carcinoma. The tumor cells are positive for CK7 and possibly positive for CK20 and CDX2.

Therapeutic Impact

Accurate T and N staging is essential for optimizing therapy in esophageal cancer. In patients with potentially operable disease, the median survival strongly correlates with disease stage (AJCC). EUS-FNA allows for stratification based on locoregional staging. Patients with locally advance disease (T3 or greater) and/or evidence of lymph node involvement generally will be treated with neoadjuvant chemotherapy and radiation (Fig. 6.3d). Those patients with locally limited disease as determined by EUS-FNA (T2 or less without LN) potentially would be offered curative resection.

6.2.3.2 Squamous Cell Carcinoma

The annual incidence of squamous cell carcinoma does not exceed 5 per 100,000 in males and 1 per 100,000 in females [28]. It is 2–3 times more frequent in blacks [28]. The median age is 65. Contrary to the rise of esophageal adenocarcinoma, squamous cell carcinoma has decreased in incidence significantly [29]. Etiologies include tobacco, alcohol, nutrition, hot beverages, and HPV.

Diagnostic Challenges and Techniques

Similar diagnostic dilemmas exist in accurately diagnosing this disease [29]. While intestinal metaplasia can be clearly visualized in the esophagus, squamous dysplasia may be very subtle and require adjuvant imaging techniques. Endoscopy may reveal polypoid, flat, or ulcerative masses (Fig. 6.4a). Using iodine staining with the pink sign as a diagnostic index for endoscopy to diagnose high-grade intra-epithelial squamous neoplasia and squamous cell carcinoma, sensitivity is 91.9 % and specificity is 94.0 % [30]. Most cases can be diagnosed by cytology [31]. Biopsy and cytologic screening (brushing or esophageal balloon)

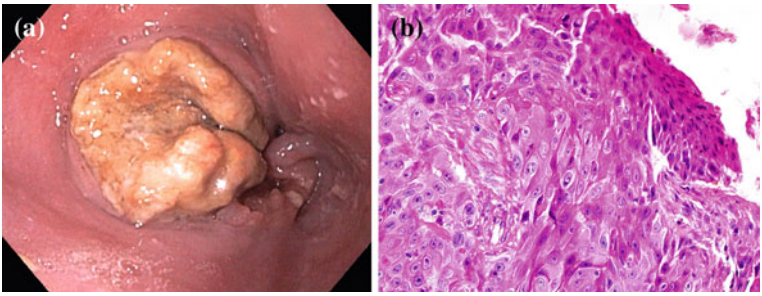

Fig. 6.4 Esophageal squamous cell carcinoma. **a** Endoscopy image. **b** Biopsy, H and E stain, 400×

of high-risk populations could decrease the mortality by facilitating early detection of the disease (Fig. 6.4b) [32].

Application of Cytology in Diagnosis of Esophageal Squamous Cell Carcinoma

Squamous cell carcinoma can be keratinizing or non-keratinizing. Keratinizing SCC is characterized by the presence of single- or three-dimensional clusters of marked pleomorphic (polygonal, bizarre, and spindle in shape) keratinizing squamous cells (Fig.6.4b). Non-keratinizing squamous cell carcinoma is characterized by the presence of single- or three-dimensional clusters of polygonal or spindle cells arranged like streams of fish or swirls and with high nuclear to cytoplasm ratios. Basaloid squamous cell carcinoma is characterized by the presence of cohesive three-dimensional clusters of cells with basaloid appearance (small, round to oval, hyperchromatic nuclei, and scant cytoplasm). Spindle squamous cell carcinoma is characterized by the presence of spindle-shaped non-keratinizing squamous cell carcinoma cells.

Differential diagnosis includes poorly differentiated adenocarcinoma, reactive/reparative squamous cells, radiation and chemotherapy effects, and squamous cell dysplasia. Basaloid squamous cell carcinoma should be distinguished from small cell carcinoma. Spindle squamous cell carcinoma should be distinguished from sarcoma or GIST. Immunohistochemical markers, p63, CK5/6, and K903 can be used to confirm squamous cell carcinoma.

Squamous cell dysplasia and carcinoma in situ are the precursors of invasive squamous cell carcinoma. Endoscopy may reveal normal mucosa, white plaques, or red areas. Using iodine staining, endoscopy can diagnose high-grade squamous dysplasia with high sensitivity [30]. Biopsy can accurately evaluate the degree of dysplasia. Brushing cytology may show atypical squamous cells with no tumor diathesis. Differential diagnosis includes reactive/reparative squamous cells, chemotherapy or radiation effect, and squamous cell carcinoma.

Combination of brushing cytology and molecular analysis targeting tumor suppressor genes and oncogenes as well as microRNA will increase the sensitivity and specificity to early diagnose precursor lesions (dysplasia and carcinoma in situ).

Therapeutic Impact

The role of EUS in squamous cell carcinoma of the esophagus is similar to adenocarcinoma as described above. Adequate locoregional staging has similar impact on management and ultimate outcome.

6.2.3.3 Other Esophageal Neoplasms

Benign or malignant mesenchymal tumors (e.g., leiomyoma, leiomyosarcoma, gastrointestinal stromal tumor (GIST), etc.), low- and high-grade endocrine neoplasms (carcinoid, endocrine carcinoma, and small cell carcinoma), low- and high-grade lymphomas, melanoma, and metastases (e.g., carcinoma, sarcoma, thymic carcinoma, etc.) can be seen in esophagus or paraesophageal lymph nodes or tissue, which should be included in the differential diagnosis based on the cytomorphology. IHC stains should be ordered based on the cytomorphology and clinical information (history, imaging studies, etc.).

Diagnostic Challenges and Techniques

The other neoplasms seen in the esophagus can be divided into mucosal and submucosal lesions. The mucosal lesions are generally endoscopically visible and diagnosis made with mucosal biopsy and/or brushing cytology. Submucosal lesions provide a diagnostic dilemma as they are generally not amenable to simple mucosal biopsy or brushing cytology. EUS-FNA is essential in obtaining tissue for diagnosis and IHC [33]. Submucosal lesions are easily identified by EUS and amenable to FNA. The most common clinical scenario is distinction between leiomyoma and GIST. This requires demonstration of not only a spindle cell neoplasm but also adequate IHC for desmin versus CD117 and CD34. The sensitivity of EUS-FNA for diagnosis of GIST is 78 % [33]. The addition of core biopsy does not appear to significantly improve diagnostic yield [33]. The introduction of tunneled jumbo biopsies in ulcerated lesions can be very effective [34].

Therapeutic Impact

EUS-FNA diagnosis and immunohistochemical studies are essential in management of submucosal lesions of the esophagus. EUS-FNA and biopsy may facilitate

preoperative diagnosis of these tumors. Application of cytology (cytomorphology and ancillary tests) and therapeutic impact will be discussed in the following section.

6.3 Stomach

6.3.1 Non-Neoplastic Conditions

6.3.1.1 Gastritis

Diagnostic Challenges and Techniques

Gastritis is generally discovered on upper endoscopy. The majority of causes of gastritis can be diagnosed with standard mucosal biopsy or brushing. Most gastroenterologists utilize mucosal biopsy to make the diagnosis of *H. Pylori* gastritis or other causes. Occasionally, there may be an infiltrative cause of gastritis (e.g., eosinophilic gastroenteritis or amyloidosis). In these circumstances, jumbo biopsy forceps may improve diagnostic yield.

Cytology Application in Gastritis

Brushings and exfoliate cytology are not commonly used in gastric lesions. EUS-FNA and endoscopic biopsy are the techniques for tissue acquisition.

H. pylori infection is an important factor of acute and chronic gastritis, peptic ulcer, gastric adenocarcinoma, and gastric MALT lymphoma [35]. Gastric brushing cytology provides a sensitive, inexpensive, accurate, and easy technique for rapid detection of *H. pylori* infection, although it is not often used in diagnosis of gastritis [36]. Sensitivity of brushing cytology to identify *H. pylori* (95 %) was higher than that of biopsy histology (80.5 %) and rapid urease test (RUT) (72 %) [36]. *H. pylori* is 1–3-μm-curved or "seagull"-shaped bacteria. Markedly increase in neutrophils and/or lymphocytes, and a reactive/reparative glandular epithelium can be seen in brushing cytology, and superficial acute and chronic inflammation, erosion or an ulcer can be seen in gastric biopsy. The differential diagnosis includes Gastrospirillum hominis, which is longer and more tightly coiled. IHC stain for *H. pylori* or special Giemsa stain should be ordered for suspicious cases.

If abundant neutrophils are seen on smears from brushing and FNA specimens without the presence of atypical epithelial, mesenchymal or lymphoid cells, acute gastritis of varied causes is favored. If abundant lymphoid cells are seen, differential diagnosis includes chronic gastritis and lymphoma. If atypical lymphoid cells are identified, an aliquot of sample should be sent for flow cytometry analysis for lymphoma and a biopsy specimen should be obtained for histology

examination and possible IHC studies. The sensitivity of gastric brushing cytology in diagnosis of gastric lymphoma was approximately 48 % in one study, all of which were large B cell lymphoma [37]. If abundant foamy macrophages are seen, differential diagnosis should include atypical mycobacteria infection (MAI) and xanthelasma, and an aliquot of the specimen should be sent for culture or special studies for mycobacteria. If inflammatory cells as well as atypical epithelial or mesenchymal cells are seen, a biopsy or FNA specimen for cellblock should be obtained for histologic examination and possible IHC studies to distinguish neoplasia from reactive/reparative atypia due to inflammatory processes. IHC studies can also be performed on smear slides.

Therapeutic Impact

While endoscopy with biopsy generally can provide a diagnosis of gastritis and its etiology, only 63 % of patients biopsied for gastritis truly had histologic evidence of gastritis [38]. Therapy is dependent on histologic or cytologic assessment. Early eradication of *H. Pylori* is essential to decrease risk for gastric cancer and MALT lymphoma [38].

6.3.1.2 Polyps

Gastric polyps are divided into non-neoplastic and neoplastic [39] and the majority are benign in nature. Most of them measure less than 1 cm [40]. Hyperplastic polyps were the most frequent (71.3 %), whereas fundic gland polyps accounted for 16.3 % and adenomatous polyps for 12.4 % [40]. Hyperplastic and adenomatous polyps were primarily single, whereas fundic gland polyps tended to be multiple [40]. Adenocarcinoma was detected in 0.9 % of hyperplastic polyps and in 10.5 % adenomatous polyps [40]. High-grade dysplastic foci were found in 21 % adenomatous polyps [40]. Histopathological identification is not possible by endoscopic impression thus diagnosis and treatment will depend on biopsy results [40].

Diagnostic Challenges and Techniques

As polyps are mucosal in nature, they are amenable to mucosal biopsy or endoscopic polypectomy. Cytology is seldom used in evaluation of gastric polyps. In general, there is limited challenge with gastric polyps. If the polyp is large or has any suspicion for adenoma, it can be removed in its entirety with snare polypectomy or endoscopic mucosal resection [41].

6.3.2 Neoplastic Conditions

6.3.2.1 Gastric Adenocarcinoma

Gastric cancer was the second commonest cancer in the world, but its incidence and mortality rate declined steadily worldwide [42]. The incidence increases with age in both males and females [42]. The etiology includes diet, bile reflux, *H. pylori* infection, excessive cell proliferation, oxidative stress, interference with antioxidant functions, and DNA damage [42].

Role of EUS-FNA in Diagnosis and Staging of Gastric Adenocarcinoma

EUS is an essential component of T and N staging in gastric adenocarcinoma. This has become increasingly important with the advent of endoscopic submucosal resection in the eastern world. The accuracy of T and N staging is near 90 % in gastric adencocarcinoma [43]. Similar to esophageal cancer staging, EUS-FNA is excellent for locoregional staging and LN sampling (Fig. 6.5c). It is essential to combine EUS-FNA with CT imaging to assess for metastatic disease. Endoscopy may show polypoid, fungating, or ulcerated lesions (Fig. 6.5a). It tends to occur on the greater curvature of the stomach.

Cytology Application in Gastric Adenocarcinoma

Cytology is seldom used in the diagnosis of gastric adenocarcinoma. Endoscopic biopsy and EUS-FNA are the techniques to acquire specimens for diagnosis (Fig. 6.5b). The diagnostic accuracy in gastric malignancy was 75 % for brushing alone, which was significantly lower than touch smear (87.5 %) and endoscopic biopsy (87.5 %) [27]. The diagnostic yield for crush smear was 81.25 %. A combination of touch smears and biopsy had a diagnostic yield of 100 %; it was 93.75 % for combined brushings and biopsy [27].

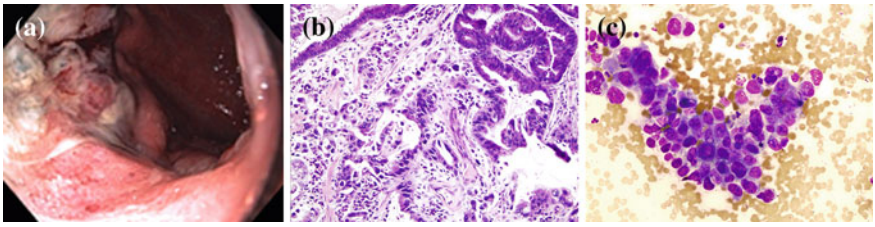

Fig. 6.5 **a** Endoscopy image of fungating gastric adenocarcinoma. **b** Biopsy of severe dysplasia and invasive gastric adenocarcinoma, H and E stain, 200×. **c** EUS-FNA smear of an enlarged 1.5-cm peri-gastric lymph node of metastatic gastric adenocarcinoma, Diff Quik stain, 400×

Brushing and FNA cytomorphology of gastric adenocarcinoma is similar to that seen in esophageal adenocarcinoma. Tumor cells are positive for CK7, possibly for CK20 and CDX-2.

Differential diagnosis includes reactive atypia, dysplasia, endocrine neoplasm, and metastatic adenocarcinoma. Presence of single, viable dysplastic epithelial cells, and tumor diathesis is helpful to establish a diagnosis of adenocarcinoma.

Therapeutic Impact

Tissue acquisition and accurate staging are important in the management of gastric cancer. This includes endoscopic and surgical resection for early lesions and chemotherapy and radiation for more advanced lesions. Perhaps the biggest diagnostic dilemma in gastric cancer lies in patients with premalignant conditions. There is a constant struggle in patients with gastric intestinal metaplasia and/or atrophic gastritis with surveillance [44]. It remains unclear what the optimal biopsy strategy and surveillance interval should be in these patients [45].

6.3.2.2 Lymphoma

The gastrointestinal tract, particularly the stomach, is the most common extranodal site of non-Hodgkin lymphoma, particularly B cell types [46]. Stomach lymphomas are considered primary if the main bulk of disease is present in the stomach. Gastrointestinal tract lymphomas account for 4–18 % of all non-Hodgkin lymphoma in Western countries, 25 % of cases in the Middle East, and 10 % of all gastric malignancies [46]. The majority of gastric lymphomas are high-grade B-cell lymphomas, some of which have developed through progression from low-grade lymphomas of mucosa associated lymphoid tissue (MALT) [46]. MALT lymphoma is most common in patients over 50 years at presentation. Predisposing factors include *H. pylori*, immunocompromised states, celiac disease, and chronic inflammatory bowel disease [46].

Role of EUS-FNA in Diagnosis and Staging of Gastric Lymphoma

The diagnosis of gastric lymphoma is generally made by mucosal biopsy. However, CT and upper endoscopy do not allow for accurate locoregional staging. EUS has been shown high accuracy in assessing gastric wall thickness and involvement by gastric lymphoma and for assessment of treatment response (Fig. 6.6a). In addition, utilization of FNA for cytologic analysis improves accuracy in nodal staging in advance disease (Fig. 6.6c) [47].

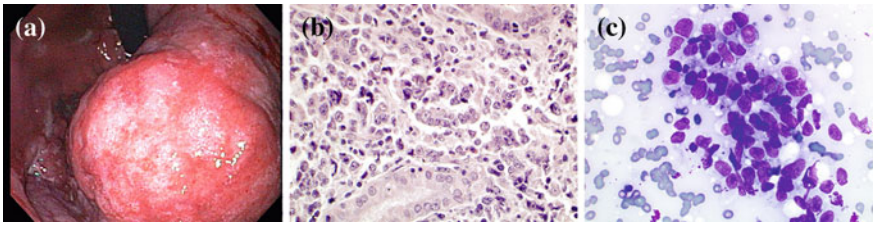

Fig. 6.6 Gastric large B cell lymphoma. **a** Endoscopy image. **b** Biopsy, H and E stain, 600×. **C** EUS-FNA cytology of metastatic gastric large B cell lymphoma in an enlarged peri-gastric lymph node, Diff Quik stain, 600×

Cytology Application in Gastric Lymphoma

Biopsy is a more commonly used technique than FNA and brushing cytology to obtain tissue for diagnosis (Fig. 6.6b). EUS-FNA can also obtain tissue for diagnosis of gastric lymphoma [48, 49]. FNA cytology of MALT lymphoma is characterized by the presence of monotonous to polymorphic, small to medium-sized lymphoid cells with minimal nuclear membrane irregularity, scant to abundant cytoplasm, plasmacytoid or monocytoid features [48, 49]. Large cell transformation is characterized by the presence of large atypical lymphoid cells with irregular nuclear membranes, vesicular chromatin and one or multiple prominent nucleoli, and moderate cytoplasm (Fig. 6.6b). Necrosis, mitotic, DNA strings, and apoptotic figures may be seen.

Differential diagnosis includes chronic gastritis, endocrine neoplasms, and poorly differentiated adenocarcinoma. FNA specimens can be used to make cell-block for histologic examination, immunohistochemistry or molecular studies, or submitted for flow cytometry analysis. MALT lymphoma cells are positive for CD20, CD19, CD79a, PAX-5, and CD43, while negative for CD5, CD10, and CD23. Molecular studies demonstrate 11:18(q21:q21) translocation, which can be detected by FISH on smears or cytospin slides. Large cell lymphoma cells are positive for CD19 and CD20, and possibly positive for CD10 and BCL-6. Immunohistochemistry and in situ hybridization for Kappa and Lambda light chain may show light chain restriction. Flow cytometry may not show a clonal proliferation of abnormal lymphoid cells in large B cell lymphoma, and thus may be not useful in the diagnosis of large B cell lymphoma.

Therapeutic Impact

Mucosal biopsies are generally obtained for diagnosis of MALT lymphoma. However, similar to other lymphomas for optimal staging, additional sampling (cytology with brushing or EUS-FNA) may improve overall yield. Detecting the presence of *H. Pylori* is essential for both diagnostic and therapeutic purposes. Adequate tissue sampling along with proper staging (EUS and endoscopy) allows

for patients to be treated appropriately from simple *H. Pylori* eradication to systemic chemotherapy for advanced disesase [50].

6.3.2.3 Endocrine Neoplasms

Most endocrine tumors of the stomach are well differentiated, nonfunctioning enterochromaffin-like cell carcinoids arising from oxyntic mucosa in the corpus or fundus [51]. Carcinoid of stomach is composed of three types: (1) type I, associated with autoimmune chronic atrophic gastritis; (2) type II, associated with multiple endocrine neoplasia type-1 (MEN-1) and Zollinger–'Ellison syndrome; and (3) type III, sporadic, i.e., not associated with hyper gastrinaemia or autoimmune chronic atrophic gastritis. Carcinoid is the second most common epithelial tumor of the stomach and accounts for 11–41 % of all gastrointestinal carcinoids [51]. Gastric carcinoids are often multiple. Early diagnosis of carcinoid tumors in the gastrointestinal tract allows for possible endoscopic mucosal resection. EUS examination can determine the depth of this subepithelial lesion and determine if it is amenable for endoscopic mucosal resection or surgery. Endoscopic biopsy is much more commonly used technique than EUS-FNA for gastric endocrine tumor. The FNA cytology characteristics of endocrine tumors are discussed in the pancreas section.

6.3.2.4 Subepithelial Mesenchymal Neoplasms

Stomach is the most common site for gastrointestinal mesenchymal neoplasms [52]. The most common gastrointestinal mesenchymal neoplasms are GIST or smooth muscle neoplasms [52].

Diagnostic Challenges and Techniques

Submucosal lesions provide a diagnostic dilemma as they are generally not amenable to simple mucosal biopsy or brushing cytology. Submucosal lesions are easily identified by EUS and amenable to FNA. EUS-FNA is essential in obtaining tissue for diagnosis and immunohistochemistry [33, 48]. The sensitivity of EUS-FNA for diagnosis of GIST is 78 % [33]. The addition of core biopsy does not appear to significantly improve diagnostic yield [33]. The introduction of tunneled jumbo biopsies in ulcerated lesions can be very effective [34].

Cytology Application in Subepithelial Mesenchymal Neoplasms

GIST

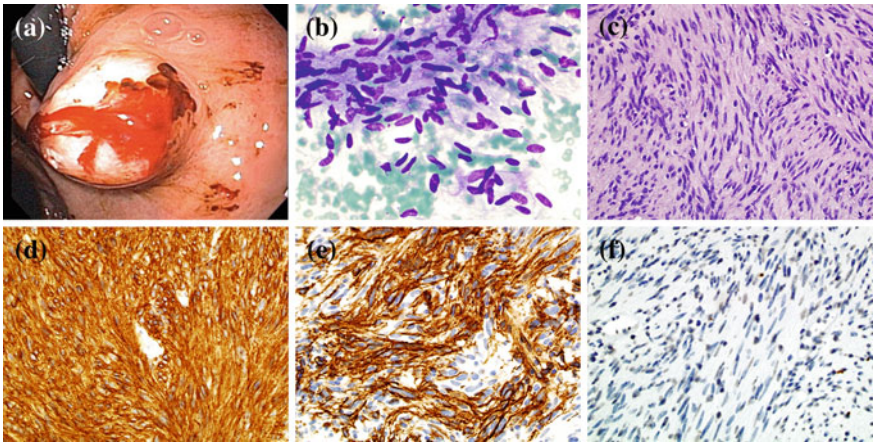

Fig. 6.7 Gastrointestinal stromal tumor of stomach. **a** Endoscopy image. **b** Touch preparation of a percutaneous needle core biopsy of 4 cm mass attached to gastric wall of a 58-year-old male patient, Diff Quik stain, 600×. **c** Needle core biopsy, H and E stain, 400×. **d** IHC stain for CD117, 400×. **e** IHC stain for CD34, 400×. **f** IHC stain for desmin, 400×

GIST accounts for 2.2 % of malignant gastric tumors with no gender preference. Patients are generally older than 60 years of age. The sensitivity of EUS-FNA cytology to diagnose gastric GIST was 84.4–100 % and is influenced by size, location, shape, and layer of origin [33, 48, 53]. Diagnostic FNA cytology samples were characterized by spindle cells (about 86 %), spindle and epithelioid mixture (7–12 %) and epithelioid cells (7 %) that present in either loosely cohesive or tight aggregates with irregular borders [33, 53]. Tumor cells are mostly well organized in one direction and focally palisading with ill-defined cytoplasmic borders (Fig. 6.7) [53]. The nuclei are either spindle or ovoid, and contain fine and evenly distributed chromatin and inconspicuous nucleoli, with or without atypia. Tumor cells are positive for CD117(c-kit), CD34, and vimentin. They are occasionally positive for S100 and smooth muscle actin. Grading of GIST depends on the tumor size and mitoses/50 high power fields [52]. The differential diagnosis includes normal smooth muscle, smooth muscle neoplasms, and fibromatosis.

Smooth Muscle Neoplasms

Well-documented true gastric leiomyomas and leiomyosarcomas are infrequent [52]. They are usually asymptomatic. The cytologic features of EUS-FNA smears and touch preparations of core biopsy are the presence of spindle cells that are well organized in one direction, have "cigar-shaped" nuclei containing fine evenly distributed chromatin, and scant to moderate cytoplasm without clear cytoplasmic borders. Hypercellularity, atypical mitoses, necrosis, and markedly pleomorphism can be seen in leiomyosarcoma. Smooth muscle neoplasms are positive for desmin and smooth muscle actin, and negative for CD117 and CD34. Differential diagnosis includes normal smooth muscle, fibromatosis, and GIST.

Other Mesenchymal Neoplasms
Glomus tumor, Schwannomas, lipoma, granular cell tumor, and Kaposi sarcoma can occur in the stomach.

Therapeutic Impact

Differentiation between leiomyoma and GIST is essential for targeted therapy of subepithelial neoplasms of the stomach. Adequate tissue for IHC is essential to make this distinction. In general, GIST is offered surgical resection or therapy with tyrosine kinase inhibitor, Gleevac. In addition to tissue sampling, EUS staging allows for assessment of size and intralesional features that may predict malignancy [54].

6.4 Pancreaticobiliary System

6.4.1 Non-Neoplastic Diseases: Benign Biliary and Pancreatic Strictures

Benign biliary and pancreatic strictures can be caused by primary sclerosing cholangitis (PSC), pancreatitis, cholangitis, and other inflammatory processes. Benign strictures may mimic ductal adenocarcinoma clinically.

6.4.1.1 Role of ERCP and Brushings in Benign Strictures

Brushings of the common bile duct and pancreatic duct are important in distinguishing benign from malignant disease. Brushing cytology has had low sensitivity (42–88 %) and high specificity (97–100 %) [55]. This can be problematic in inflammatory conditions such as PSC. Atypical brushings may result from regenerative atypia. As a result, other factors are incorporated into this diagnosis such as serum tumor markers (CA19-9). The recent improvement in techniques of peroral cholangioscopy has allowed for direct and improved sampling. However, longitudinal studies are still underway to delineate the role of cholangioscopic directed tissue sampling in benign biliary and pancreatic strictures. In the setting of pancreatitis, endoscopic tissue sampling has a limited role. CT-guided FNA can be important in identifying infection in patients with necrotizing pancreatitis. In chronic pancreatitis, brushing cytology is again utilized to assess for malignant progression, however, is fraught with limitations similar to that seen with PSC. Further investigations are underway to assess the role of both cholangioscopy and pancreatoscopy in improving diagnostic yield in benign disease.

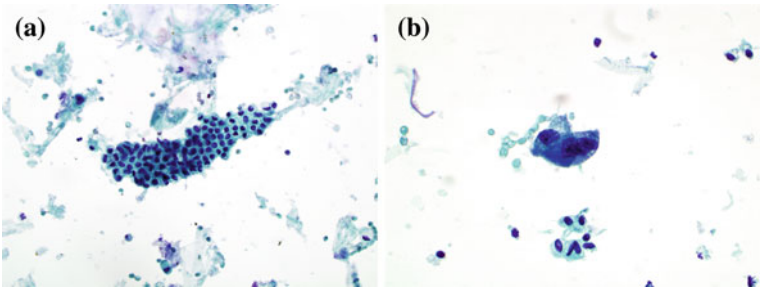

Fig. 6.8 **a** Brushing cytology image of benign stricture of common bile duct, ThinPrep, Pap stain, 400×. **b** Brushing cytology of cholangiocarcinoma of common bile duct, adenocarcinoma cells and benign bile duct epithelial cells, ThinPrep, Pap stain, 600×

6.4.1.2 Cytology Application in Benign Biliary and Pancreatic Strictures

Brushing cytology, biopsy, and FNA are commonly used in evaluation of common bile duct or pancreatic duct stricture. The cytologic features of benign stricture in brushing and FNA cytology are the presence of glandular epithelial cells with reactive atypia that are present in cohesive flat "honeycomb" sheets with maintenance of polarity (Fig. 6.8a). The nuclei may be hyperchromatic, vesicular, degenerative, enlarged, and varied in size, contain prominent single or multiple nucleoli and evenly distributed chromatin, and show smooth nuclear membranes. Normal mitotic figures and inflammatory cells can be seen. The differential diagnosis of reactive atypia includes dysplasia and adenocarcinoma.

6.4.1.3 Therapeutic Impact

In inflammatory conditions such as PSC and chronic pancreatitis, there is a small but true increase in the incidence of malignancy. Identification of cholangiocarcinoma in PSC would potentially allow a patient to be considered for liver transplant. Identification of early malignancy in chronic pancreatitis would potentially allow a patient to be considered for curative pancreaticoduodenectomy.

6.4.2 Cystic Lesions of the Pancreas

Pancreatic cystic lesions are often found incidentally and divided into non-neoplastic (pseudocyst, lymphoepithelial cyst, retention cysts, mucinous non-neoplastic cyst, etc.) and neoplastic lesions (mucinous cystic neoplasms (MCNs), intraductal papillary mucinous neoplasms (IPMNs), serous microcystic adenoma,

solid pseudopapillary cystic neoplasm, teratoma, etc.). Endocrine neoplasms and acinar cell carcinoma can occasionally present as a cystic mass.

6.4.2.1 Role of EUS-FNA in Diagnosis of Cystic Lesions of the Pancreas

A combination of clinical and radiologic features, analysis of the cyst fluid, FNA biopsy, immunohistochemistry, and molecular studies is useful in differentiating the various types of pancreatic cysts [56]. The mainstay of assessment of pancreatic cysts is CT and MRI [57]. Accurate differentiation of cystic neoplasms of the pancreas is essential when considering surgical management. Preoperative distinction between mucinous and non-mucinous, and benign and malignant neoplasms is essential for planning an appropriate treatment strategy [56]. Endosonography allows for detailed characterization of cystic neoplasms and with the addition of FNA provides fluid for biochemical analysis and cytology. EUS examination allows for accurate assessment of size, presence of septation, presence of mural nodularity, and calcification. Increasing size and presence of any of these EUS features are more predictive of malignancy. ERCP is useful to determine the relationship between cystic lesions and the pancreatic duct.

Fluid analysis is generally sent for CEA, amylase, and cytology. A CEA level exceeding 192 has a specificity of 84 % for mucinous neoplasm [58]. Cytologic evaluation in general has had low sensitivity but high specificity for mucinous neoplasms. Recently, cytology has been coupled with molecular analysis. The addition of molecular analysis (e.g., KRAS and LOH) increases sensitivity and specificity of cyst fluid analysis significantly [56]. All patients with pancreatic cystic lesions must be thoroughly investigated to ascertain the underlying nature of the cyst. Small asymptomatic cysts (<3 cm) with no suspicious features on imaging or FNA may be safely followed up [57]. Follow-up should continue for at least 4 years, with a repeat FNA if needed [57].

6.4.2.2 Role Of Cytology in Cystic Lesions of the Pancreas

Pseudocyst

Pseudocyst results from pancreatitis, and is the most common cystic lesion in the pancreas (up to 75 %) [57]. Pseudocysts mostly occur in the tail, are usually unilocular, and lack lining epithelial cells (Fig. 6.9). Clinical presentation and image studies may mimic pancreatic cancer. The FNA-aspirated cyst content is low viscosity, hemorrhagic, dark and opaque [56], and has high amylase and low CEA [56]. The features of FNA cytology are the presence of rare benign appearing or reactive ductal epithelial cells or no epithelial cells, macrophages, hemosiderin/bile pigment, necrotic amorphous debris, inflammatory cells, calcification, fatty necrosis, cholesterol crystals, granulation tissue, and fibrous tissue (Fig. 6.9). Islet

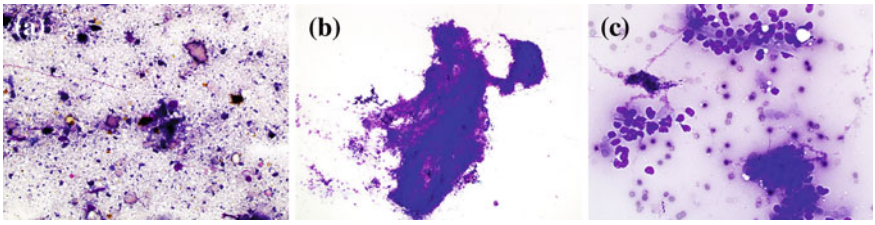

Fig. 6.9 Pancreatic pseudocyst, EUS-FNA. **a** Cyst aspiration, Diff Quik stain, 400×. **b** Fibrous tissue fragment containing chronic inflammation, islet cells and benign bile duct cells, Diff Quik, 100×. **c** Two clusters of islet cells and one cluster of benign bile duct cells, Diff Quik stain, 400×

cells may be present due to hyperplasia, which should not be misinterpreted as endocrine neoplasm. Atypical reactive and reparative ductal cells aspirated from chronic pancreatitis should be distinguished from dysplasia or ductal adenocarcinoma. Atypical reactive and reparative mesenchymal cells and histiocytes should not be mistaken for sarcoma.

Lymphoepithelial Cyst

Lymphoepithelial cyst of the pancreas is a rare non-neoplastic cystic lesion that occurs predominantly in male adults (usually in the 5th and 6th decade) with or without symptoms [59, 60]. The features of FNA cytology are presence of benign squamous cells, keratinous debris, predominantly small and mature lymphocytes, histiocytes, germinal center fragments, multinucleated giant cells, and cholesterol crystals [60, 61] (Fig. 6.10). Differential diagnosis includes benign cystic teratomas including dermoid cyst and epidermal cyst, squamous cell carcinoma [59], and adenosquamous carcinoma.

Mucinous Non-Neoplastic Cyst

Mucinous non-neoplastic cyst is a recently described pancreatic cystic lesion that presents as an isolated unilocular or multilocular mucinous cyst. The cyst is lined by single layer of cuboidal to columnar mucinous epithelium supported by hypocellular stroma and does not communicate with pancreatic ducts [62]. The features of FNA are presence of flat nests or sheets of cuboidal to columnar mucinous cells and abundant background mucus (Fig. 6.11). The mucinous cells have basally located small round to oval nuclei containing fine granular chromatin, and possible small nucleoli. Slightly nuclear membrane irregularity, nuclear grooves, and intranuclear inclusions may be seen. The epithelial cells are positive for MUC-1 and MUC5AC, and negative for MUC-2. Cyst fluid CEA levels are >500 ng/ml.

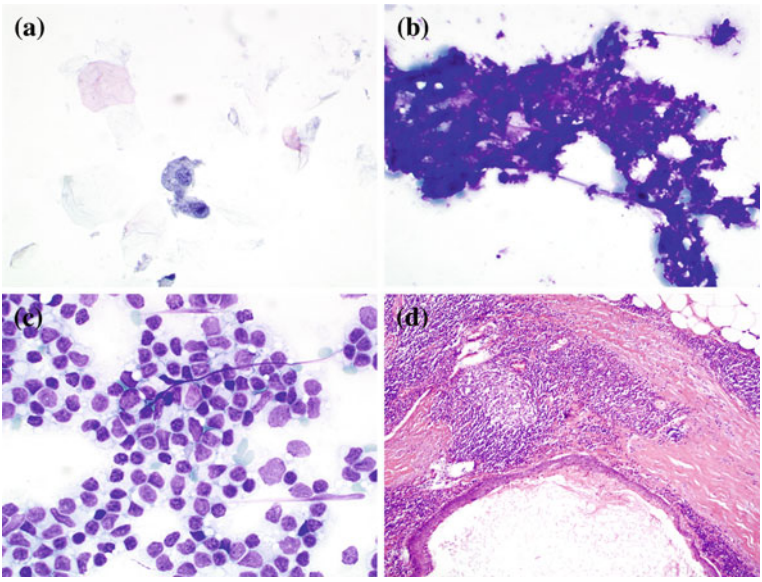

Fig. 6.10 Pancreatic lymphoepithelial cyst, EUS-FNA and excision of cystic mass. **a** Nucleated and anucleated squamous cells, Pap stain, 600×. **b** Necrosis, Diff Quik stain, 600×. **c** Polymorphous lymphocytes, predominantly small mature lymphocytes, Diff Quik, 600×. **d** Excision of cystic mass, H and E stain, 100×

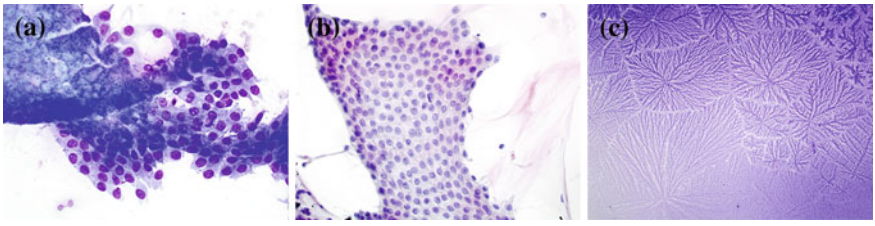

Fig. 6.11 Pancreatic mucinous non-neoplastic cyst, EUS-FNA. **a** Diff Quik stain, 400×. **b** Pap stain, 400×. **c** Extracellular mucin, Diff Quik stain, 100x

Serous Cystic Neoplasms

Serous cystic neoplasms (serous microcystic adenoma, serous oligocystic adenoma, and serous cystadenocarcinoma) are cystic epithelial neoplasms composed of glycogen-rich, ductular-type epithelial cells that produce a watery fluid similar to serum [63]. Most are benign with or without clinical symptoms, and may occur as part of the von Hippel-Lindau syndrome. FNA gives clear fluid. Serous epithelial cells were identified in <20–100 % of FNA specimens with a sensitivity ranging from 3.6 to 100 % [64–66]. The features of FNA cytology are the presence of loose flat nests or "honeycombed" sheets of bland cuboidal epithelial cells with

small and round nuclei containing fine chromatin and possibly containing nucleoli [64, 67]. The cytoplasm is moderate in amount and clear to finely granular or vacuolar with indistinct cell borders. Mitotic figures and necrosis are absent. CEA levels are <5 ng/ml, CA-19.9 levels are low, and amylase levels range from 11 to 90 U/L [64, 67].

Mucinous Cystic Neoplasms

MCNs are composed of columnar, mucin-producing epithelium supported by the ovarian-type stroma with no communication with the pancreatic ductal system. MCNs are rare and usually found in the pancreatic body or tail of middle-aged women [57]. The aspirated cystic contents are usually thick, but may be hemorrhagic or necrotic [57]. The features of FNA cytology are the presence of nests, sheets, or papillae of cohesive mucinous columnar cells with or without atypia and loss of polarity, and abundant extracellular mucin (Fig. 6.12). Stroma seen in cellblock or core is typically ovarian-type stroma, which may be immunohistochemically positive for ER, PR, and inhibin and is useful to separate mucinous neoplasm from mucinous non-neoplastic cysts and IPMN. The epithelial cells are positive for CDX-2 (67 %), PDX-1 (100 %), CK7 (83 %), and CK20 (100 %), which is similar to ovarian mucinous cystic neoplasm of intestinal type [68].

Intraductal Papillary Mucinous Neoplasm

IPMNs commonly occur in old men, and most commonly involve the main pancreatic duct but may also affect the branches or a combination of the two [57]. There are three subtypes such as intestinal, pancreaticobiliary, and gastric foveolar subtype. Invasive adenocarcinoma arising from intestinal subtype has better prognosis than that arising from pancreaticobiliary subtype. Gastric foveolar subtype is seen in branch-type IPMN, which rarely progresses toward frank malignancy [69]. Communication with the pancreatic duct may be visualized on

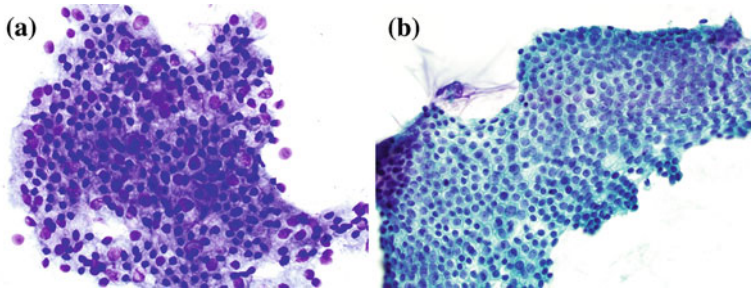

Fig. 6.12 Pancreatic mucinous cystic neoplasm, EUS-FNA. **a** Diff Quik stain, 400×. **b** Pap stain, 400×

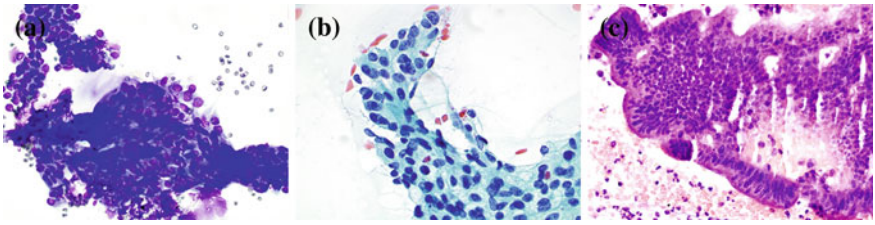

Fig. 6.13 Pancreatic intraductal papillary mucinous neoplasm, EUS-FNA. **a** and **b** EUS photos. **a** Diff Quik stain, 400×. **b** Pap stain, 400×. **c** Cellblock, H and E stain, 400×

endoscopic retrograde cholangiopancreatography (ERCP), magnetic resonance cholangiopancreatography (MRCP), or EUS [70]. The EUS-FNA technique in diagnosing IPMNs has been recognized to be of great importance in recent years. The features on FNA cytology are presence of papillae or sheets of cuboidal to columnar cells with round to ovoid, basally or eccentrically located nuclei containing fine evenly distributed granular to irregularly distributed coarse chromatin and small to prominent nucleoli (Fig. 6.13) [71]. Irregular clustering, complex papillae, and discohesiveness with single atypical cells were features of borderline and highly suspicious malignant lesions. Micropapillary clusters and single markedly atypical cells were seen in malignant cases [71]. The abundant background mucus obtained from IPMNs is thicker and more gelatinous than the watery mucin associated with a normal duodenal epithelium. Three main patterns of IHC stains with CDX-2, MUC-1, and MUC-2 are noted depending on the subtypes of IPMNs [71, 72]. Increased cytoplasmic expression of caspase-3 is seen in IPMNs with invasive adenocarcinoma [73].

6.4.2.3 Therapeutic Impact

Despite increased utilization of EUS-FNA for classifying pancreatic cystic lesions, a majority are managed conservatively. Serous cystadenomas are managed conservatively unless they become symptomatic or have demonstrated propensity to grow between serial imaging studies. In general, mucinous cystadenomas should be resected due to malignant potential. A distal pancreatectomy is performed for lesions in the body and tail and pancreaticoduodenectomy for lesions in the head and uncinate process. IPMN lesions which are main duct in nature should undergo surgical resection. This may require localized resection or even total pancreatectomy in severe cases. IPMN lesions limited to side branches of the pancreatic duct can be monitored. However, significant growth between imaging studies should prompt resection [74].

6.4.3 Solid Pancreatic Neoplasms

Pancreatic ductal adenocarcinoma, endocrine neoplasm, acinar cell carcinoma, solid pseudopapillary neoplasm, pancreatoblastoma, mesenchymal tumors, lymphoma, and metastatic malignancies are commonly present as a solid mass, but some of them may be present as a cystic mass or with cystic degeneration.

6.4.3.1 Ductal Adenocarcinoma

Ductal adenocarcinoma is the most common type of pancreatic cancer, representing 85–90 % of all pancreatic neoplasms, mostly affects older patients (60–80 years), and is mostly found in the head of the pancreas (60–70 %) [75].

Role of FNA and Brushing Cytology in Diagnosis and Staging of Pancreatic Ductal Adenocarcinoma

FNA biopsy is a more sensitive and reliable diagnostic technique than endoscopic brushing cytology and biopsy. The needle can be guided with a variety of diagnostic imaging techniques, including endoscopic retrograde cholangiopancreatography (ERCP), percutaneous transhepatic cholangiography (PTC), ultrasound, computed tomography, and MRI. EUS-FNA has provided significant improvements in locoregional staging for pancreatic adenocarcinoma. In direct comparison to Helical CT, EUS-detected significantly more tumors (97 vs. 73 %), more accurately determines respectability (91 vs. 83 %), and is more sensitive for assessment of vascular invasion (91 vs. 64 %) [76]. Addition of FNA increases the accuracy of acquiring tissue for diagnosis. In contrast to CT-FNA or other imaging-guided tissue sampling, a significant risk of tumor seeding is not seen. A majority of seeding occurs in the skin which is not traversed with EUS-FNA. The advent of EUS-FNA has significantly improvement staging and diagnosis of pancreatic adenocarcinoma. Brushing cytology is an alternative technique to obtain sample for superficial mucosal lesions or lesions involving mucosa [2–4]. The sensitivity, specificity, positive predictive values, negative predictive value, and diagnostic accuracy are 53–96.6 100, 100, 25–96.2, and 56–97.6 %, respectively, which are similar to those of biopsy and can be improved by simultaneous biopsy [2, 4, 77, 78].

Cytology in Diagnosis of Pancreatic Ductal Adenocarcinoma

FNA cytology characteristically presence of single, “drunken honeycomb” sheets or loosely cohesive two- or three-dimentional glandular clusters of pleomorphic polygonal, cuboidal or columnar cells with loss of polarity (Fig. 6.14). The nuclei are enlarged and pleomorphic, show irregular and thicken nuclear membranes, and

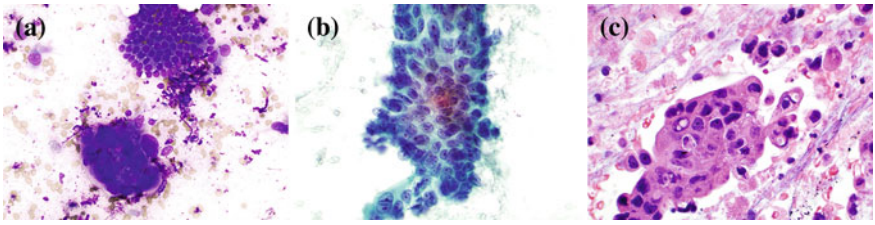

Fig. 6.14 Pancreatic ductal adenocarcinoma, EUS-FNA. **a** EUS image. **b** Diff Quik stain, 400×. **c** Pap stain, 600×. **d** Cellblock, H and E stain, 400×

contain hyperchromatic, coarse, or vesicular, unevenly distributed chromatin and prominent nucleoli. The cytoplasm is delicate, vacuolated or squamoid, is variable in amount, and may contain mucin. Bizarre cells, signet ring cells, binucleated or multinucleated cells, osteoclast-like giant cells, mitoses, necrosis, and mucin can be seen.

FNA cytology of mucinous (colloid) adenocarcinoma shows single or small clusters of malignant ductal cells floating in extensive extracellular mucin. Clear cell variants of ductal carcinoma is not uncommon, and FNA cytology shows clear cytoplasm, which should be separated from mucin production. FNA cytology of adenosquamous carcinoma shows glandular and squamous differentiation. Giant cell carcinoma is characteristically by presence of significant proportion of bizarre, pleomorphic, multinucleated tumor giant cells. Presence of abundant osteoclast-like giant cells raises the diagnosis of undifferentiated carcinoma with osteoclast-like giant cells.

Differential diagnosis includes degenerative changes, reactive atypia, mucinous cystadenocarcinoma, endocrine neoplasm, and metastatic adenocarcinoma. The tumor cells are positive for CK7, CK20, CK19, and rarely CDX-2.

Therapeutic Impact

The introduction of EUS-FNA in staging pancreatic malignancies has significantly altered management. Resectable lesions in the tail are amenable to distal pancreatectomy while lesions in the head to pancreatico-duodenectomy. Increasing accuracy for vascular invasion and lymph node metastasis has allowed for significant improvements in detection of respectability. In one study, EUS and EUS-FNA precluded surgery in 27 % patients with a cost-reduction of $3300 per patient [79]. Preoperative staging combining CT and EUS-FNA provides the most effective method to assess patients for surgical resection or chemotherapy and radiation.

6.4.3.2 Pancreatic Endocrine Neoplasms

Pancreatic endocrine neoplasms predominantly occur in older adults. They may present as part of MEN, and may be associated with a variety of clinical syndromes, depending hormones secreted.

Role of EUS-FNA in Diagnosis and Staging of Endocrine Neoplasms

EUS is also essential in localization of pancreatic endocrine tumors. EUS has been demonstrated have 82 % sensitivity, 95 % specificity, and 90 % accuracy for localization of these tumors [80, 81]. In addition, FNA can be done in lesions as small as 5 mm to obtain tissue diagnosis of pancreatic endocrine tumors when desired.

Cytology in Diagnosis of Pancreatic Endocrine Neoplasms

The FNA cytology of endocrine neoplasms is characterized by the presence of single, loosely cohesive sheets or clusters, and rosettes of monotonous cells that contain round or oval and eccentrically located nuclei (plasmacytoid) with characteristic "salt and pepper" chromatin, smooth nuclear membrane, and no or small nucleoli (Fig. 6.15). The cytoplasm is small-to-moderate in amount, finely granular, or delicate. Abundant naked nuclei with granular background are often seen and occasionally, significant pleomorphic cells (endocrine atypia) can be seen. Number of mitotic figures, tumor size measured by CT, and degree of nuclear atypia are related to the grade of the endocrine neoplasm. Small cell carcinoma is rare in pancreas.

Tumor cells are positive for pan-cytokeratin, CD56, chromagranin, synaptophysin, NSE, and hormones. Electron microscopy can demonstrate dense core neurosecretory granules.

The differential diagnosis includes islet cell hyperplasia, solid pseudopapillary neoplasm, acinar carcinoma, ductal carcinoma, metastatic small cell carcinoma, and malignant lymphoma including plasmacytoma.

6.4.3.3 Solid Pseudopapillary Neoplasm

Solid pseudopapillary neoplasm is a rare, usually benign neoplasm with predominant manifestation in the pancreatic body or tail of young women [82]. The diagnostic accuracy of EUS-FNA is 75 % [83]. The characteristics of FNA cytology are the presence of single or dyscohesive clusters of plasmacytoid cells surrounding branching vasculature (pseudopapillae) and myxoid stroma (metachromatic matrix) (Fig. 6.16) [83–85]. The relatively uniform cells have scant to moderate, delicate or granular cytoplasm with occasional many small or large clear

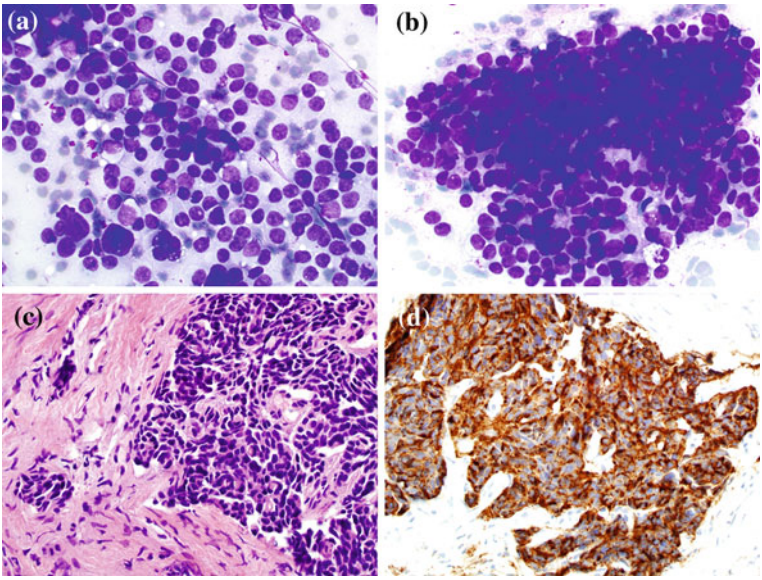

Fig. 6.15 Pancreatic endocrine neoplasm, percutaneous needle core biopsy. **a** and **b** Touch preparation, Diff Quik stain, 600×. **c** Core, H and E stain, 400×. **d** IHC for chromogranin, 400×

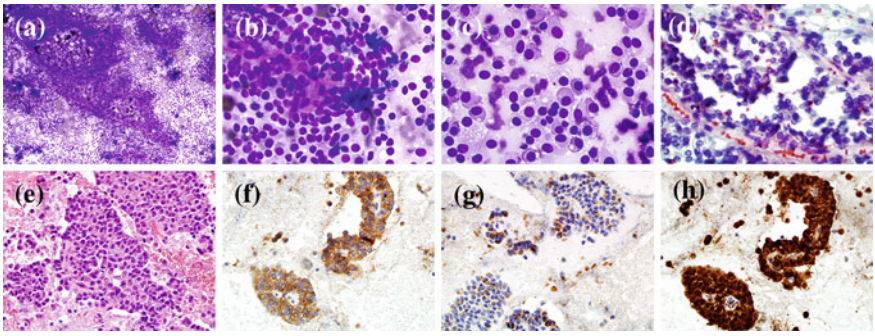

Fig. 6.16 Pancreatic solid pseudopapillary neoplasm, EUS-FNA. **a** Diff Quik stain, 100×. **b** and **c** Diff Quik stain, 600×. **d** Pap stain, 600×. **e** Cellblock, H and E stain, 400×. **f** IHC for CD10, 400×. **g** IHC for chromogranin, 400×. **i** IHC for β-catenin, 400×

cytoplasmic vacuoles and ill-defined cytoplasmic borders, and round or oval nuclei containing fine, evenly distributed chromatin and small nucleoli, showing smooth, regular nuclear membrane, and rarely showing nuclear grooves [84, 85]. Macrophages and necrosis may be seen [85]. Differential diagnosis includes endocrine neoplasms [83]. Large, clear cytoplasmic vacuolation, and IHC stains can be used to separate solitary pseudopapillary neoplasm from endocrine neoplasms [83, 84]. Tumor cells are positive for CD10, vimentin, beta-catenin, NSE, α-1

antitrypsin, CD56, and progesterone receptors, and focally positive for cytokeratin, chromogranin, and synaptophysin.

6.4.3.4 Acinar Cell Carcinoma

Acinar cell carcinoma is composed of relatively uniform neoplastic cells that are arranged in solid and acinar patterns and produce pancreatic enzymes [86]. Acinar cell carcinomas represent 1–2 % of all exocrine pancreatic neoplasms in adults with a mean age of 62 and may arise in any portion of the pancreas [86]. The FNA cytology is characterized by presence of abundant single or loosely cohesive clusters of malignant epithelial cells with vaguely acinar and trabecular formations (Fig. 6.17) [87, 88]. The pleomorphic nuclei show fine granular chromatin and small or prominent nucleoli. There are scant to moderate amounts of granular cytoplasm. Scattered, strikingly large tumor cells with giant nuclei, prominent mitoses, associated necrosis, and granular background released from cytoplasm due to preparation artifact may be seen. The differential diagnosis includes endocrine neoplasm, solid pseudopapillary neoplasm, and benign acinar cells from needle track. Tumor cells are positive for trypsin, chymotrypsin, lipase, and elastase. Tumor cells may also be focally positive for chromgranin, synaptophysin, CEA, B72.3, and AFP.

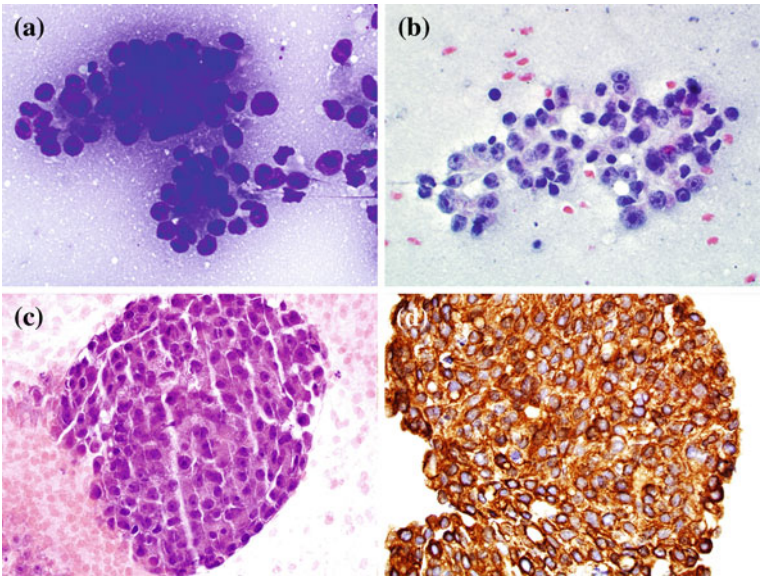

Fig. 6.17 Pancreatic acinar cell carcinoma, EUS-FNA. **a** Diff Quik stain, 600×. **b** Pap stain, 600×. **c** Cellblock, H and E stain, 400×. **d** IHC for CK7, 400×

6.5 Extrahepatic Biliary Tract: Cholangiocarcinoma

6.5.1 Role of ERCP Brushings and EUS-FNA in Diagnosis of Cholangiocarcinoma

Tissue acquisition in cholangiocarcinoma has been a longstanding dilemma. Brushing cytology has low accuracy of 65 % in malignant strictures [89]. In combination with tumor markers (CA19-9), sensitivity and specificity increase to 88 % [90]. The advent of EUS-FNA has allowed for improvements in patients with locally advanced disease and lymph nodes or overt mass lesions. Peroral cholangioscopy appears to significantly improve tissue diagnosis in cholangiocarcinoma in preliminary investigations. This allows for direct visualization of the stricture or mass lesion and targeted biopsies. Ultimately, the utilization of cholangioscopy and enhanced ductal imaging techniques will significantly improve diagnosis in cholangiocarcinoma.

6.5.2 Cytology in Diagnosis of Cholangiocarcinoma

FNA cytology and brushing cytology of the extrahepatic biliary tract, common bile duct and pancreatic duct, can be used in the diagnosis of cholangiocarcinoma. The characteristics of FNA and brushing cytology of cholangiocarcinoma are similar to those of pancreatic ductal adenocarcinoma (Fig. 6.8).

6.5.3 Therapeutic Impact

In general cholangiocarcinoma has a poor 5-year survival of 5–10 %. Respectability rates for distal, intrahepatic, and perihilar lesions are 91, 60, and 56 %, respectively [91]. Even in patients undergoing resection, tumor-free margins are obtained in only 20–40 % of proximal and 50 % of distal tumors [92]. Preoperative ERCP not only allows for tissue diagnosis but provides opportunity for biliary decompression and ultimately may be the only treatment option. In unresectable cholangiocarcinoma, chemotherapy and radiation is an option, but has very limited efficacy.

6.6 Other Pancreaticobiliary Malignancies

6.6.1 Role of ERCP Brushings and EUS-FNA in Diagnosis of Other Pancreaticobiliary Malignancies

ERCP and EUS can be effective in patients with other pancreaticobiliary malignancies. ERCP with brushings and or cholangioscopy with targeted biopsies are very effective in patients with metastatic malignancy and biliary strictures. EUS-FNA allows for tissue sampling with FNA and core biopsy sampling. In cases of suspected, lymphoma, core biopsy provides large samples for flow cytometry and proper lymphoma staging. In general, EUS-FNA is very effective in obtaining tissue diagnosis is non-adenocarcinoma pancreatic mass lesions and for metastatic malignancies with lymphadenopathy adjacent to the gastrointestinal tract.

6.6.2 Cytology in Diagnosis of Other Pancreaticobiliary Malignancies

Malignancies of the lung, kidney, breast, liver, gastrointestinal cancers, melanoma, and sarcoma may metastasize to pancreas. Pancreatic involvement by malignant lymphoma is almost always secondary rather than primary. Rare entities and metastases should be considered once cytomorphology and IHC stains do not support the diagnosis of common primary pancreatic neoplasms. Communication with clinicians and imaging study results are important to make a definitive diagnosis.

6.6.3 Therapeutic Impact

Adequate tissue diagnosis allows for appropriate therapeutic strategies. This is especially important in lymphoma. The diagnosis of lymphoma by EUS-FNA would preclude unnecessary surgery and allow for proper chemotherapy. EUS-FNA diagnosis of metastatic adenocarcinoma can provide the primary diagnosis and allow again for selection of the appropriate chemotherapeutic agents.

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