

Introduction

Lumbar puncture (also known as spinal tap) was first introduced toward the end of the nineteenth century, and it has since become a mainstream procedure in medicine in general, and neurology in particular. Initially, the focus was aimed at diagnosing infections of the meninges, especially tuberculous meningitis, but now, the cerebrospinal fluid (CSF) is examined for a range of nonneoplastic conditions and, of course, certain malignancies. Brain smears were introduced during the first few decades of the last century and are now used in many neurosurgical centers around the world for rapid diagnosis, especially intraoperatively. Frozen sections are preferred by some units, and it is useful for both techniques to be available as there are some situations where one is superior for diagnosis over the other. For example, hard or fibrotic lesions may be impossible to spread satisfactorily, and so frozen sections may be essential for diagnosis.

Anatomy and Physiology of the CSF

CSF is produced by the choroid plexus, a specialized part of the ependyma in the lateral ventricles. From the local lateral ventricles, it moves through the foramina of Monro into the third ventricle and via the aqueduct of Sylvius into the fourth ventricle. The foramina of Magendie and Luschka allow the fluid to pass from the fourth ventricle to the subarachnoid space. The fluid circulates in a closed system within the subarachnoid space bounded by the arachnoid mater externally and the pia mater internally which is closely applied to the brain. CSF is resorbed by the arachnoid granulations within the dural sinuses, mainly the superior sagittal sinus. The granulations act as a valve allowing CSF into the blood but not vice versa. The intimate but discrete barrier which separates the CSF from the blood is called the blood–brain barrier. The total volume of fluid is around 150 mL in the absence of disease, but this can be

increased or decreased if there is a pathological alteration in the brain, spinal cord or membranes. About 500 mL of fluid is produced per day.

The arachnoid layer extends to the midsacral level while the cord ends at around L2 (or 3). Beneath this, the filum terminale is enclosed in the space containing CSF, and therefore, the fluid is aspirated in this region to avoid damage to the cord. The routine lumbar puncture involves passing a long needle through the disc space between L3 and L4 and collecting the fluid. The latter will usually be split into samples for microbiology, cytopathology, and biochemistry and sometimes also for immunology.

Occasionally, CSF may be removed via the fontanelles in children during operations, the cistern magna (especially at autopsy) or via a burr hole or shunt. It is usually handled in the same manner whatever the sampling method, but of course, if the amount of fluid is limited, it is prudent to send the material to the most important of the laboratories first. Any fluid leftover may be passed to the other laboratories afterward.

Clinical Indications

In common with many other organ systems, cytology of the CSF is most useful in identifying or excluding a wide range of infections; a variety of primary tumors, metastases, and hematological malignancies; and a limited number of other nonneoplastic conditions such as hemorrhage, infarcts, and degenerative or demyelinating conditions. CSF may also be examined to determine the effects of treatment when involvement of a particular condition has already been established.

Before the lumbar puncture is performed, one should consider whether the procedure is in the patients' best interests. There is a range of relative contraindications to lumbar puncture mostly related to bleeding disorders and raised intracranial pressure, the latter potentially leading to fatal herniation. The procedure itself will not be described in any detail here.

The cells present are frequently nonspecific and do not provide a specific cytological diagnosis, but the results may be very helpful in excluding neoplasia, and in some cases,

Table 2.1 Findings in normal and pathological specimens of CSF (Bell 1994)

Clinical situation	Protein	Glucose	Cells
Normal	0.1–0.45 g/L	2.5–4 mmol/L	<5 × 10 ⁶ /L lymphocytes
Bacterial infection	0.5–5 g/L	0–2 mm/L	Polymorphs
Viral infection	0.5–1.5 g/L	As normal	Polymorphs early, lymphocytes later
Tuberculosis	0.5–2 g/L	1.2–3 mmol/L	Polymorphs early, lymphocytes later
Multiple sclerosis	Oligoclonal IgG	As normal	Lymphocytosis

the appearance does point to a specific cause (Table 2.1). Several things are important to bear in mind, especially whether there has been previous surgery or a shunt has been introduced. The cytologist should be aware of these to avoid misinterpreting a normal finding as pathological. If diagnostic uncertainty is present, then a request for a repeat sample should be considered. This course of action is also appropriate for those cases where additional material may allow further studies such as immunohistochemistry.

Specimen Handling

CSF is a normally a clear, colorless liquid. The macroscopic appearance should be recorded and a note made if the sample is blood stained, cloudy, or clear. The volume received should also be recorded and any tissue fragments hooked out for histology. An aliquot of the sample may be sent to the hematology department for flow cytometry, if it is important to establish a more detailed assessment of the cell content and cellular composition, particularly if CNS involvement by lymphoma or leukemia is suspected.

It is well recognized that cells in CSF degenerate rather rapidly, and hence, all of those involved in the procedure should be encouraged to transport the specimen to the cytology reception as soon as possible. Likewise, the specimen should be accepted and handled within 30 min if at all possible. FNA material may be received on preprepared slides or in a liquid medium, often containing a form of fixative.

Several preparation techniques are available: cytocentrifuge, cytospin, membrane filtration, and direct spreads. Every effort should be made to utilize the sample as optimally as possible as the cells present may well be very limited in number. Often, only one slide can be prepared, but ideally, up to four slides are made for staining. As usual, the stains of choice are either Papanicolaou or H&E if the material is fixed or Giemsa if it is air-dried.

Whenever it is possible, and especially with FNA material, a cell block should be considered to optimize the subsequent immunocytochemistry that may be required.

Normal Fluid Findings

CSF is usually crystal clear because it contains very few, if any, cells. There should be fewer than 5 cells/mm³ (mostly lymphocytes) within a lumbar puncture sample, as a general rule, but in neonates, there may be up to 10 cells/mm³. The cells present may include a few lymphocytes, macrophages and polymorphs, integral CNS cells such as ependymal or glial cells, and contaminant cells from the cutaneous puncture such as squamous cells. Blood is frequently present, and it may include a few incorporated white blood cells (Fig. 2.1).

As mentioned above, the fresher the sample the better to preserve cell morphology and avoid degenerative alterations which will hamper interpretation. The volume provided is not crucial as long as the diagnostic material is present, but as a rule, 3 mL is better than 1 mL if at all possible. If there is going to be a short delay, then refrigeration should be considered. If the delay is likely to be longer, then one should consider adding a fixative. One recommended fixative is Saccomano carbowax. Most units perform cytopspins, using 0.2 mm³ spun for 5 min at 600 rpm. The slides are air-dried and fixed in methanol for a few minutes before staining.

A recurrent problem is determining whether cells in the CSF, which often contains a component of peripheral blood, are significant. For example, blood constituents such as white cells may be just circulating leukocytes rather than representing meningitis or even leukaemic cells associated with genuine leukaemic involvement of the meninges. Other contaminant cells may also cause a degree of confusion, including squamous cells. Resident cells that may appear in CSF and have no significance include ependymal cells, arachnoid cells, choroid plexus cells, meningeal fragments, and cartilaginous intervertebral disc material (Fig. 2.2). White matter may be seen, especially in shunt samples (Fig. 2.3). Corpora amylacea may be sampled, and it is important that these are not misinterpreted as pathogenic yeasts. Sometimes, the local cells look odd for “innocent” reasons such as reactive or postradiotherapy changes, and they should not be over-interpreted. Starch from gloves may also be present although starch-free gloves are recommended. They can be recognized by their classical Maltese cross appearance on polarization.

Inflammatory/Infective Conditions

Any organism can gain access into the CNS, including bacteria, viruses, fungi, parasites, and a range of other rare infectious organisms. They may cause meningitis, encephalitis, or both. If infection is suspected, it is crucial to obtain a sample for microbiology, although of course special stains including Gram, silver, and ZN can be done on the cytology sample. Usually, the organism finds its way to the CNS via the blood, but it can be through direct spread from a local infection such as in the ear, nose, or sinus. Access can also be via the peripheral nervous system, and this is the most common route by which viruses such as herpes penetrate the CNS.

Fig. 2.1 CSF sample with contaminant blood. This should not be mistaken for primary intrameningeal hemorrhage or meningeal inflammation

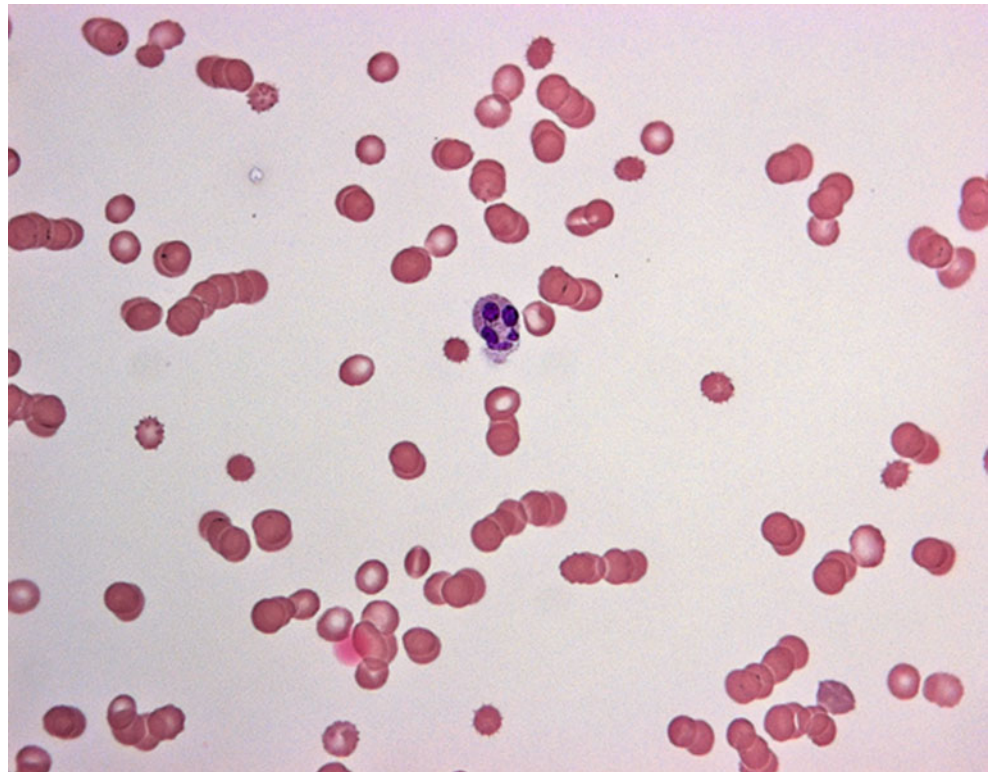
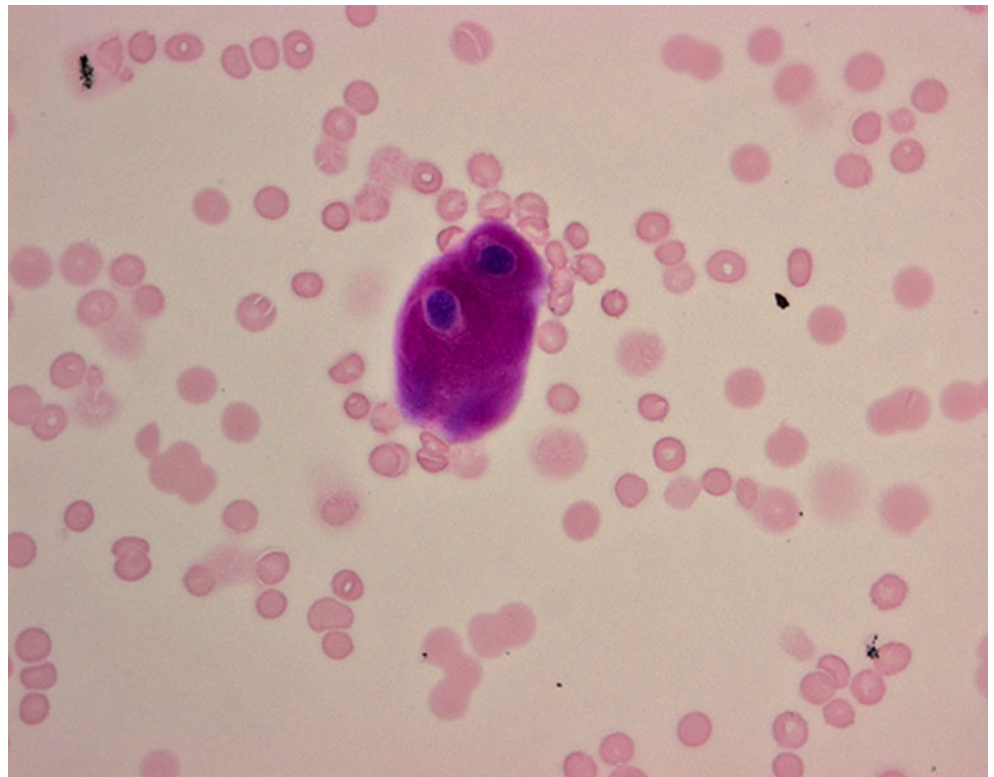


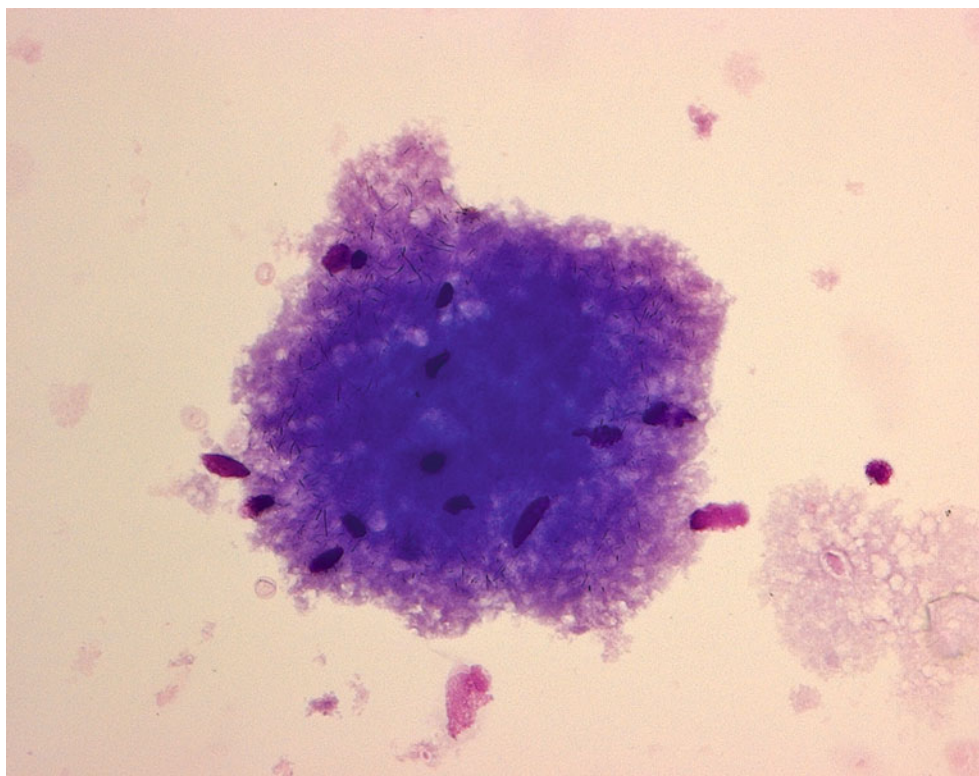
Fig. 2.2 Chondrocytes may be present in a normal CSF sample



With bacterial meningitis, the organism may be predicted by the age of the individual infected, with *Neisseria* species commonly found in adolescents, *Streptococci* most prevalent

in the young and elderly, and *E coli* most frequent in neonates. These organisms all produce a pyogenic form of meningitis with their well-recognized clinical warning signs, which

Fig. 2.3 CSF taken through a shunt may have fragments of white matter



contrast with the milder symptoms and lymphocytosis of conditions associated with viruses. *Mycobacterium tuberculosis* may cause meningitis or one or more parenchymal lesions within the brain. It usually follows a primary infection elsewhere in the body that has spread to the brain via the bloodstream. It can present acutely and needs to be diagnosed rapidly to avoid serious late complications. Lumbar puncture may be performed (unless papilloedema is present), and treatment is often started before the results are available. The presenting symptom may be headache, cranial nerve abnormalities, and/or meningism, and the differential is with other causes of meningitis or, in the case of a tuberculoma, with other CNS mass lesions. Occasionally, the presentation may be more indolent, and untreated chronic basal meningitis may lead to local fibrosis which interferes with the flow of CSF and cause hydrocephalus.

Generally, infections of the meninges produce the same population and range of cells with no specific features (Fig. 2.4). However, one can occasionally get clues from the cell profile – bacterial infections, including TB, are said to produce neutrophils early and monocytes later with accompanying plasma cells. Lymphocytes may also point to TB, but they feature more prominently with viruses. Lymphocytes may be the only hint to a local viral infection as most commonly this is due to echovirus and Coxsackie virus, which produce no cellular or nuclear features and, in particular, no recognizable inclusions. Eosinophils may be present if there are parasites locally, but they also accumulate in the CSF

after surgery. Rarely, one may actually see the organism in the specimen, but this is infrequent (Fig. 2.5). When lymphocytes are present in significant numbers, it is quite possible to mistake a reactive population for lymphoma, especially if odd forms and mitoses are present (Fig. 2.6). If the patient is known to have leukemia, any atypical cells present may be just contaminants of the accompanying blood. In this situation, it is best to repeat the lumbar puncture after a gap of several days. Flow cytometry and immunocytochemistry may be useful if they demonstrate uniform monoclonality in all of the cells present.

A range of infections may be present in the setting of immunosuppression (especially HIV). Many of these are opportunistic and include *Candida*, *Cryptococcus*, and CMV. On rare occasions, these infections may occur without established immunodeficiency. Often, there is a lymphocytosis, and if this is the case, then lymphoma needs to be considered and differentiating the two may be problematic, as discussed above. The organisms may be detected by their conventional morphology, with owl's eye inclusions of CMV and Cowdry inclusions of herpes simplex or zoster sometimes visible. Appropriate clinical background will clearly offer some suspicion of the underlying infection, but, in general, the infective organism is more likely to be recognized on smear (see later). Routine CSF analysis in patients with progressive multifocal leukoencephalopathy is usually normal, but PCR for the JC viral antigen on the fluid obtained during the procedure can be very helpful in establishing the diagnosis.

Fig. 2.4 Mixed inflammatory cells in CSF are likely to indicate meningitis

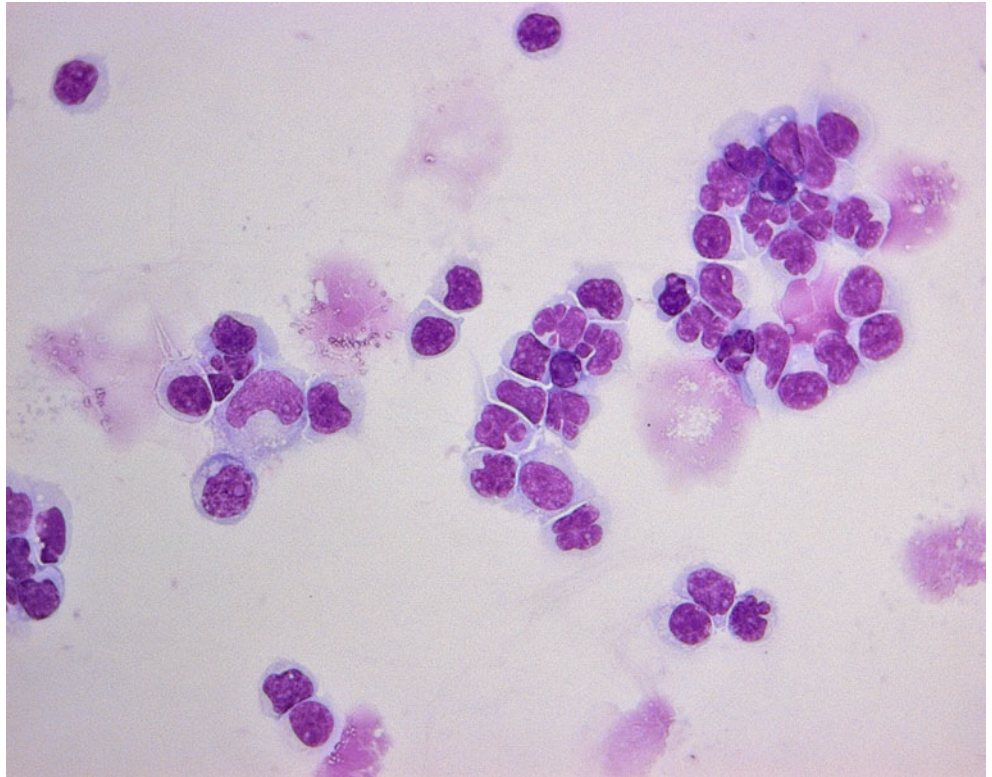
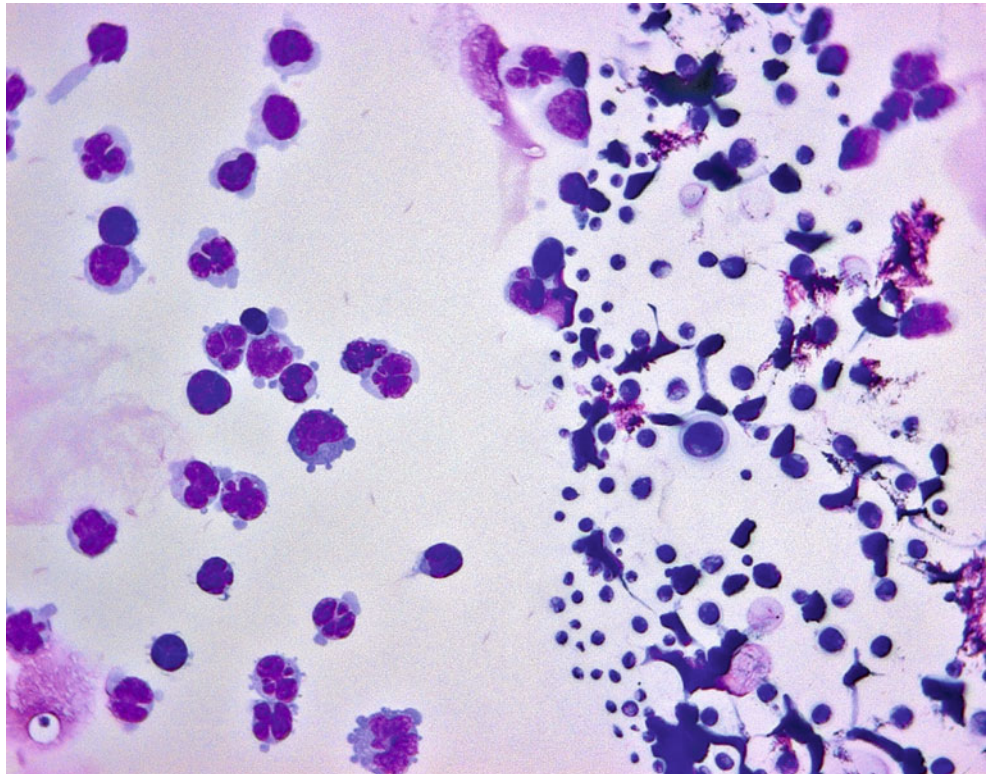


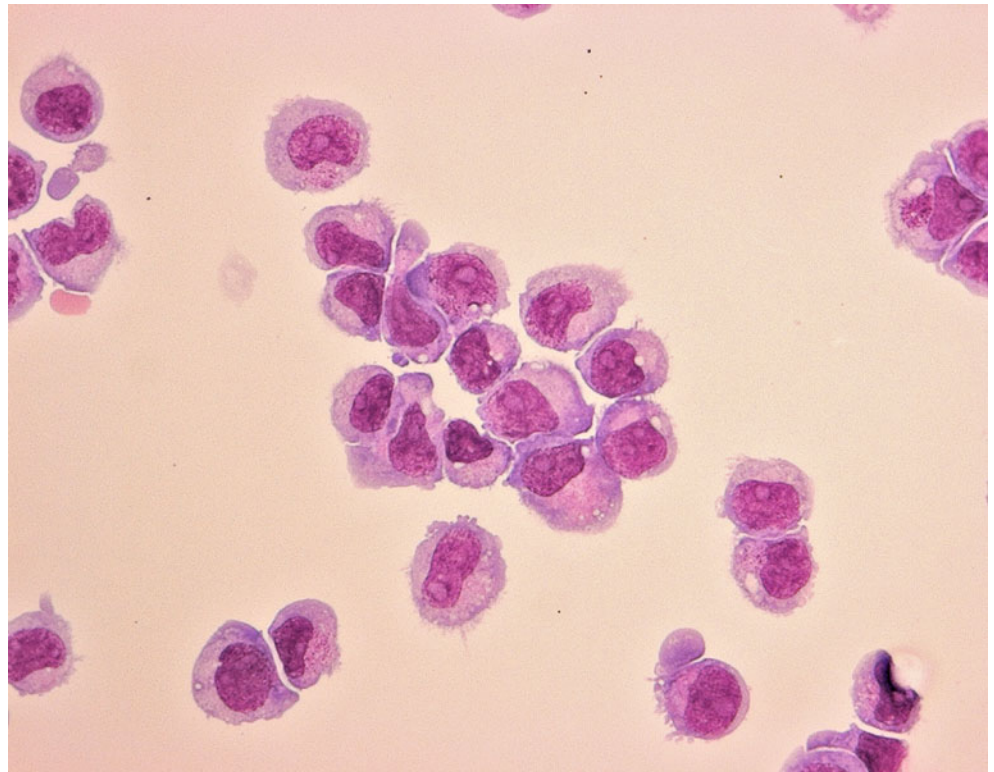
Fig. 2.5 Sometimes the inflammatory cells and organisms are present in the same sample. Note the Cryptococci on the right



Toxoplasmosis is not uncommon in immunosuppressed individuals, causing foci of inflammation and necrosis in the deep gray matter. Symptoms may be nonspecific but include

headache and other features of hydrocephalus. The organism may be seen in a CSF sample, but this is very rare and more likely with a ventricular sample than one obtained by lumbar

Fig. 2.6 Increased numbers of lymphoid cells may indicate chronic bacterial infection, viral infection, mycobacterial infection, or lymphoma



puncture. One may see the 4–6- μm tachyzoite lying extracellularly or within phagocytes or the cyst forms may be identified. These are more likely to be detected through PCR on the CSF sample or by searching for the organism on a brain smear.

Cryptococcus neoformans on the other hand does present more frequently in CSF samples. This fungus occurs as a yeast form, about 15 μm in diameter, which are refractile; have a thick mucopolysaccharide capsule that is positive with mucin stains; and characteristically show thin-necked, asymmetrical budding (Figs. 2.7 and 2.8). Although organisms may be plentiful, inflammatory cells may be inconspicuous. Sometimes, the organisms are not readily identifiable, and they may be hidden within histiocytes. Several stains may aid visualization including PAS, Alcian Blue, and India ink. Rarely, air trapped beneath the coverslip looks refractile, and one should not be misled by this. Starch particles contaminating the sample may also appear similar, but the fungi do not have the Maltese cross of starch when polarized light is used. The India ink test is still used for detection of meningitis caused by *Cryptococcus neoformans*, but the cryptococcal antigen (CrAg) test has a higher sensitivity.

Small spores and pseudohyphae of *Candida* are recognized in less than 50 % of cases of candidal meningitis. The latter can be seen as a complication of CNS shunts. *Candida* also produces microabscesses within the deeper brain tissue

in disseminated candidiasis. When identified, the fungi have the typical size (4–8 μm) and appearance of candidal infections seen elsewhere. Culture or CSF antibody levels will identify more cases than routine CSF cytology.

If eosinophils are present, one of course needs to think about parasites and the background clinical setting. If appropriate, one should specifically look for *Entamoeba histolytica*, *Naegleria fowleri* and *Acanthamoeba culbertsoni*, or *Trypanosoma rhodesiense*, the causative organism of sleeping sickness. However, eosinophils may simply reflect repeated aspiration or be associated with drug reactions or shunts.

Some of the mass lesion-producing organisms do not readily gain access to the CSF. Sometimes, only reactive histiocytes are present in infections, and this can be troublesome for two reasons. The first is the fact that the underlying cause is not apparent. The second is the problem with histiocytes resembling large pale metastatic adenocarcinoma cells. The same is true for enlarged virally infected cells. Sometimes, there are no organisms, but an aseptic meningitis is present. Mollaret disease is a particular form of this and is a recurrent self-resolving “meningitis.”

CSF can and should be sent to the microbiology laboratory for various types of smears and cultures to diagnose infections. Polymerase chain reaction (PCR) has been a great advance in the diagnosis of some types of meningitis. It has high sensitivity and specificity for many infections of the CNS, is fast, and can be done with small volumes of CSF.

Fig. 2.7 Cryptococcal infection. Note the characteristic capsule on MGG

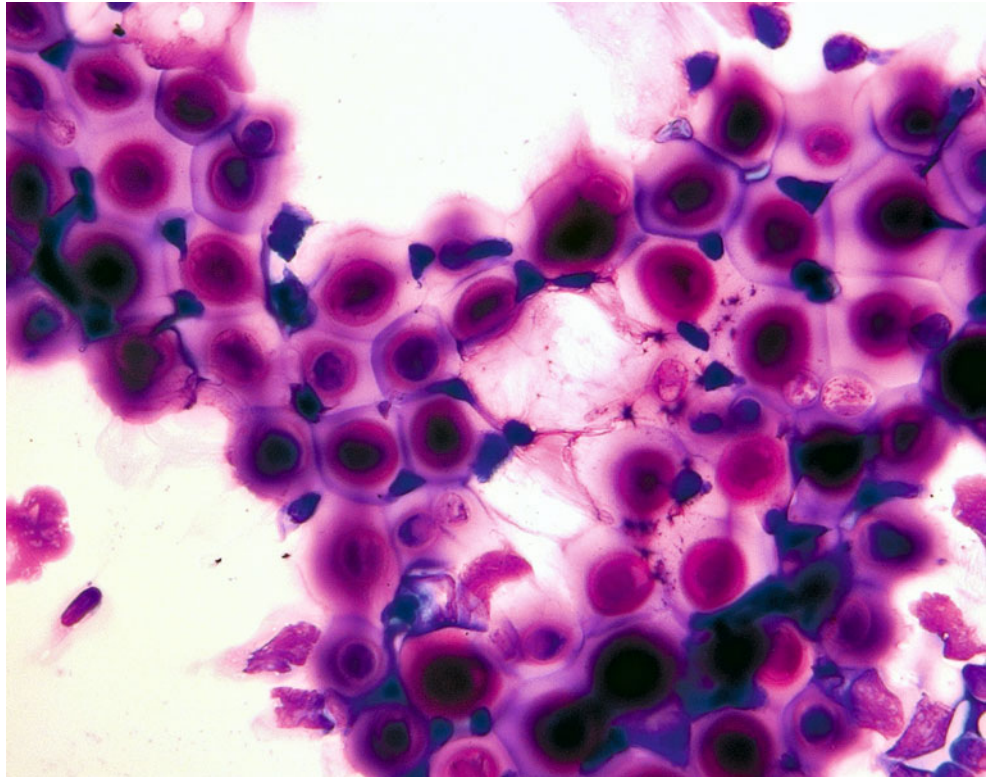
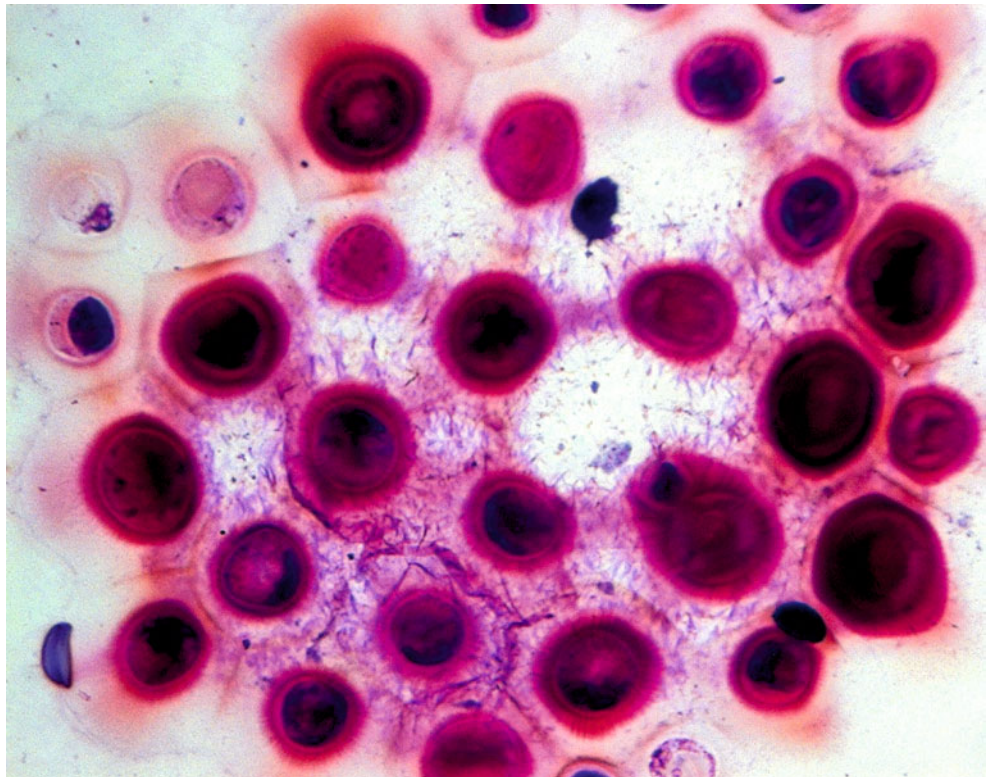


Fig. 2.8 Cryptococcus highlighted by PAS stain



Numerous antibody-mediated tests for CSF are available in some countries: these include rapid tests for antigens of common bacterial pathogens, treponemal titers for the

diagnosis of neurosyphilis, and similar tests for Lyme disease and *Coccidioides*. Other tests are also available that search for the antibody to the infective organism.

Other Tests on CSF

In addition to cytology and microbiology, samples are routinely sent to clinical biochemistry, and the cytologist should be able to interpret the significance of biochemical alterations in the CSF. Here, the levels of protein (or albumin) and glucose are often measured and compared to those in a blood sample taken at the time of the lumbar puncture. CSF protein is usually very low, and glucose is about two-thirds that of the blood. Changes in total protein content of cerebrospinal fluid can result from pathologically increased permeability of the blood-cerebrospinal fluid barrier or obstruction of CSF circulation such as with meningitis, neurosyphilis, brain abscesses, subarachnoid hemorrhage, polio, autoimmune disease, or Guillain-Barré syndrome, leakage of CSF, increased intracranial pressure, or hyperthyroidism. Very high levels of protein may be seen in tuberculous meningitis or spinal cord compression.

Glucose is usually present in the CSF; the level is usually about 60 % that in the peripheral circulation. A fingerstick or venepuncture at the time of lumbar puncture should therefore be performed to assess peripheral glucose levels in order to determine a predicted CSF glucose value. Glucose may be normal, high, or low. Decreased glucose levels can be seen with fungal, tuberculous or pyogenic infections, lymphoma, leukemia, CNS involvement by mumps, or systemic hypoglycemia. A glucose level of less than one-third of blood glucose levels in association with low CSF lactate levels is typical in hereditary CSF glucose transporter deficiency. Increased glucose levels in the fluid can indicate underlying diabetes, but this still needs to be clarified by referring to the blood glucose level.

Measurement of chloride levels may aid in detecting the presence of tuberculous meningitis. It is slightly higher in normal CSF compared to serum, but in tuberculosis, it is often much lower.

The enzyme lactate dehydrogenase can be measured to help distinguish meningitides of bacterial origin, which are often associated with high levels of the enzyme, from those of viral origin in which the enzyme is low or absent. Increased levels of glutamine may point toward liver disease such as hepatic encephalopathy or Reye's syndrome, and this may also be seen with hypercapnia. Lactate is high in certain CNS tumors, multiple sclerosis, heritable mitochondrial disease, low blood pressure, low serum phosphorus, respiratory alkalosis, idiopathic seizures, traumatic brain injury, cerebral ischemia, brain abscess, hydrocephalus, hypocapnia, or bacterial meningitis.

IgG levels are assessed, also with reference to serum IgG and total protein levels. These may be elevated in immune disorders such as multiple sclerosis, transverse myelitis, and certain other conditions including Behcet's disease and neuromyelitis optica of Devic. Oligoclonal bands may be present, and these may be used to assess disease activity.

Neoplasia

The main role of CSF cytology is to establish whether cancer is present or there is another nonneoplastic condition that is specifically diagnosable on cytology. Sometimes, a benign tumor will be present, but most tumors diagnosed on CSF cytology are malignant. If the cells present are clearly neoplastic, then the next stage is to differentiate between a primary CNS tumor and metastasis. In contrast to the cytology of many other sites, the cellularity and presence of inflammatory cells in CSF are not generally as useful in determining whether infection or neoplasia is present. Low numbers of cells can be found in cancer, and inflammatory cells might mask tumor cells especially if the tumor is a lymphoma or leukemia.

Leukemia

The incidence of involvement of the CNS by ALL is increasing (around 4 % in current studies), and symptoms are not a good predictor of the presence or absence of CNS tumor. Cells are thought to access the CSF via the blood through the intracranial veins. There may be many cells, and they may be so numerous that hydrocephalus develops. Therapy, which may be prophylactic, is introduced into the CSF often, as this reduces the incidence of recurrence from 60 to 10 %, and this offers the opportunity for sampling of the fluid.

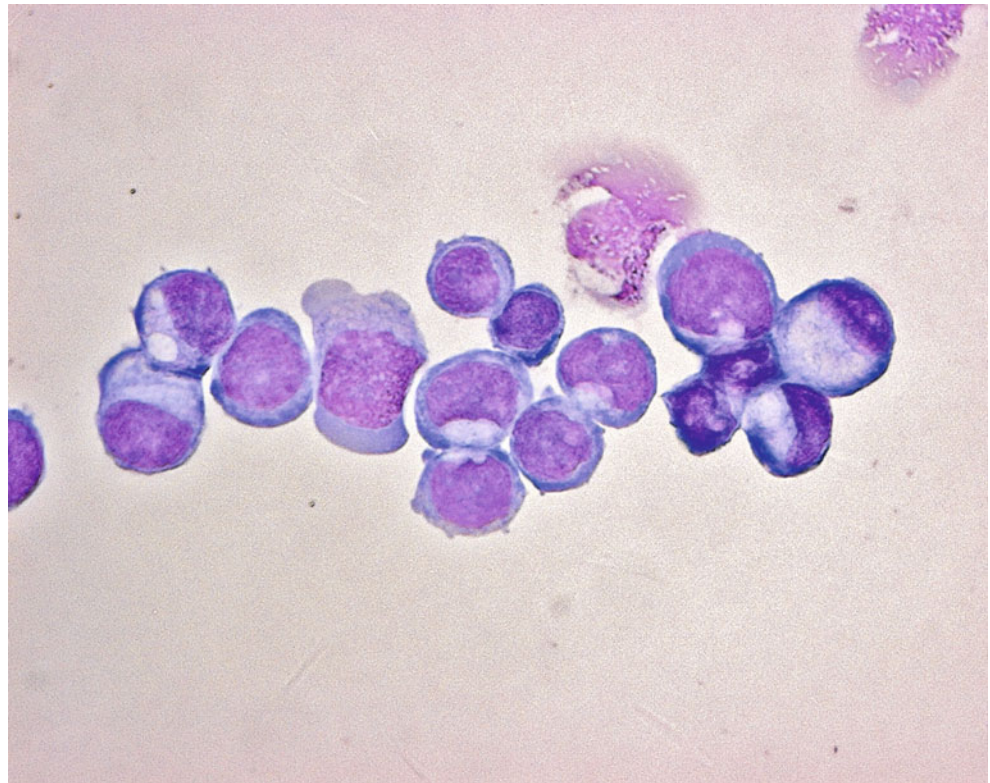
The differentiation between ALL and AML may be very difficult and, as mentioned previously, the possibility of introducing leukaemic cells during procedure from blood needs to be considered. Cellularity is not necessarily a helpful feature as reactive processes may produce many cells, and involvement by tumor may generate only a few. One may be able to identify Auer rods, especially in AML M3, but still it may be impossible to be sure that the CNS is definitely involved by the leukemia.

CNS involvement by chronic leukemias is rare. If the CSF contains leukocytes, these are more likely to represent infections than CSF involvement by CML even if the polymorphs look a little odd. When leukemic involvement is present, the cells present look immature, especially on MGG, and flow cytometry may have a role in establishing the correct diagnosis.

Lymphoma

CSF involvement by lymphoma is not common but is reported to be mostly from extradural deposits leading to spinal cord compression. For example, NHL shows brain involvement in 5 % of cases with leptomeningeal involvement in 3.7 %. High-grade tumors involve the brain more

Fig. 2.9 Metastatic breast cancer in a CSF specimen



often than low grade, and involvement by Hodgkin's disease is uncommon. The association of primary high-grade lymphoma and HIV infection is now well established. Symptoms may be due to compression by a local tumor mass or destroyed skeletal structure, direct tumor invasion, space occupation in the cranium, an encephalopathy, neuropathy, cerebellar degeneration, or polymyositis. Apart from direct tumor-related neurological pathology, there may be consequent infections such as PML or HZV, and there may also be bacterial meningitis, irradiation change, drug-induced changes, or hemorrhage.

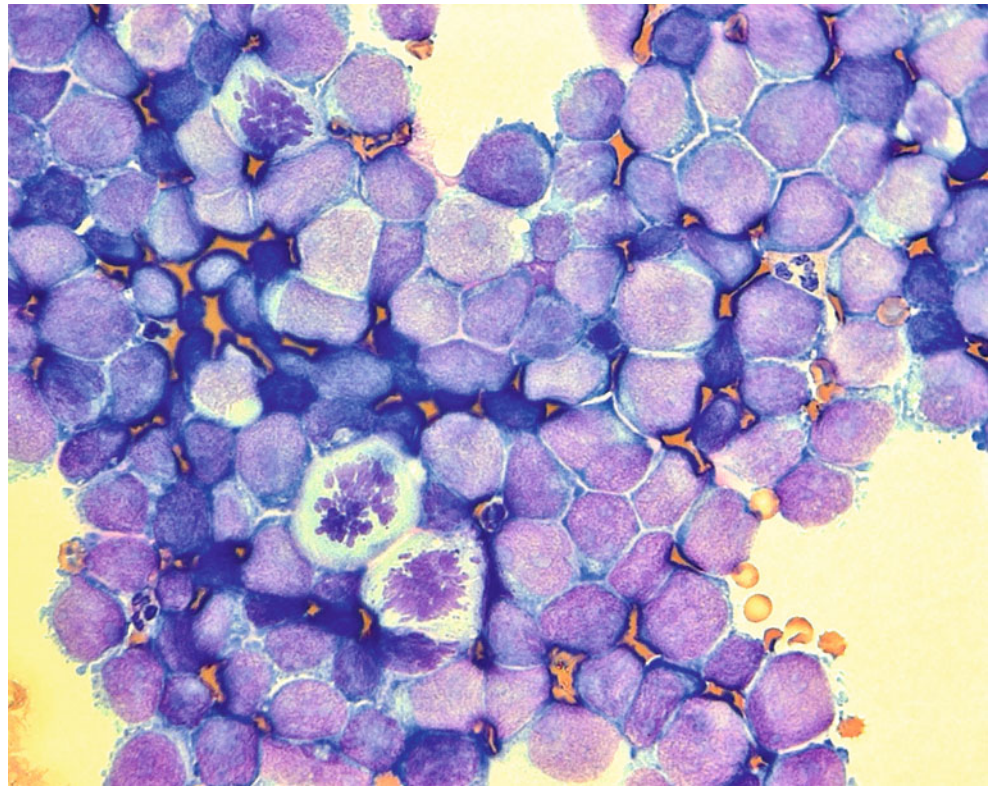
The cytologist needs to look for cellular monotony, large nucleoli, and nuclear irregularity. Because CNS lymphomas are usually high-grade tumors, so the cytological appearance is one of dissociated large cells. T and B cell immunoprofiling may help since B dominant populations are more likely to be lymphoma, while principally T cell infiltrates tend to be reactive. Light chains may also help to differentiate between lymphoma and a reactive lymphocytosis in the B cell proliferations. Flow cytometry is possible if 8–12-mL fluid is available. The differential diagnosis includes metastatic small cell carcinoma of the lung, retinoblastoma, and medulloblastoma – clues include cell molding and aggregation, but cytopsin does encourage lymphoid cells to aggregate, so one needs to be careful. CD56 and TTF1 immunocytochemistry may be helpful in this situation, as these markers would be positive in metastatic small cell lung carcinoma.

Metastatic Tumors to the CNS

A large number of intracranial tumors are metastases rather than primary CNS neoplasms. It is reported that about 20–40 % intracranial tumors are in fact metastases. Almost all peripheral solid tumors have been recorded to spread to the CNS, but melanoma and a limited range of carcinomas (lung, breast, and stomach) are the most common primaries in common with other metastatic sites (Fig. 2.9). Certain cancers, such as breast cancers, are often already diagnosed, but it is not infrequent for some tumors to present with brain metastases as a first manifestation of the disease. Some tumors are recognized to spread to the brain relatively early in their course, including choriocarcinoma and pancreatic adenocarcinoma.

Cells usually present in CSF samples when there is meningeal involvement rather than solid parenchymal tumor deposits. The latter are occasionally aspirated during surgery. The cells may be clustered or dispersed. Adenocarcinoma cells can be identified by their eccentric nuclei, large nucleoli, cytoplasmic vacuoles, irregular nuclear contours, and clumped chromatin. Lung cancer is the most common source of metastases, but it is not usually possible to predict the primary origin of the adenocarcinoma. Rarely, intracytoplasmic vacuoles and Indian filing may point to spread from a lobular breast carcinoma. Mucin may be recognized in adenocarcinoma, either on routine stains or with PASD.

Fig. 2.10 Metastatic small cell carcinoma shows typical molding and speckled chromatin



Small cell carcinoma may have typical cellular features such as nuclear molding, speckled chromatin, and indistinct nucleoli (Fig. 2.10). Pigment may be present in melanoma cells with their typical macronucleoli and intranuclear inclusions. If there is no pigment, the tumor cells look very much like adenocarcinoma. Immunocytochemistry may be possible if there are enough cells in the sample. Usually, CSF samples produce limited cellular material so one needs to be sensible, wise, and selective. Cytokeratins, CD3, CD20, and GFAP may help in focusing the diagnosis on a particular tumor group but ER, TTF1, S100, melan A, HMB45, and PSA can provide a more specific diagnosis.

There are sometimes difficulties in confidently confirming metastatic tumor involvement. For instance, the differential diagnosis of metastatic melanoma from melanosis cerebri may be problematic if pigment is present, but the associated cells are relatively bland. Ependymal cells may resemble metastatic adenocarcinoma, and this may be very difficult to distinguish. Another particularly awkward situation that introduces a frustratingly wide differential is when the cells present are individual and dissociated. Often, isolated cells tend to round up and lose their cytoplasmic processes which causes considerable confusion. In this situation, it may not be possible to confidently tell the difference between metastatic carcinoma, high-grade lymphoma or glioblastoma. Clearly, it may still be possible to predict the true nature or origin of the tumor by correlating with the clinical and

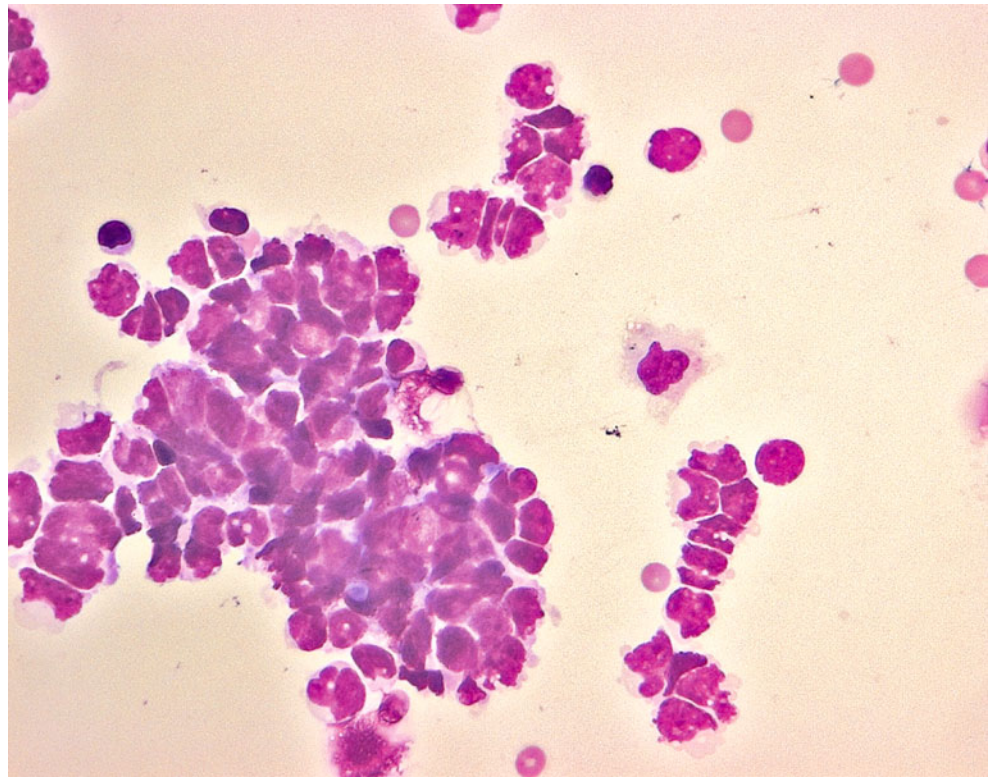
radiological features, and immunocytochemistry may prove fruitful.

Primary Tumors

There is an extensive list of primary CNS tumors which are generally better appreciated cytologically on smears, frozen sections, or FNAs than on CSF findings. Cells are said to shed into the CSF quite often, but interpretation is tricky. Grading is based on a range of microscopic features that will not be available in the fluid sample (see below). The majority of tumors are malignant, but occasionally benign tumors may be encountered. The difficulties found in differentiating between well-differentiated astrocytomas and reactive astrocytosis on histology are equally true for cytology. Glioblastomas are the tumors most likely to be diagnosed on CSF, but distinguishing these high-grade tumors from high-grade lymphoma and metastases can be problematic especially as glioblastoma cells often look epithelioid and lose their long cytoplasmic processes in cytological samples. Medulloblastoma is probably diagnosable, but it is not too commonly seen, and cytology may be useful for recurrence (Fig. 2.11). Lower-grade tumors such as oligodendroglioma do not usually present in CSF.

Ependymal tumors have an epithelioid appearance and therefore may be difficult to differentiate from metastatic adenocarcinoma. Well-differentiated tumors may look like

Fig. 2.11 Medulloblastoma sometimes presents in the CSF



normal columnar cells of the ependyma or choroid plexus; others may resemble adenocarcinoma. Ependymoblastoma resembles the other small round cell tumors.

Tumors located in the vicinity of the pineal gland are uncommon, but there are several well-described types. These are usually easily recognized when the site of sampling is appreciated. They include germ cell tumors or primary pineal tumors or very occasionally gliomas. Primary pineal tumors may either be pinealocytoma, which is more regular and epithelioid and which looks like ependymoma or adenocarcinoma. Pineoblastoma is more primitive and blastic and resembles medulloblastoma. Most germ cell tumors are seminomas with their large spheroidal or vesicular nuclei, prominent nucleoli, and background lymphocytes.

Choroid plexus cells often exfoliate, but this is not necessarily because tumor is present, and they are often seen with ependymal cells in hydrocephalus. These epithelioid cells look indistinct compared to metastatic carcinoma cells and are larger than lymphocytes with inconspicuous nucleoli and rather pale staining of the nucleus and cytoplasm. One needs to be careful however that one is not dealing with a metastasis.

Pituitary tumors very rarely present in CSF, but they may be aspirated or smeared. On occasion, they may present in CSF as medium-sized cuboidal or columnar cells, and of course, clinical and radiological correlation should help to determine the correct diagnosis.

Very rarely, chordomas may present in the CSF. They are typified by the large epithelioid physaliphorous cells containing characteristic bubbly cytoplasm. Immunocytochemistry shows the expected result with positivity for cytokeratin, S100 and EMA, together with a positive result using mucin stains.

The differential diagnosis is similar with all of these lesions and focusses around difficulties differentiating between primary CNS tumors and lymphoma, leukemia, and a range of metastatic deposits.

Other Conditions and Cell Types

Intracranial hemorrhage may be confirmed on CSF cytology, although of course a traumatic or bloody tap needs to be considered. One useful tip is that traumatic taps will settle to leave a clear supernatant while genuine subarachnoid hemorrhage (SAH) produces a xanthochromia and spectroscopy may detect hemoglobin and/or bilirubin. The appearance of the red cells may help to determine the time frame of the hemorrhage with frank blood (fresh red blood cells) present early on and erythrophagocytosis and hemosiderin pigment later. It is important to weigh up the risks versus the benefits before performing a lumbar puncture on a patient with a suspected SAH as the accompanying raised intracranial pressure usually precludes the investigation as it may precipitate coning. Infarcts may produce subtle CSF abnormalities

including a few mixed inflammatory cells, but these are nonspecific and are unlikely to be helpful in confirming the diagnosis. Radiology is probably more useful, and of course, a cytological diagnosis should be correlated with the radiological features in any case.

A range of degenerative and demyelinating conditions may be present without any abnormality of the CSF, but there may be a few nonspecific cells especially lymphocytes and histiocytes and possibly even polymorphonuclear neutrophils. Sometimes, plasma cells are obvious, and clearly, the differential diagnosis in this situation includes multiple myeloma, viral or tuberculous meningitis, syphilis, or Lyme disease.

Eosinophils may be seen with parasites and cysticercosis in the CNS, but they may also be present when there is malignant meningitis, drug instillation, or a shunt is present, or the sample is removed at the time of a repeat lumbar puncture. Eosinophils may be admixed with histiocytes in cases of Langerhans cell histiocytosis. The latter should have the usual grooved nuclei, and immunomethods may be used for CD1a, Langerin, or S100.

Very rarely, foamy macrophages may be an indicator of an underlying storage disorder.

Sensitivity and Specificity

CSF examination in a general cytology department is dependent on several factors including the volume of the specimen, timely transportation to the laboratory, and the use of fixative in case of delayed transportation. Often, the cytology department will receive the remainder following microbiological and/or biochemical testing on the same sample, and this also affects the results of cytology. As a result, the cytology may be nondiagnostic with many acellular samples reported, and this is not surprising. However, sensitivity is reported to be as high as 60 % in malignant meningitis which can be improved upon with multiple samples. The false-positive rate is said to be approximately 2.6 %. False positives usually turn out to be the cases one would predict and are mainly overdiagnosis of lymphomas and leukemia, and rarely other malignancies, due to lymphocytosis, blood contamination, inoculated cells, or other material such as extraneous or keratin.

Aspiration of "Cysts"

An intracranial lesion with a lucent center may be a benign cyst, an abscess, granulomatous disease, or a necrotic tumor. Usually, the nature of the lesion is appreciated on radiology, but sometimes a pathological diagnosis is required. The material aspirated should be processed as for a conventional fluid sample as discussed previously and either spread

directly, cytopun, or processed by liquid-based methodology or a cell block. A genuine benign cyst will probably contain clear fluid, and the cytological sample comprises only macrophages. This appearance could also be found with a cystic astrocytoma or cystic hemangioblastoma without the lesional cells represented. If turbid and full of neutrophils, then the aspirate is probably from an abscess, especially if there is necrosis (microbiology may be crucial in this case). In the cytology laboratory, one may perform a fungal or other specific stain to pick up the causative organisms. If the patient is immunosuppressed, one should look for unusual organisms such as *Nocardia*, *Mucor*, *Candida*, and *Aspergillus* with the appropriate stains. If granulomatous features are present, one must search for mycobacteria or *Toxoplasma gondii* as described earlier.

If squamous cells are present and conspicuous, one should think about an epidermoid or dermoid cyst, depending on the location of the lesion. Another possibility is an adamantinoma (craniopharyngioma) which will usually be located in the suprasellar region. Clues include a predominance of anucleate squames in craniopharyngioma with foreign body-type giant cells and cholesterol crystals, although one may not be able to tell which of these possibilities is correct on cytology alone. Metastatic squamous cell carcinoma can also present as a "cystic lesion" in the brain (Fig. 2.12).

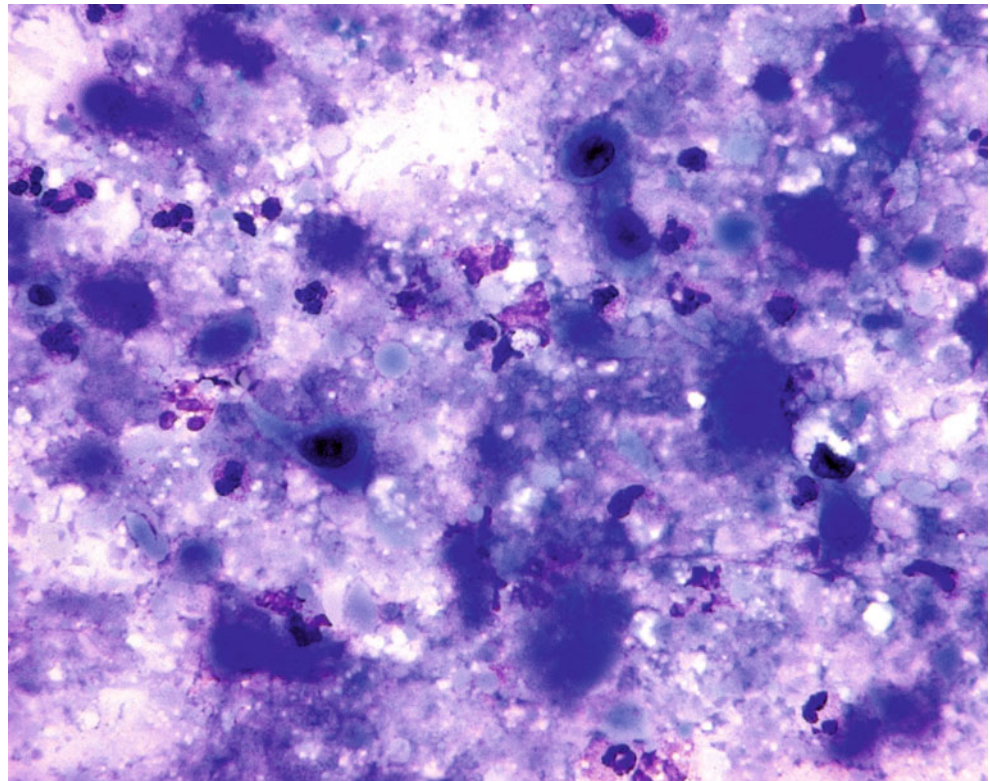
If just necrosis is present, one cannot be confident of the diagnosis, in particular exclusion of malignancy, and the sample should be considered nondiagnostic. The clinician may well consider reaspiration.

Brain Smears

CNS cytology, by direct sampling of a lesion in the brain, may be appropriate for a range of primary and secondary tumors and a variety of infections, especially in immunocompromised persons. Viral, bacterial, fungal, and mycobacterial infections can be diagnosed by this technique and also several types of demyelinating conditions. Most studies suggest that brain smears have an accuracy of over 90 % and have the great benefit of allowing immediate confirmation that pathological material has been sampled.

In fact, brain smears of intracranial tumors are usually carried out intraoperatively to determine whether lesional material is present. The pathologist may be able to identify the tumor type and grade in some cases. Cystic lesions may also be aspirated during neurosurgical procedures. If the amount of tissue is small and likely to be used up in a smear, the pathologist needs to decide whether to smear the material or keep for histology and other subsequent studies. Most lesions in the brain either do not need to be removed or cannot be resected. Intraoperative sampling does not usually lead to removal, but it does direct subsequent management, and appropriate chemoradiotherapy relies on an accurate diagnosis. Intrinsic lesions and metastases are usually

Fig. 2.12 This is a cystic metastasis from a squamous cell carcinoma of the lung. Note the atypical squamous cells



soft and accompanied by edema which makes them ideal for smearing. This is particularly useful for CNS samples because it avoids the ice-crystal artifact of frozen sections that is commonly seen when brain tissue is frozen. Often the biopsy is obtained by a stereotactic procedure because only a local anesthetic is required, and this technique can provide good sampling of mass lesions such as tumors, cysts, and abscesses. Tissue may additionally be examined by frozen section examination, and occasionally this may be the preferred method, especially with firmer tumors such as meningiomas and nerve sheath tumors. Any surplus tissue can be retained for paraffin, but fixation should await formal diagnosis on smear in case more tissue smearing is needed. Alternatives include imprints and needle washings for cytology.

Although smears are the main sampling method, some units perform FNAs. This generally produces similar appearances to the smear but may produce fewer complications with deep-seated lesions.

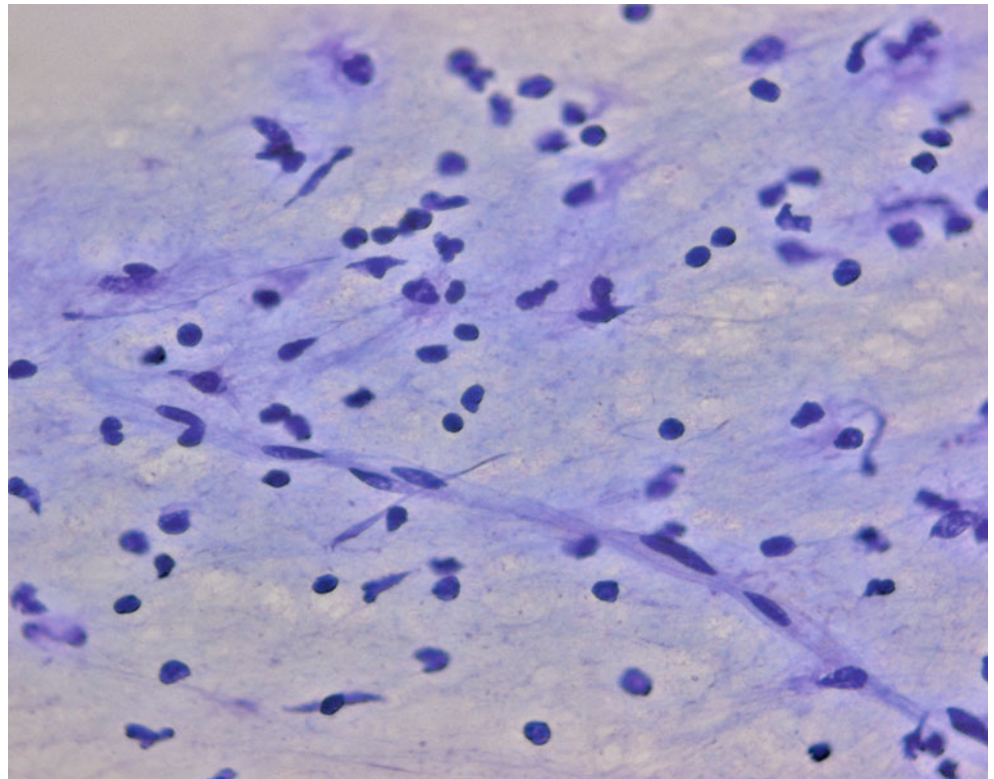
Whatever the sampling method, the typical diagnostic relationship is seen between radiology and pathology in CNS lesions though with pathology mostly confirming or refining a radiological diagnosis. This may not always be the case, and the radiology and cytology need to be correlated with the clinical picture to avoid making a mistake. Radiology, especially MRI, is good at suggesting diagnoses, but a tissue diagnosis is often crucial for accuracy and appropriate management.

It is crucial to know the appearances of normal cells, their reactive counterparts, and a variety of tumors before embarking on CNS cytology. Two well-recognized catches are the cerebellar molecular layer cells which look like blast cells and ependymal cells which are epithelioid and therefore raise the suspicion of adenocarcinoma. It is also invaluable to know the clinical data, the radiological features, and the site of sampling, all of which will focus on the differential diagnosis even before the operation has begun.

Technical Methods and Smear Technique

Sometimes, the material sent for the smear will be the only available pathological material and with small samples, a rapid decision needs to be taken as to whether it is better to smear or embed – this will depend on experience and luck. Health and safety issues are important especially with respect to HIV and CJD in terms of handling and instrument cleansing/disposal. A 1–2-mm piece of tissue is placed at one end of a labeled glass slide, and this is squashed and dragged gently along its length with another labeled slide. If necessary, several pieces may be used to prepare a number of slides. There will be a concentration of the viewable material at the primary tissue location which will tail off as one moves along the slide. The slides are fixed immediately in an appropriate fixative such as alcohol (e.g., 2 min in 95 % ethanol). They are rinsed off with tap water, and then a suitable stain is

Fig. 2.13 Normal brain smear with incorporated blood vessel



applied. Stains used depend on personal preference, but those most frequently employed include a rapid H&E, toluidine blue, or methylene blue.

Once the smears have been reported, one needs to decide what to do with the rest of the material. This may include sending some material to microbiology, fixing for paraffin embedding, and/or electron microscopy or retaining for molecular techniques. Rarely, the diagnostic smear may need to be destained and used for a special stain, immunocytochemistry, or in situ hybridization.

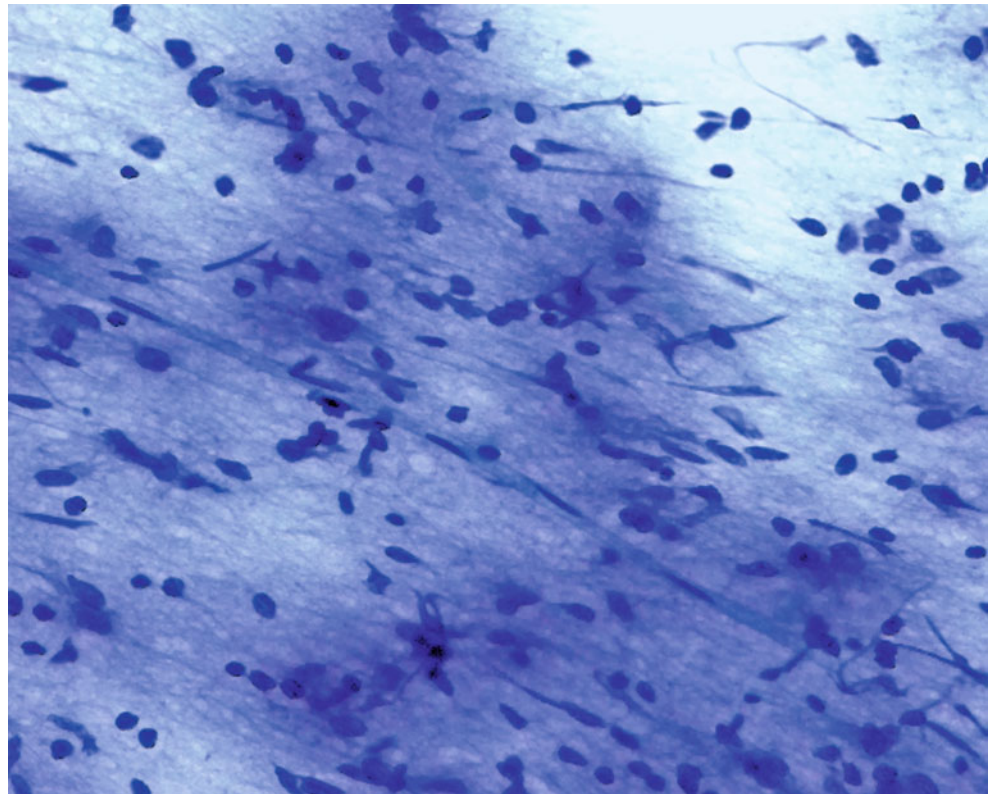
Frozen section may be used as an alternative or addition to the smear. The former is more appropriate for tougher lesions, and it may provide a better assessment of the overall architecture. On the other hand, some architectural features are appreciable on the smear, especially in the thicker areas, and the cell detail is much better. Frozen section appearances are not described any further here as this enters the realms of histology.

Normal Appearances

Normal brain tissue typically spreads thinly and uniformly across the slides, in contrast to the irregularity of reactive and pathological states. One can usually make out certain normal resident cells and background matrix with relative ease including neurons, glial cells, and blood

vessels (Fig. 2.13). Neurons are generally the largest cells with triangular outlines, elongated cytoplasmic processes, large nuclei, and single prominent nucleoli, as well as cytoplasmic Nissl substance. Glial cells (especially astrocytes and oligodendrocytes) may be difficult to differentiate from each other as they may lose their cytoplasm and appear as bare nuclei. However, astrocytes typically have ovoid nuclei with stippled chromatin and a stretched cell body with cell processes. Oligodendrocytes are round cells and have darker chromatin and scanty cytoplasm. Cerebellar tissue gives a distinctive appearance with small granular neurons from the granular layer and larger paler cells from the molecular layer admixed with occasional dendritic Purkinje cells which are located between the other two layers. Other normal structures occasionally seen include ependymal cells and choroid plexus cells. Capillaries are seen to traverse the smear. These have smooth outlines and are made up of elongated endothelial cells. They may branch and appear papillatoid, but they are always thin walled and delicate. Microglia shares the same histiocytic appearance as macrophages present elsewhere although they often appear more spindly and “rod shaped,” these are rarely conspicuous on smear preparations. Very occasionally, normal arachnoid granulations and choroid plexus are sampled in smears which can be mistaken for meningiomas and papillomas, respectively.

Fig. 2.14 The cellularity is increased in reactive gliosis which may cause difficulty in differentiating from a low-grade glioma



Reactive/Gliosis

In this situation, the spread material is noticeably more cellular than normal (Fig. 2.14). Of course, reactive changes can be seen around benign and malignant space-occupying lesions, including the edge of an astrocytoma where they blend with normal, and this differential can be very difficult. Reactive astrocytes are larger than normal with more obvious cell processes, and gemistocytic forms (with eccentric nuclei and plentiful eosinophilic cytoplasm) are often seen. They are usually quite uniform and tend to cluster away from vessels, with bigger nuclei and nucleoli than normal, and possibly also mitoses (but the latter are not atypical). Microglial proliferation typically produces “rod-shaped” nuclei, and inflammation may be present in the background. Capillaries may appear increased with larger endothelial cells, and macrophages may be present, sometimes with hemosiderin pigment. Astrocytoma cells cluster close to local proliferating capillaries, and this may help to differentiate from reactive gliosis.

Inflammation, Infarction, or Demyelination

A variety of patterns may be seen with infections, pyogenic, chronic, or granulomatous with neutrophils, lymphocytes,

or macrophages respectively predominating. One may see the organism responsible if toxoplasmosis or viral infection is present, but bacteria not usually seen. The main space-occupying lesions are abscesses and encephalitis. Typically, the former shows numerous neutrophils and debris, but tumor necrosis must not be overlooked. If the abscess capsule is fibrotic, it may be difficult to smear. With encephalitis, there tends to be perivascular cuffing with lymphocytes and plasma cells, although one can see this around tumors as well. Tissue should be kept for microbiology if not already sent. Viruses can be diagnosed on smear, if the characteristic inclusions or features of HSV, CMV or PML are present, but these diagnoses may well require molecular techniques. There is often perivascular concentration of lymphocytes in these viral encephalitides with cytologically atypical astrocytes in the smear preparation which one needs to be careful not to misinterpret as malignant. Epithelioid macrophages or discrete collections of macrophages forming granulomas may be recognizable in TB or sarcoid. ZN stain is obviously appropriate in this situation. Toxoplasmosis may be recognizable on smear with the 3–6- μ m tachyzoites seen (Figs. 2.15, 2.16, and 2.17). There is often a necrotic background.

Infarcts can be acute or chronic. In both, shrunken neurons are seen in early lesions with deep-staining cytoplasm and loss of Nissl substance. The neurons often take on a

Fig. 2.15 Toxoplasmosis may be recognized by identifying the intracellular organisms

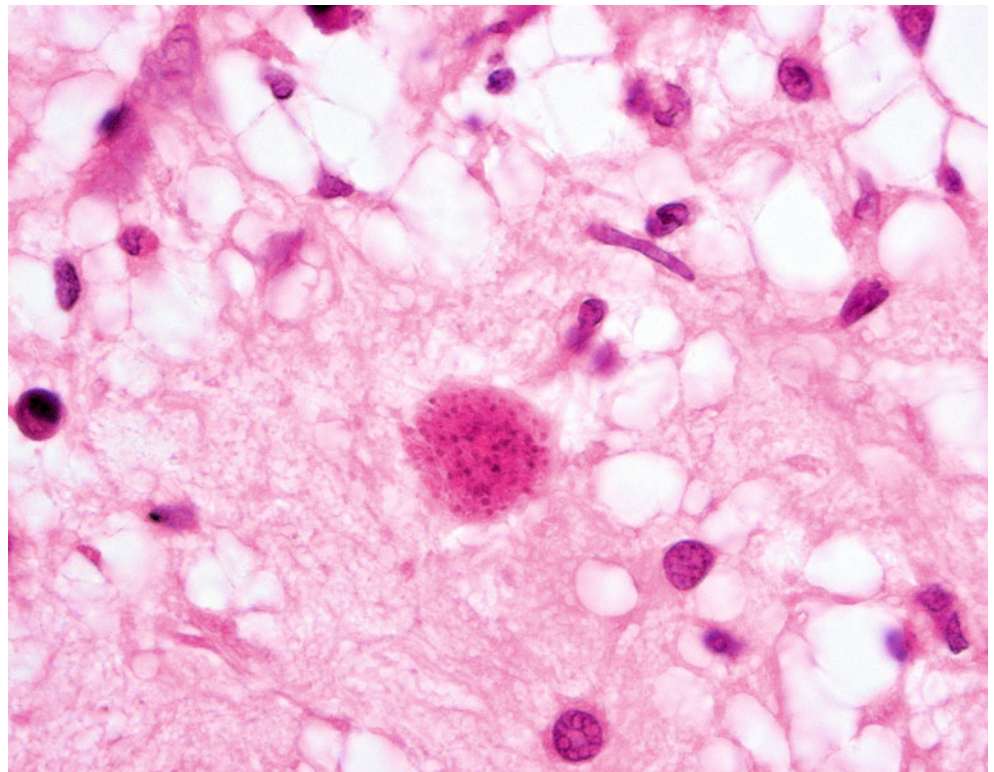
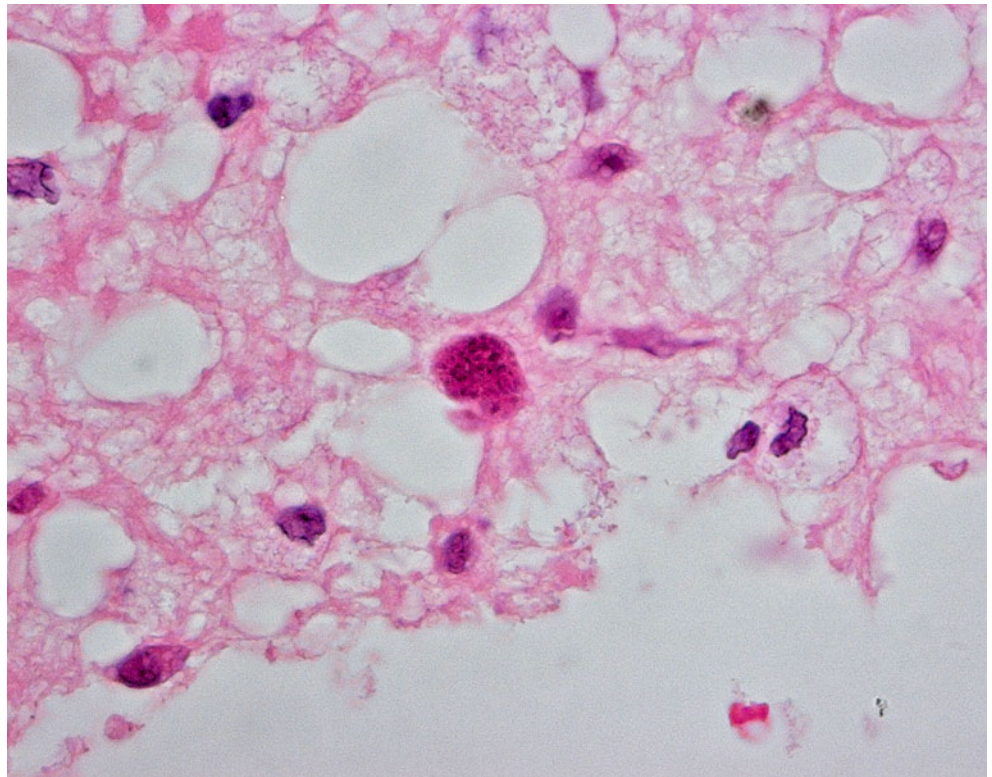
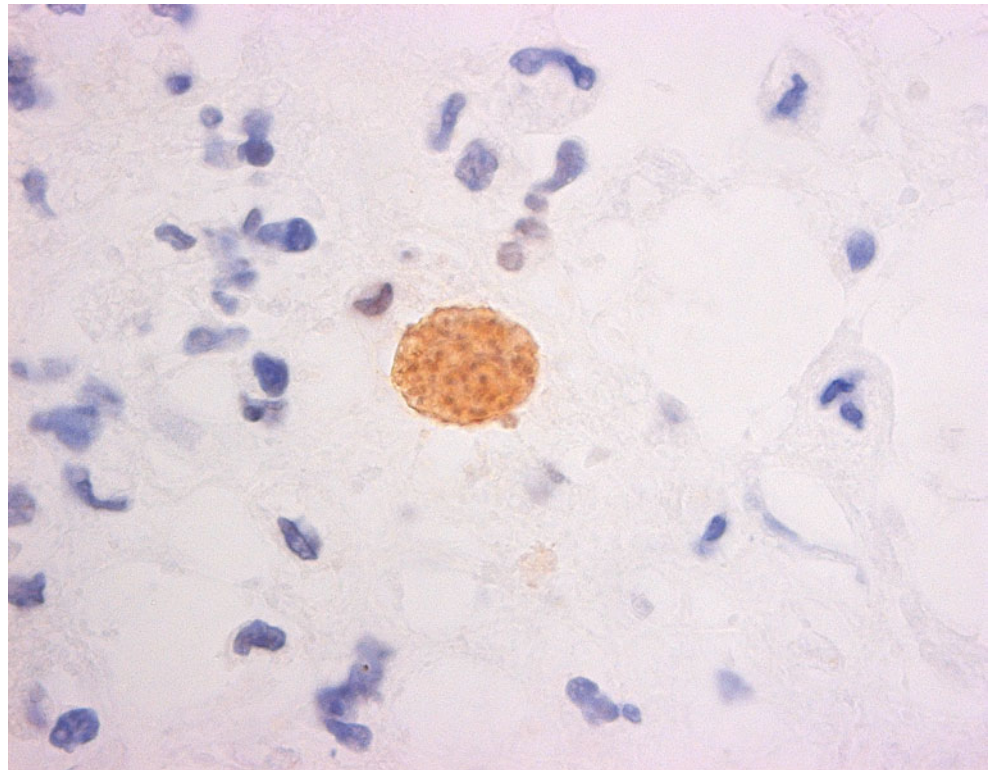


Fig. 2.16 High power of toxoplasma organisms

Fig. 2.17 Immunohistochemistry can be used to support a suspicion of toxoplasmosis



sharply outlined triangular shape. There may be local polymorphs in early lesions but with time macrophages, including lipid-laden forms, and proliferating capillaries dominate the picture. Demyelinating lesions, such as those seen with multiple sclerosis, show a lymphocytic infiltrate, again perivascular, and obvious foamy macrophages without the other features of an infarct (Fig. 2.18). In general, one does need to be careful of extreme reactive astrocytosis which may be seen around any of the above lesions.

Tumors

These are mainly primary gliomas (most commonly astrocytomas) or metastases. Many of the comments made previously for CSF samples are also valid for smeared specimens. For example, it can be very difficult to differentiate on smear between glioblastoma and metastatic carcinoma especially with high-grade tumors. Smearing encourages an artifactual impression of cytoplasmic processes as well as an apparent lack of cell cohesion. Glial tumors tend to have genuine cytoplasmic processes, an infiltrative pattern into surrounding brain tissue, and often there is necrosis with palisade of tumor cells around a central necrotic focus. If possible, immunohistochemistry for GFAP may be helpful. Lymphomas may resemble gliomas but typically show no cell cohesion, cytoplasmic processes, or the typical perivascular pattern. Carcinomas may be

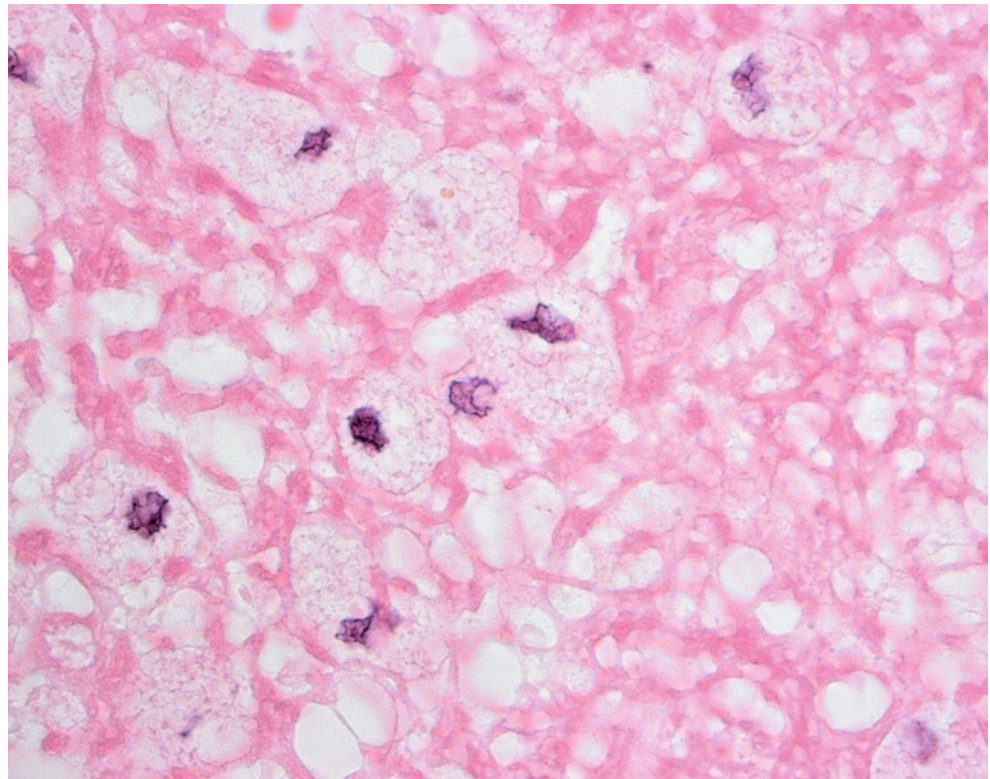
recognized by their cohesive pattern on spreading. They tend to be well circumscribed on frozen sections. Melanoma often has prominent macronucleoli. If material is available, immunocytochemistry for cytokeratins, melan A, and HMB45 may be invaluable.

Primary tumors can usually be identified and their grade predicted if the sample obtained for smear is representative, which it may not be. Grading is based on 4-tier WHO system depending on whether there is increased cellular density, nuclear atypia/pleomorphism, necrosis, mitotic activity, and endothelial cell proliferation. As mentioned earlier, the pathologist requires knowledge of the location of the biopsy, the age of the patient, and the results of the radiology to interpret the material and give the most accurate diagnosis. Most units rely on an urgent report to establish whether the material sampled is pathological or not, especially tumor versus no tumor, but if the smear is abnormal, a more specific preliminary report is desirable, importantly whether there is a specific benign condition (inflammatory or neoplastic) or whether any tumor present is primary or metastatic and the grade of the tumor if at all possible.

Types of Primary Tumor

Astrocytomas make up around 25–30 % of primary brain tumors in adults and a similar proportion of cerebellar tumors in children. There are several different types of

Fig. 2.18 Foamy macrophages may be seen in demyelinating conditions or infarcts



astrocytoma which present in particular sites and at different ages so clinical and radiological background information is essential.

Fibrillary astrocytoma is the most common type of astrocytoma and is composed of a meshwork of processes producing a striking fibrillary appearance. There may be low cellularity and only mild pleomorphism of the nuclei with PTAH-positive material in the cytoplasm. The fibrils may radiate toward the blood vessels present. There may be calcification. When the edge of the lesion is sampled, this may include neurons and make diagnosis problematic. This can be a difficult differential diagnosis, and exclusion of reactive astrocytosis may be impossible (Figs. 2.19 and 2.20). In addition to increased cellularity, one should look for nuclear pleomorphism, hyperchromasia, and whether or not the astrocytes are admixed with oligodendrocytes.

Protoplasmic astrocytomas are rare, but they have typical histological findings with a delicate web of processes and small cystic spaces with eosinophilic fluid. It is extremely difficult to confidently differentiate these from fibrillary tumors on cytology.

Pilocytic astrocytomas are low-grade tumors found in the cerebellum in a younger age group. They are composed of fusiform cells with very long wavy processes that are associated with PTAH-positive red blobs or “waxy bodies” called Rosenthal fibers. There may be calcification, and all of these features may be visible on smears.

Gemistocytic astrocytomas have large cells with abundant red cytoplasm and a pseudorhabdoid appearance with eccentric nuclei (Figs. 2.21 and 2.22). They are often accompanied by dedifferentiated cells and show perivascular lymphocyte cuffing. These are usually high-grade tumors.

Anaplastic astrocytomas are common, high-grade tumors (WHO grade 3). They may be the sole form of tumor present or are sometimes a component of one of the other types of astrocytomas described above. These produce very cellular smears with clear morphological atypia (Fig. 2.23). It may be difficult to recognize the lesion as astrocytic because of the degree of nuclear pleomorphism, but the tumor cells often have inconspicuous nucleoli in contrast to most metastatic carcinomas, melanoma, or high-grade lymphoma. There may be mitoses and/or necrosis, but unlike glioblastoma multiforme (GBM) described below, they frequently lack necrosis. It may not be possible to differentiate these tumors confidently from GBM on smear as all of the features required for this diagnosis may not be assessable.

Glioblastoma multiforme is by definition a high-grade (WHO grade 4) astrocytoma. It has extremely bizarre and pleomorphic cytological atypia and has prominent necrosis as well as endothelial and/or vascular proliferation, sometimes with glomeruloid buds. The viable tumor cells are often located around blood vessels which may help differentiate it from carcinoma (Figs. 2.24, 2.25, and 2.26).

Fig. 2.19 It may be very difficult to distinguish between reactive gliosis and a low-grade glioma. Biopsy from the leading edge of a glioma may incorporate both types of tissue

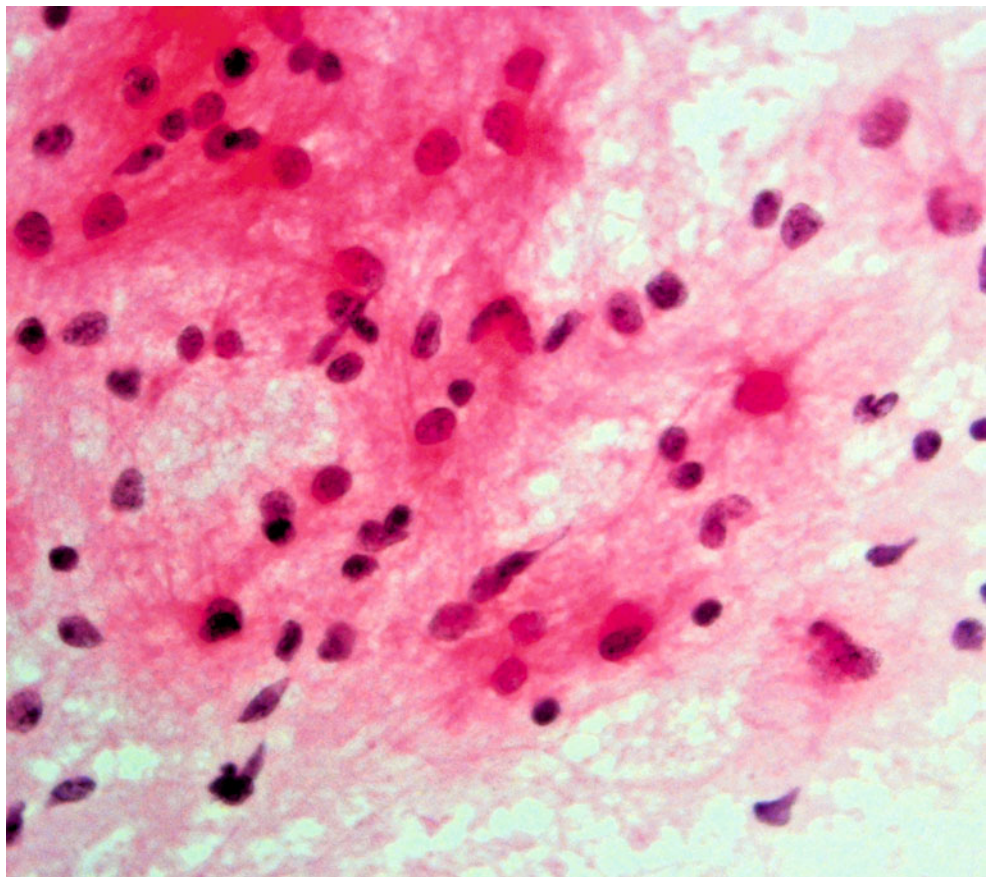
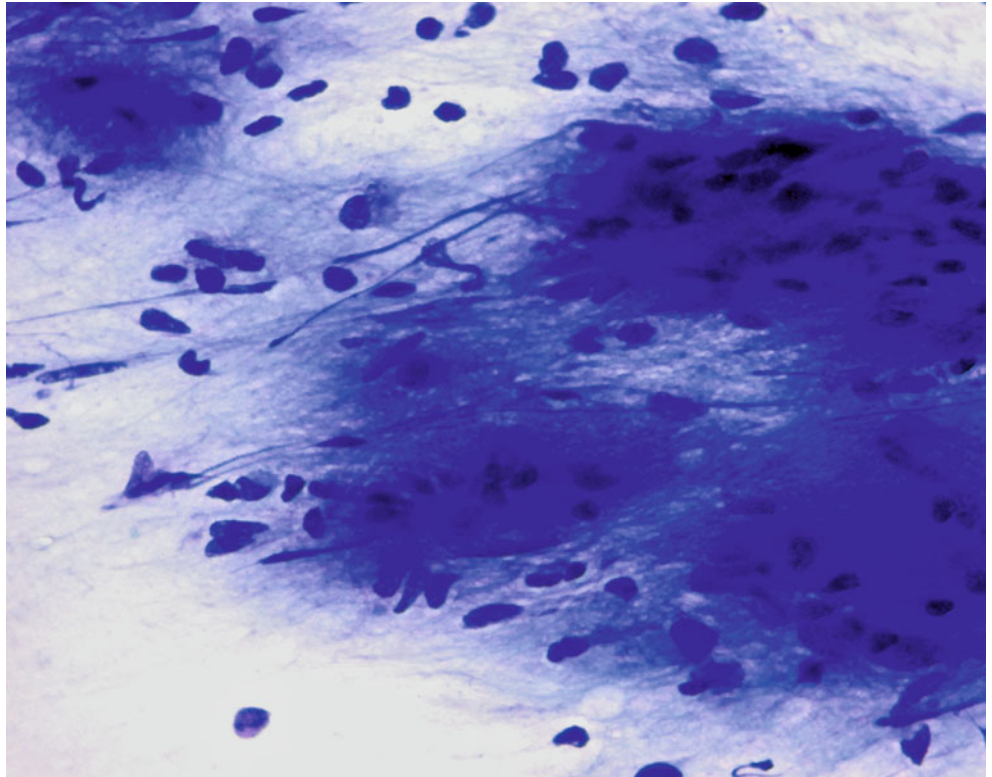


Fig. 2.20 This is the leading edge of a low-grade glioma. Note the glial processes

Fig. 2.21 Gemistocytes have eccentric nuclei and “rhabdoid” features

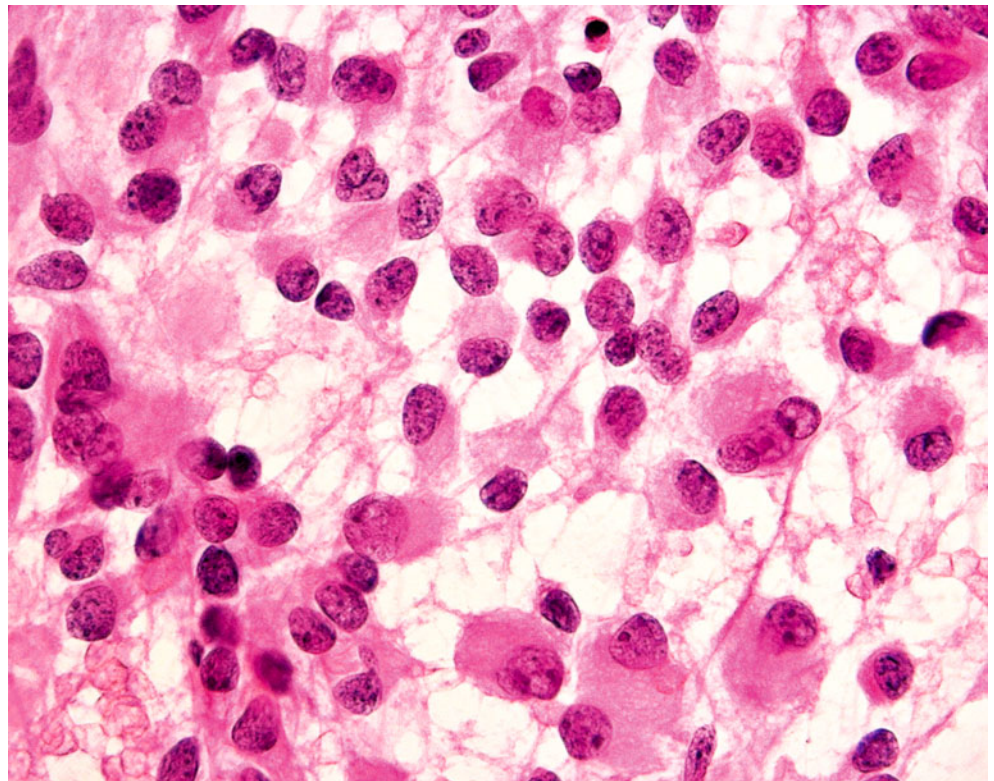
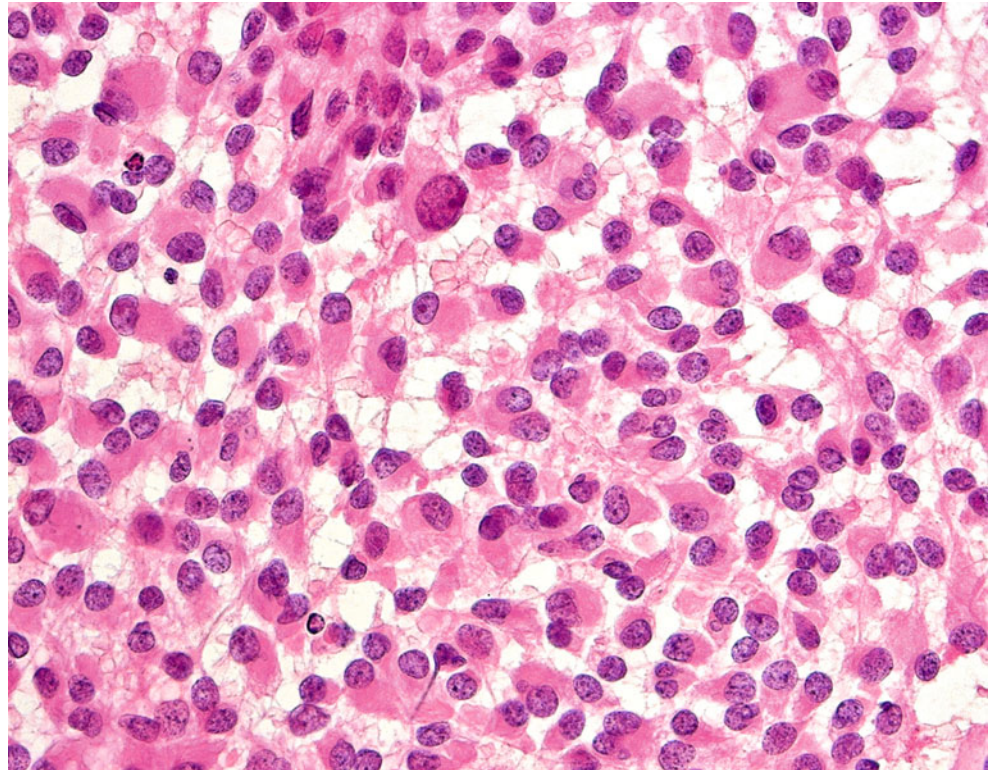


Fig. 2.22 Gemistocytic tumors are usually high grade

Fig. 2.23 There is clear atypia in this high-grade glioma

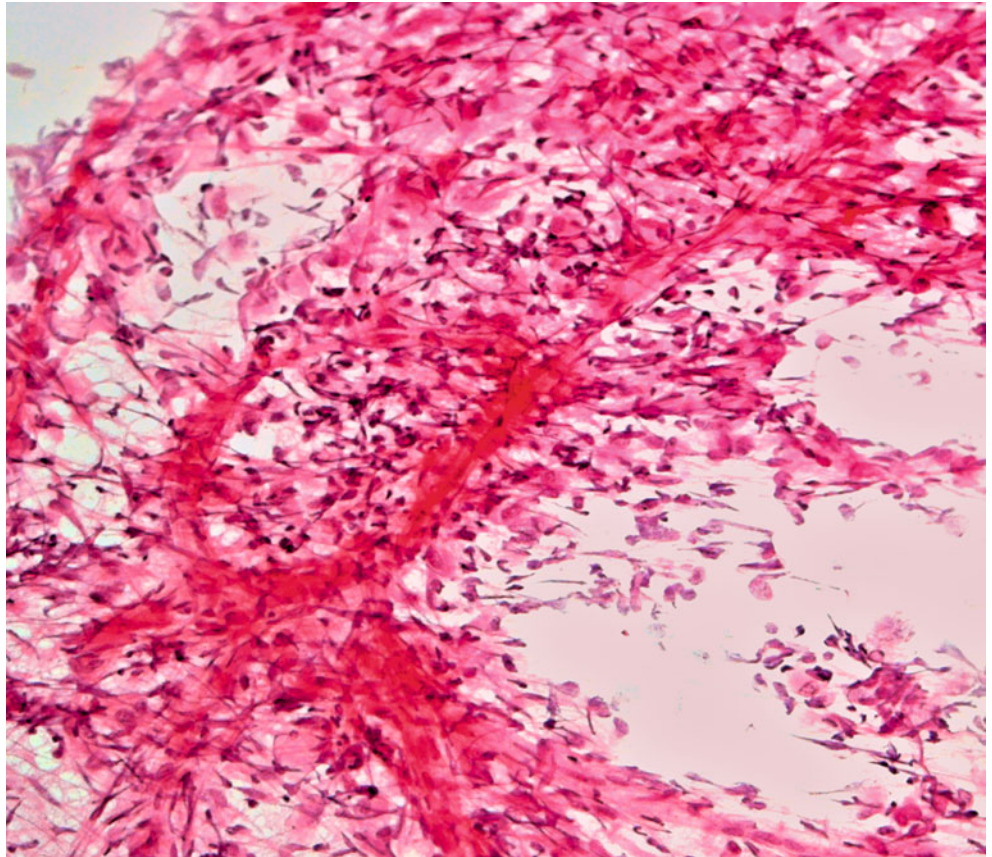
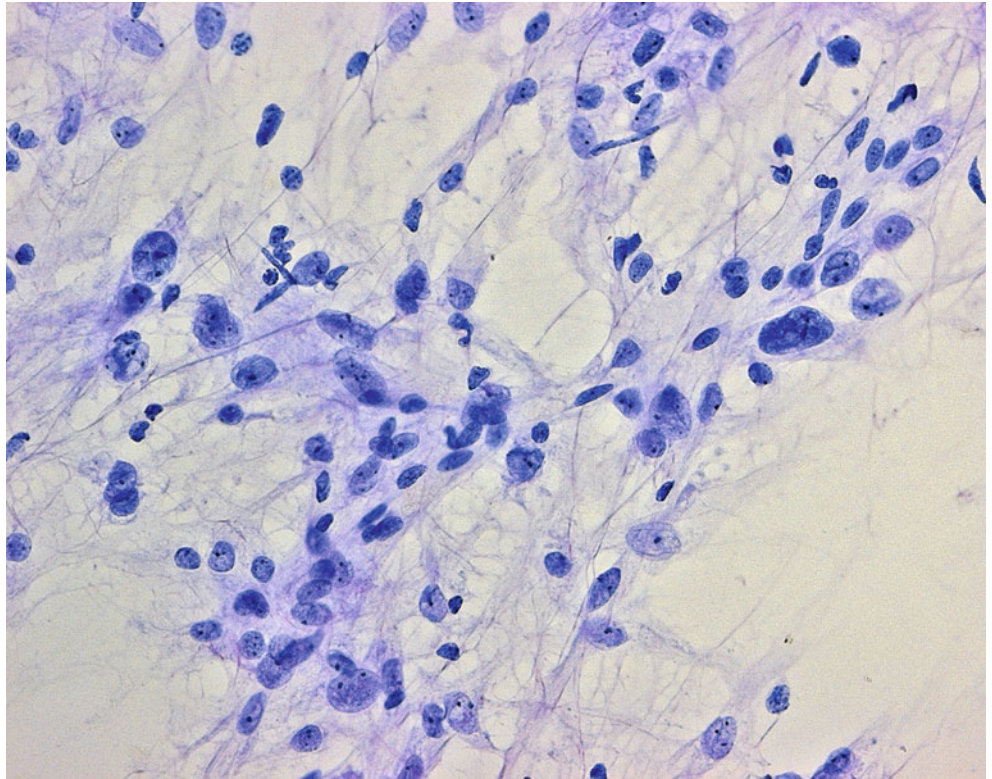


Fig. 2.24 A high-grade glioma with conspicuous vascular proliferation

Fig. 2.25 Gliomas typically show perivascular aggregation of tumor cells on smear

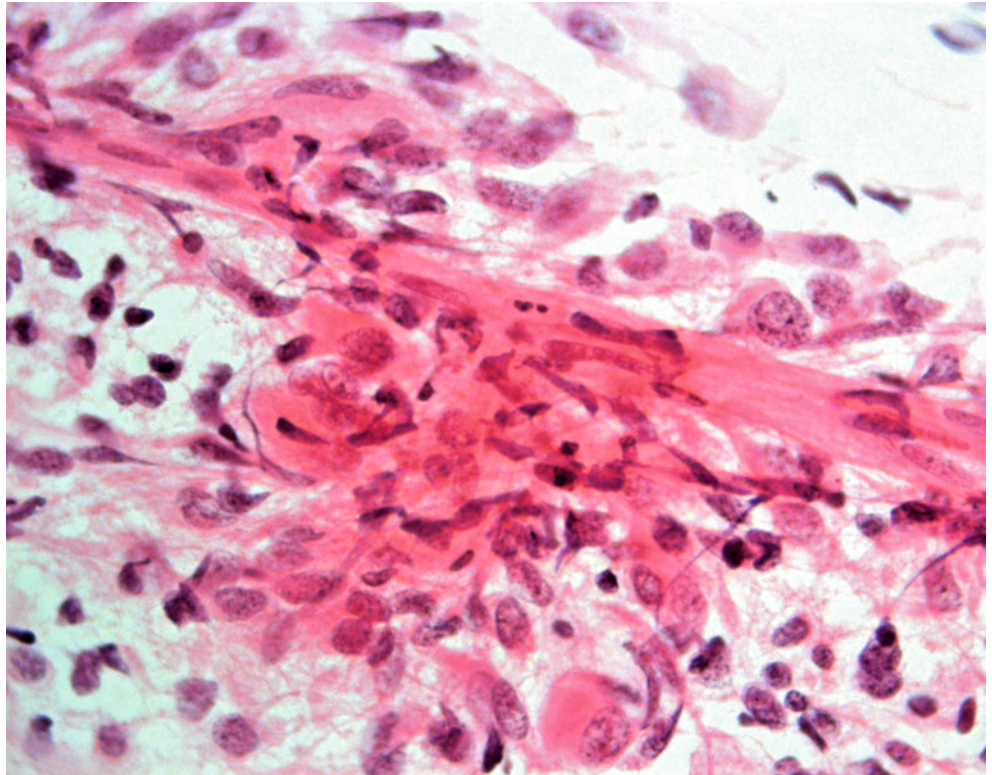
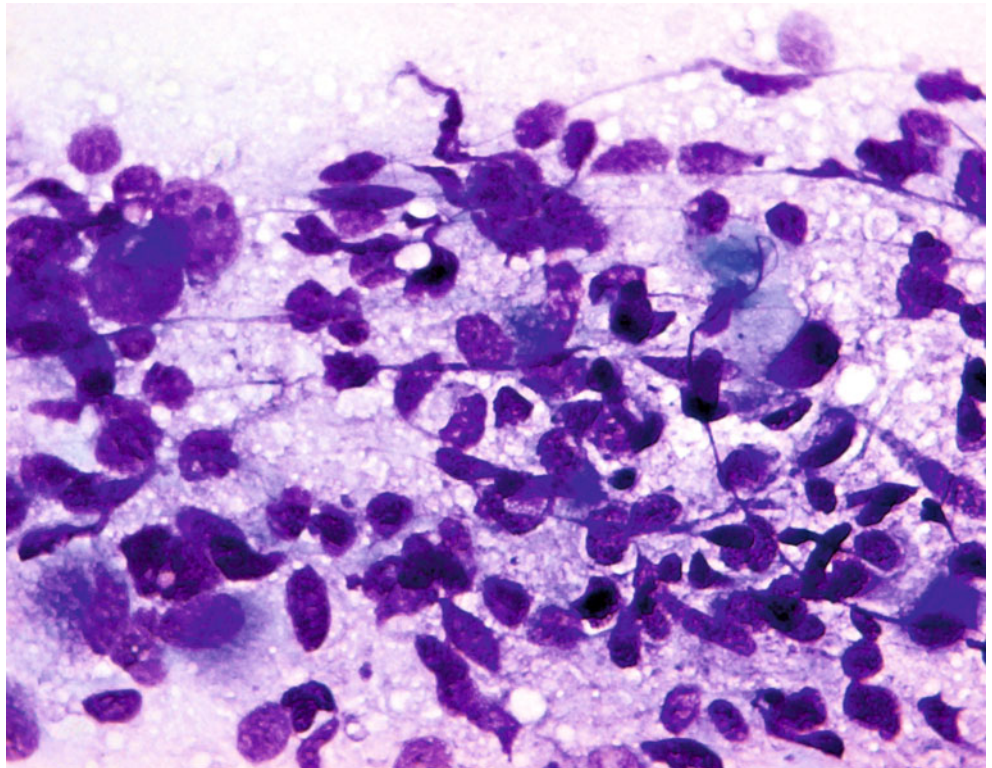


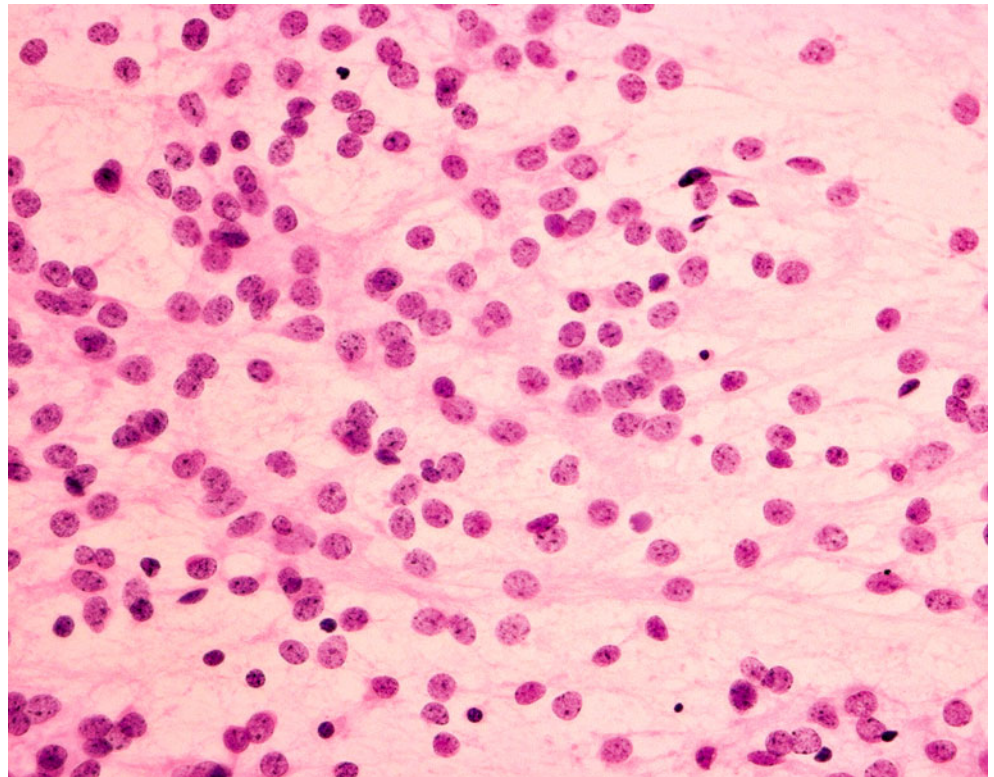
Fig. 2.26 High-grade glioma on MGG with high-grade nuclear features



Subependymal giant cell astrocytoma is a tumor that typically occurs at a young age in association with tuberous sclerosis. It most often arises in lateral ventricle wall and bulges

into the ventricular cavity. It is composed of large, often giant, and bizarre cells with eccentric nuclei, prominent nucleoli, and plenty of glassy eosinophilic cytoplasm. There

Fig. 2.27 Oligodendrocytomas have regular oval nuclei and lightly staining chromatin



is often calcification present. The tumor cells are thought to be neurons or another type of specialized subependymal cell. Morphology belies its behavior which is more indolent than one might expect, and this tumor is a classical catch for misdiagnosis as anaplastic astrocytoma.

Astroblastoma is a very rare tumor presenting in the first three decades of life. It is found in cerebral hemispheres, is highly malignant, and shows perivascular arrangements or rosettes with processes toward a central vessel. It clearly looks very similar to other blastic tumors and requires complete work up before a definitive diagnosis is possible.

Oligodendrogliomas are most often cerebral in location. They are typically slow-growing tumors with calcification. They are composed of small round or oval cells with regular nuclei, a fine chromatin pattern, and indistinct but definite nucleoli (Fig. 2.27). The most distinctive feature is the presence of clear cytoplasm giving a haloed appearance. Also, the tumor cells do not gather round blood vessels as most other tumor cells do. Nevertheless, blood vessels are usually prominent. The nuclei are slightly darker than astrocytes, and these tumors produce monotonous diffuse spreads with rare mitoses. Sometimes, the tumors dedifferentiate or are mixed oligoastrocytomas, and anaplastic/higher-grade forms of oligodendrogliomas do occur. The latter are obviously quite difficult to identify correctly on smear. Grading follows the usual principles.

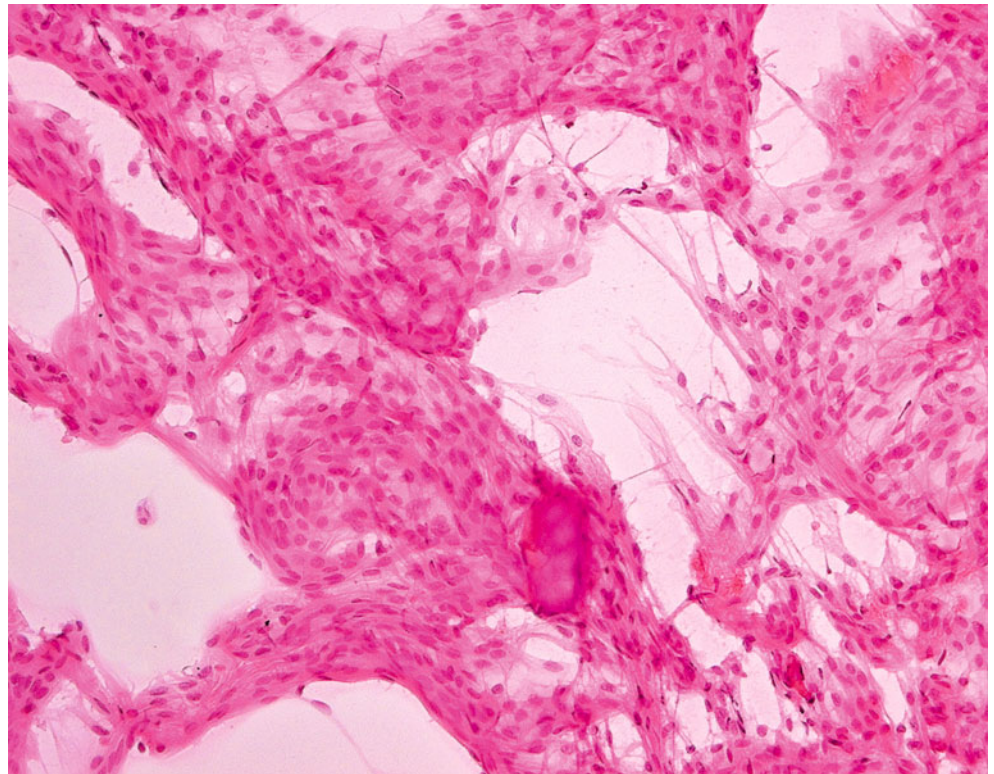
Ependymomas are most commonly located in the fourth ventricle, but they can be found in the lateral or third ven-

tricles, spinal cord, or cauda equina. Children and adults are affected. There are different types with differing patterns. Rosette-like structures (pseudorosettes) may be recognizable around blood vessels, and there may be an obvious papillary architecture. The papillae are covered by regular columnar epithelial cells. The papillae may be so conspicuous that the appearance resembles that seen with choroid plexus papillomas (see below). The bare nuclei or more cuboidal cell can look like oligodendrocytes, but they are slightly smaller and appear elongated or stretched out when compared to oligodendrocytes. Myxopapillary ependymoma is a particular form of ependymoma found especially in the cauda equina. The usual columnar or cuboidal ependymal cells with their round or oval nuclei and small nucleoli are associated with well-defined cores of hyaline material which is PASD and Alcian Blue positive. This material may also be recognized in fragments of stroma present. Rosettes are not usually present.

Subependymoma also occurs most frequently in the vicinity of the fourth ventricle. It is characterized by small nests of bland glial cells separated by a large meshwork of fibrils. They smear with difficulty and may need frozen section for diagnosis.

Choroid plexus tumors can be benign or malignant. The former is usually a papilloma originating in the lateral ventricle or fourth ventricle. The papillae are usually obvious with their fibrovascular stromal cores covered by a layer of cuboidal cells lying on a basement membrane. There is usually no calcification. There are anaplastic variants, and

Fig. 2.28 Low-power view of a meningioma with psammoma-like calcification



malignant choroid plexus tumors can be identical to metastatic adenocarcinoma which needs to be differentiated by clinical, radiological, or immunohistochemical evidence.

PNETs comprise a group of primitive tumors including medulloblastoma, neuroblastoma (including olfactory), ependymoblastoma, pineoblastoma, spongioblastoma, and others. Often, the final diagnosis relies on the site of origin of the tumor rather than its appearance. All look very similar with a high nuclear-cytoplasmic ratio, numerous mitoses and possibly a hint of differentiation. Medulloblastoma is the most frequent tumor diagnosed on smear in children although it may occur in individuals in their twenties and occasionally later. These tumors show true rosettes with cytoplasmic processes arranged toward the central lumen rather than around a blood vessel. They are often located in the posterior fossa, and this site makes this a diagnostic challenge as the tumor cells can easily be mistaken for cerebellar granular cells. However, on close inspection, the latter are more regular, and they may be admixed with Purkinje cells.

Neuronal tumors such as gangliocytoma and gangliogliomas are composed of a mixture of mature ganglion cells and glial cells. They are slow growing and may be confused with low-grade astrocytoma invading background brain with entrapped neurons. The separation can be very difficult on smear. Ganglioneuroblastomas are usually more obvious with their small dark cell component, but these are more frequently located in the retroperitoneum and periadrenal region.

Meningioma is a relatively common tumor usually arising from the arachnoid villi in the venous sinuses and their tributary veins. Half of the tumors are located close to the superior sagittal sinus, but the spinal canal, orbit, ventricles, skull, or even extracalvarial tumors are described including in the skin and lung. They most commonly arise in middle age and more often in women than men. Different types are described histologically, but smears often reveal cohesive groups of cells with regular nuclei and fine chromatin with small nucleoli. Whorls are typical and psammoma bodies are common (Figs. 2.28, 2.29, 2.30, and 2.31), but the latter are not seen in the fibrous or angioblastic types. Fibroblastic forms smear badly, and they are composed mainly of bland spindle-shaped cells which have no specific features. If no whorls are visible, the differential with neurilemmoma or astrocytoma can be difficult, particularly if the site of sampling is not appreciated (this type of meningioma is often intraventricular). Syncytial meningiomas may be more pleomorphic with mitoses, but there are also atypical and malignant varieties of meningioma which can be difficult to diagnose on cytology. The hemangioblastic type resembles hemangioblastoma on histology and often requires immunohistochemistry to differentiate as meningiomas are positive with PR and EMA, while CD34 is positive in hemangioblastoma. Schwannoma and neurofibroma are described more fully in the soft tissue chapter. They can arise from cranial nerves and present intracranially; a classic example is acoustic neuroma. The tumor is composed of

Fig. 2.29 Meningothelial cells are regular and oval

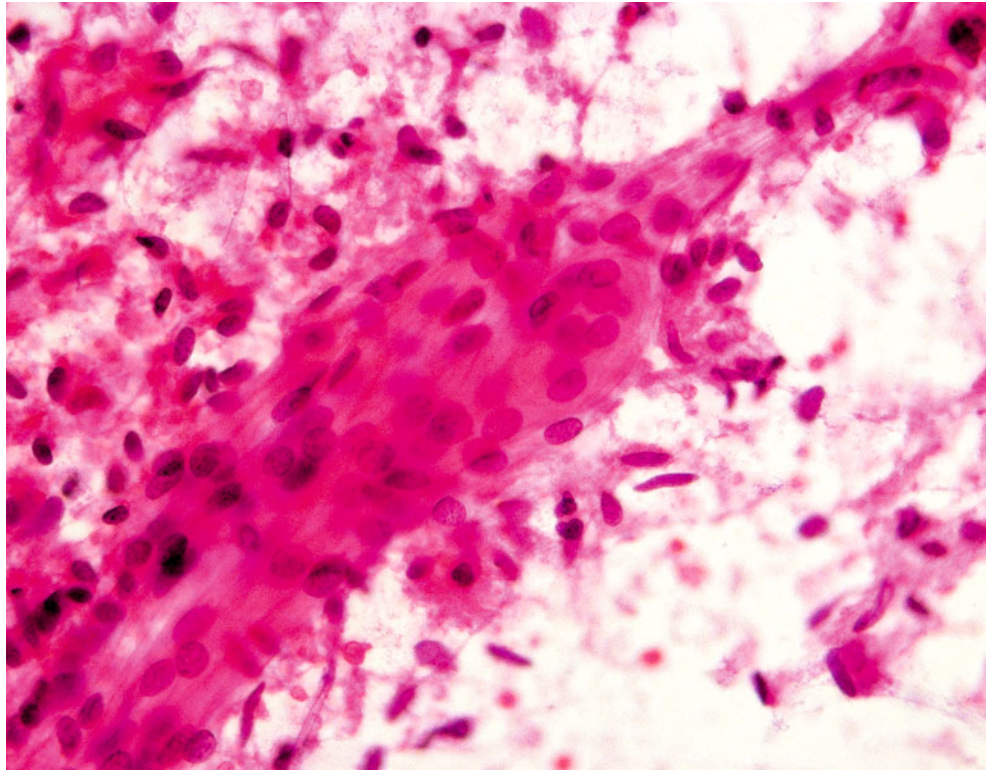
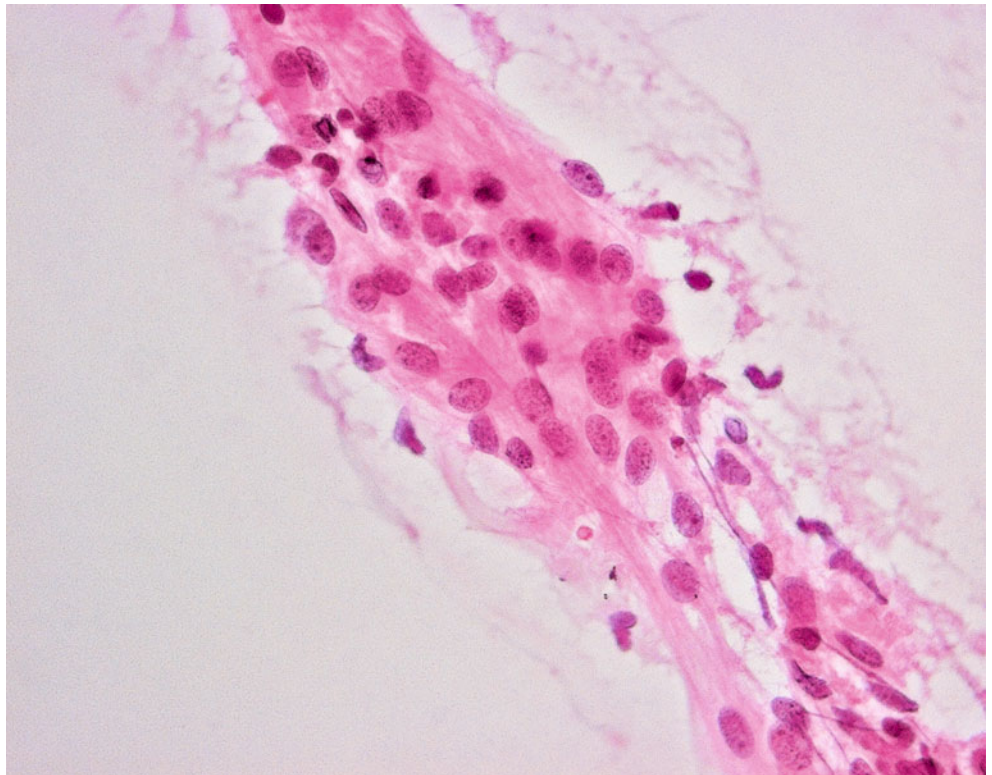


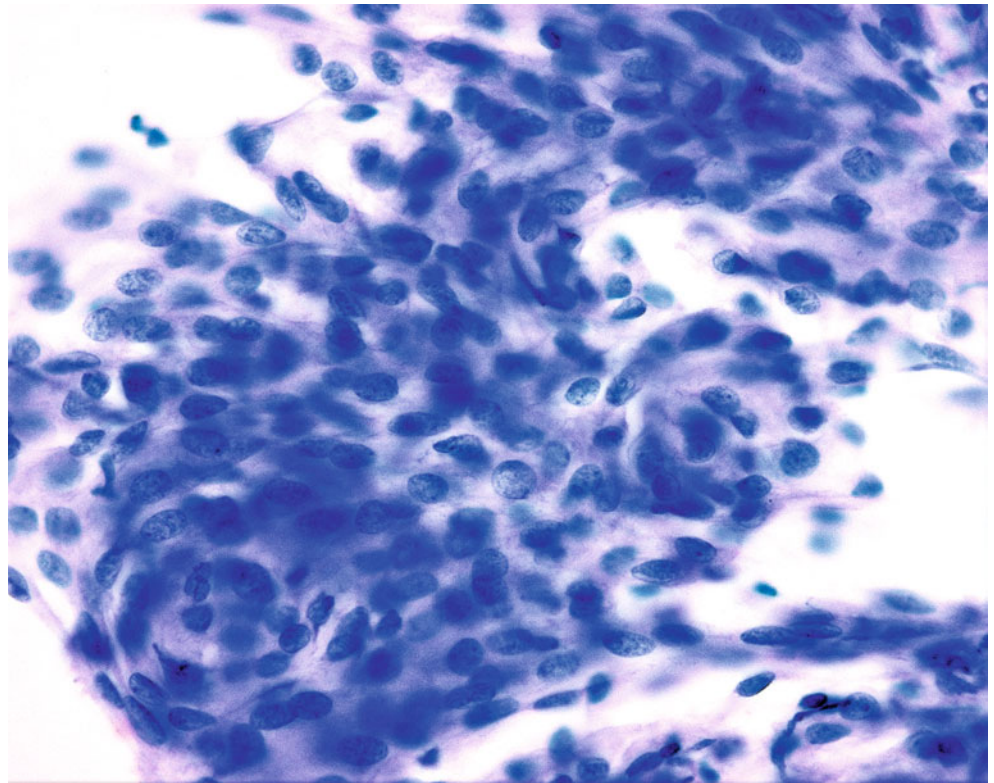
Fig. 2.30 Meningioma. The chromatin is lightly staining and processes may be observed



soft tissue, but it is noticeably a more robust material than glial tissue. There may be anastomosing bundles of bland spindle cells with small or ill-defined nucleoli with little

space between (Antoni A pattern) or larger cells with ill-defined cell borders and prominent background loose matrix (Antoni B pattern). Nuclear pleomorphism is present

Fig. 2.31 Whorls may be visible on the smeared preparation of a meningioma



when there are so-called “ancient” changes. Accompanying vessels are usually prominent and thick walled with mural hyaline material.

Pituitary gland adenomas may be nonsecretory (30 %), but the majority produce one or more hormones normally secreted by the anterior pituitary. Smears are often used for screening the tissue prior to a formal diagnosis. In this situation, a preliminary diagnosis of adenoma, normal or something else, is made with final diagnosis relying on additional studies carried out subsequently on the histological material. Adenomas are composed of cells with larger nuclei than normal pituitary cells, with nucleoli and often a degree of nuclear pleomorphism. Cellularity may help although very cellular samples may cause confusion with oligodendroglioma or nasopharyngeal carcinoma. Carcinomas of the pituitary gland occur, but they are extremely rare. They may look identical to adenomas but clinically behave like frankly malignant tumors, or they may be very atypical cytologically and impossible to differentiate from metastases.

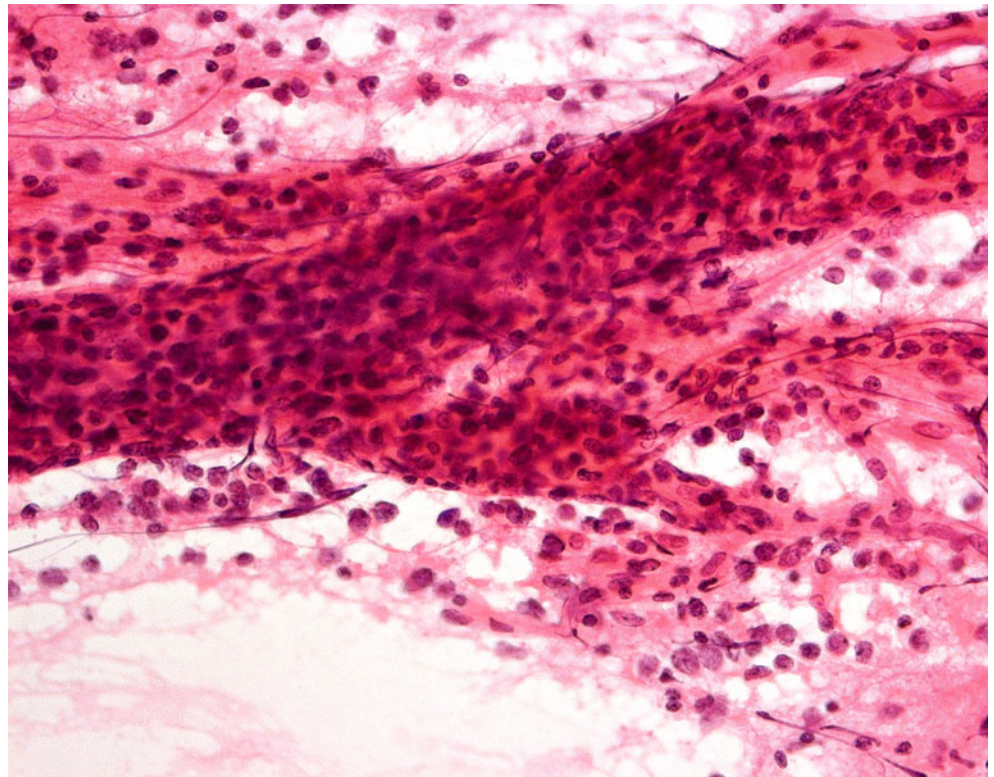
Pineal tumors may be well or poorly differentiated. The former, known as pineocytoma, have dark nuclei forming lobules radiating around a pale zone containing fine processes. There may be neuronal differentiation, and on EM, dense core granules are present. Some tumors may have astrocytic differentiation. Poorly differentiated tumors (pineoblastoma) resemble other primitive neuroendocrine tumors with dark nuclei and little cytoplasm.

Germ cell tumors also occur in pineal region and the base of the brain, especially the hypothalamus. Germinoma is identical morphologically to testicular seminoma and ovarian dysgerminoma. There are large spherical cells with vesicular nuclei and prominent nucleoli together with an associated lymphocytic population. Embryonal carcinoma, teratomas, and choriocarcinoma do occur, but they are very rare. Confident cytological diagnosis is not usually possible, especially without clinical radiological and serological confirmation.

Craniopharyngioma and epidermal cyst both produce sheets of squamous or transitional-like epithelial cells, with background cyst content mainly macrophages, hemorrhage, and cholesterol clefts. The keratin may be better appreciated on polarization. There may be surrounding gliosis so one can mistake the adjacent material for astrocytoma.

Hemangioblastoma classically develops in the cerebellum, although it may occasionally occur in the spinal cord. It is associated with von Hippel-Lindau syndrome. Cytologically, there are usually anastomosing vascular structures composed of strands and cords of elongated endothelial cells, together with foam cells and hemosiderin pigment. Lipid can be demonstrated with Oil Red O. There is often a cystic component which may be reflected in the cytological appearance (foam cells), and the surrounding gliosis that may be present must not be mistaken for a primary glial tumor, especially an astrocytoma.

Fig. 2.32 Low-power magnification of a lymphoma with a vessel filled with neoplastic cells



Primary or Systemic Hematological Neoplasms

These have been discussed earlier in this chapter, but briefly, lymphoma can be primary, especially in setting of HIV and transplantation, or part of systemic disease. Leukemia involves the CNS in a minority of patients, but prophylactic therapy can be useful in preventing CNS relapse, so it is important to identify those with it. The tumor may cause spinal cord compression, cranial nerve involvement, meningeal invasion, or a tumor mass. B cell and T cell lymphomas can both present as primary CNS tumors. The most common leukemia is acute lymphoblastic leukemia in children. Smears are often cellular, and at first sight, they may look like lymphoma or metastatic small cell or undifferentiated carcinoma, PNETs, or even reactive lymphocytosis in viral encephalitis or TB meningitis. The small and large forms of lymphoid cells on closer inspection are often irregular with atypical nuclei (Figs. 2.32, 2.33, and 2.34). Mitoses, plasma cells, polymorphs, and histiocytes may be admixed. It may be necessary to employ immunocytochemistry, flow cytometry, or molecular studies to establish the correct diagnosis.

Plasmacytoma and myeloma both commonly involve the nervous system. Vertebral tumors may directly invade the extradural space or lead to cord compression because of bony collapse which can be a medical emergency. Of course, vertebral collapse due to metastatic malignancy is an important differential diagnosis. Smears show typical plasma cell morphology, with eccentric nuclei, cartwheel nuclear content

arrangement, perinuclear hof of the Golgi apparatus, and purple cytoplasm. There may be atypical features such as multinucleation, large nucleoli, and mitoses. One may need immunocytochemistry for light chain restriction to exclude a reactive plasmacytosis.

Metastatic Tumors

Carcinomas of the lung, breast, gastrointestinal tract, kidney, and thyroid are said to be the most common origins for brain metastases. In terms of cytological presentation, the pathologist might appreciate the architecture better on frozen section rather than smear, but the smear usually provides a clearer scrutiny of the individual cell characteristics. Clumps of malignant cells in a normal background usually turn out to be metastatic carcinomas (Figs. 2.35 and 2.36). One needs to be careful with surrounding tissue which may look like an astrocytoma or encephalitis because of reactive glial changes and lymphocytic infiltration. Tumor cell aggregates are usually cohesive, with acini and cytoplasmic vacuoles in adenocarcinomas: molding, speckled chromatin, and lack of nucleoli in small cell carcinoma and pigment in malignant melanoma. Of course, the predictable differentials and considerations need to be borne in mind especially when differentiating PNET, lymphoma, and anaplastic astrocytomas from neuroendocrine carcinomas. Melanoma may present with pigment in the tumor cell cytoplasm and large nuclei

Fig. 2.33 On close inspection the typical discohesive nature of a lymphoma is usually obvious

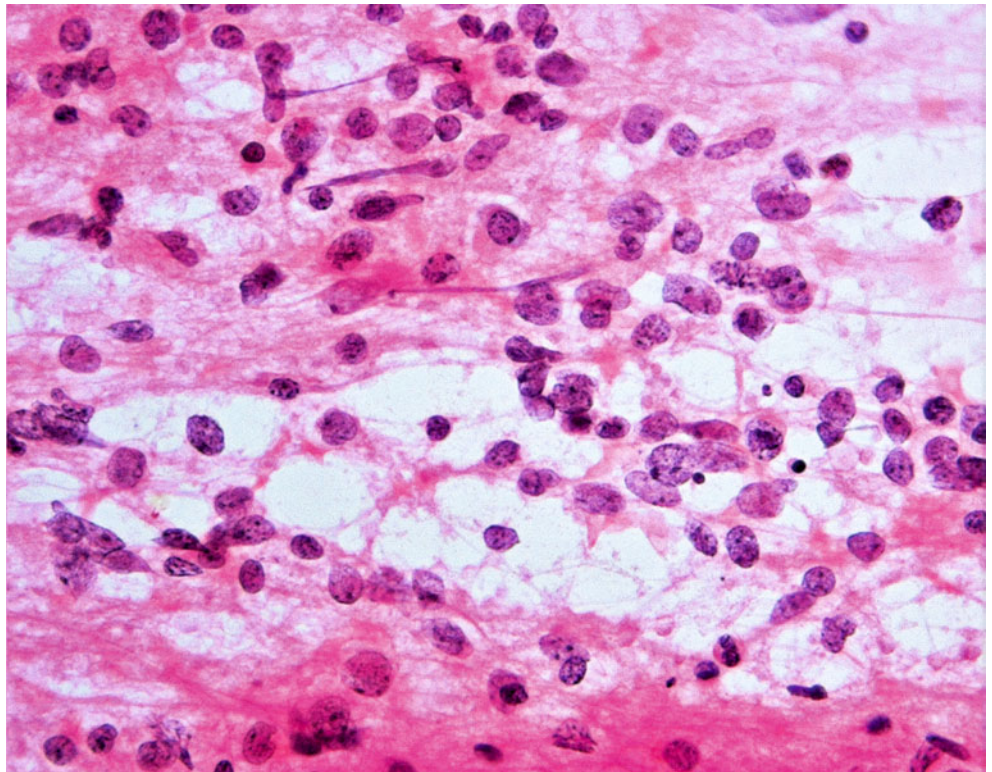
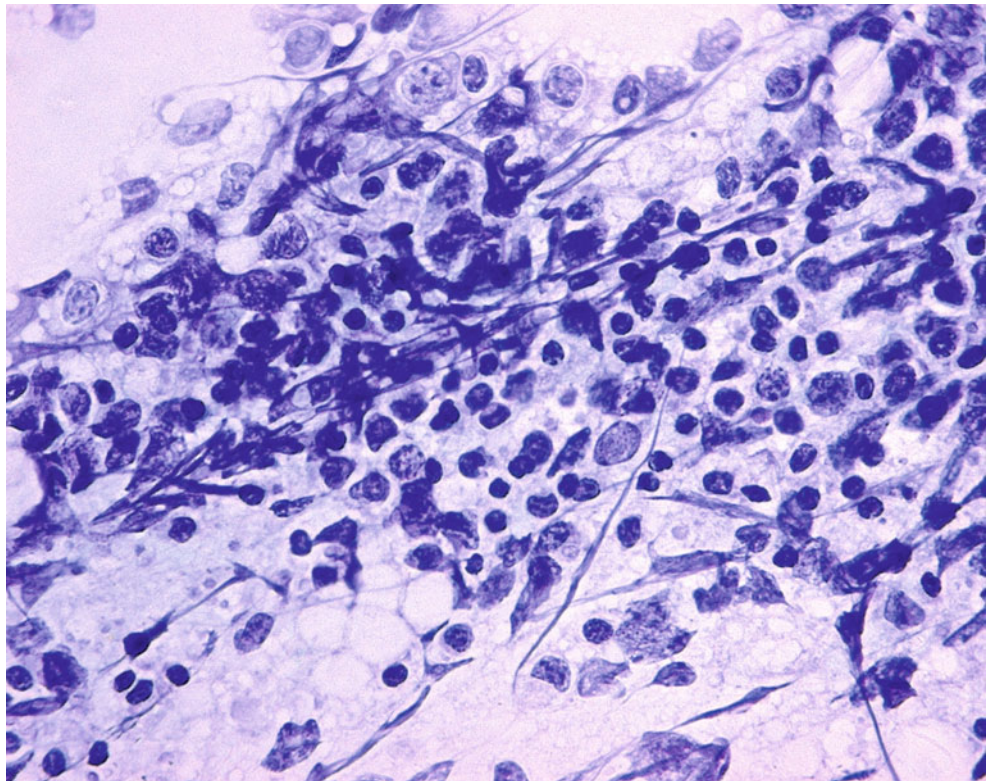


Fig. 2.34 The coarse nuclear features of high-grade lymphoma are generic and are seen wherever the tumor happens to be located



with conspicuous nucleoli, but one may not be able to tell if the tumor is a metastasis or a primary meningeal tumor. It is important however to remember that a variety of primary

CNS tumors can show pigmentation such as meningioma, schwannoma, ependymoma, medulloblastoma, and choroid plexus papilloma. With metastatic renal cell carcinoma, one

Fig. 2.35 Metastatic carcinomas tend to be more cohesive than primary brain tumors or lymphomas

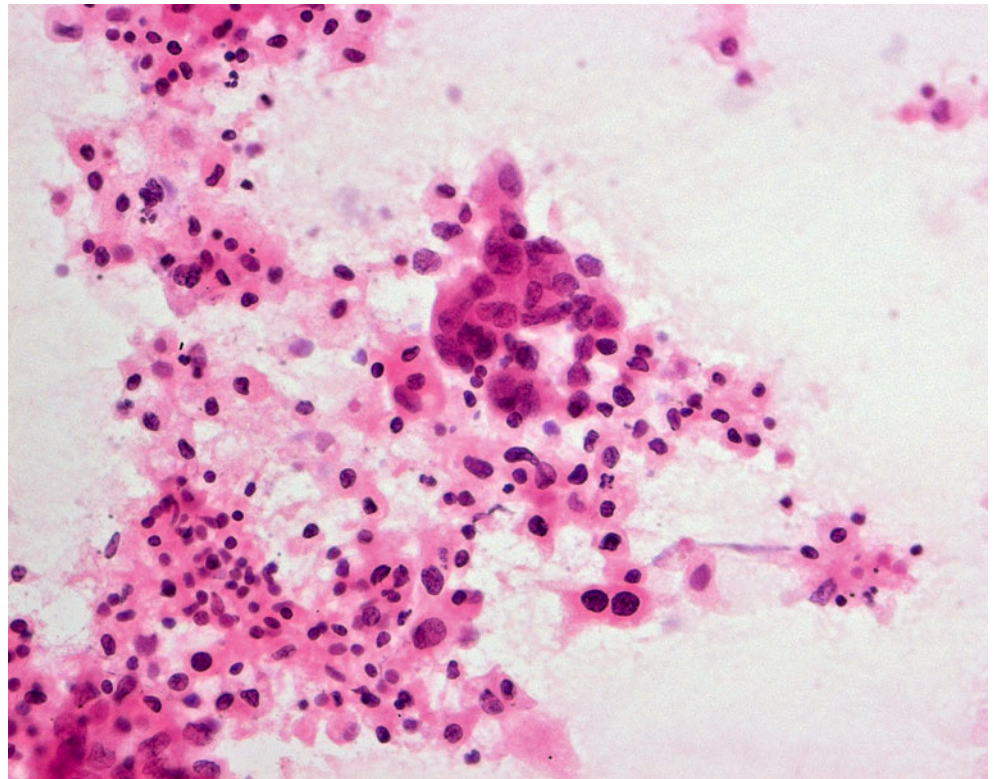
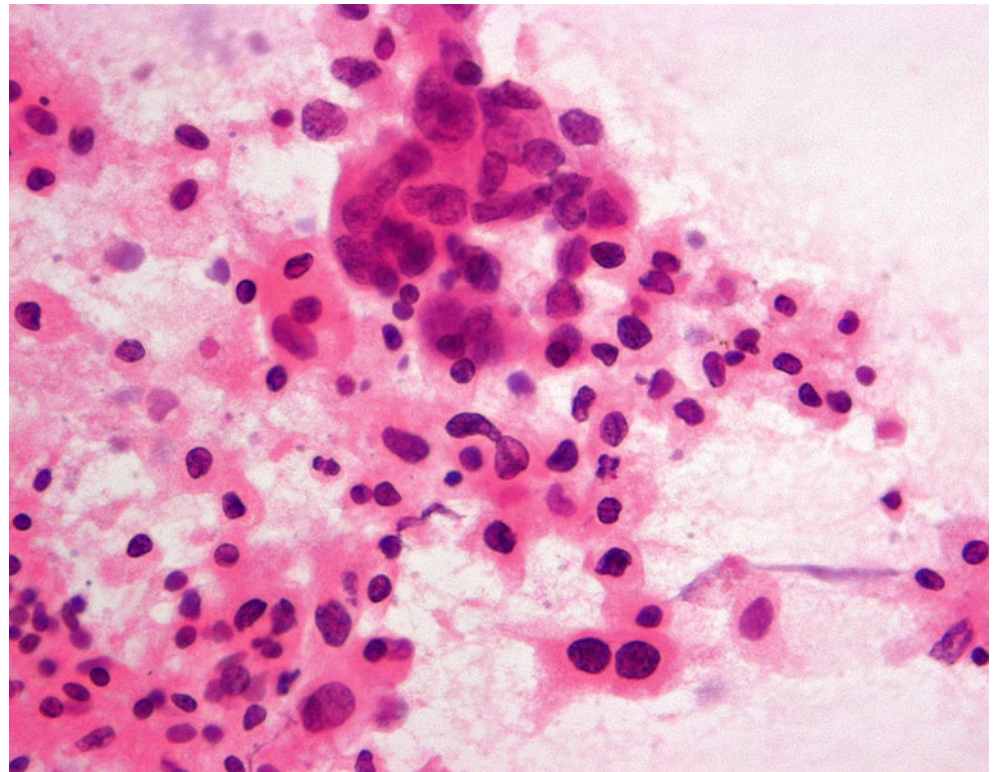


Fig. 2.36 High power of this metastatic carcinoma shows typical epithelial morphology



might find the typical vacuolated or clear cytoplasm which is glycogen positive and also positive with CD10 or RCC markers. The differential lies mainly with hemangioblastoma,

especially when located in the cerebellum. Of course, histochemistry and immunohistochemistry for a wide variety of tumor types and locations can be attempted, but CD56 and

S100 may be tricky to interpret in brain cytological samples as normal CNS cells may be decorated by the antibodies. Cytokeratins, TTF1, napsin A, hormone receptors, HMB45, melan A, and a range of other markers may prove useful when used appropriately.

Local Tumors with Direct Invasion of the Brain and Spinal Cord

In addition to metastases from distant sites, a variety of tumors at nearby sites may involve the nervous system through direct infiltration. Nasopharyngeal carcinoma may look obviously squamous with the usual features including keratinization, but it may be undifferentiated and resemble lots of other tumors including lymphoma.

Penetrating skin tumors from the scalp can invade the brain including cylindroma, basal cell carcinoma, and squamous cell carcinoma. Other appendageal tumors can also directly grow into head including sebaceous carcinoma – the respective descriptions of these are outlined in the skin chapter. Clearly, the cytology in these cases needs to be correlated with the clinical and radiology findings to reach the correct diagnosis.

Chordomas have been discussed earlier in this chapter and also in the soft tissue chapter. They can be diagnosed by the presence of the typical physaliphorous cells containing vacuolated cytoplasm, together with the extracellular mucin identified on PAS and Alcian Blue. Cytologically, they may resemble cartilaginous tumors or they may be dedifferentiated. Immunocytochemical positivity for S100, EMA, and cytokeratin aids diagnosis.

Glomus jugulare is a paraganglioma located near to the tympanic membrane that may invade local structures. Other paragangliomas may also invade the posterior fossa or cauda equina. Typically, they have regular round or oval nuclei with

little cytoplasm, and they may look epithelioid. They have fine, granular cytoplasm, and although one may suspect the diagnosis cytologically, one probably needs tissue sampling to be sure of the diagnosis.

Osteosarcoma and Langerhans cell histiocytosis may present as a CNS mass by direct involvement or metastatic or multisite involvement. The former might arise on a background of Paget's disease in skull, but this is extremely rare, or after radiotherapy. Osteoclasts, multinucleate giant cells, and other features may be present as outlined in the bone and soft tissue chapter. LCH may involve the skull or deeper soft tissue, such as the pituitary, in children and young adults. Aspirates or smears reveal large histiocytes and eosinophils. The histiocytes have typical folded or grooved nuclei and small nucleoli. Mitoses and multinucleate forms may be present. If the diagnosis is suspected, that one may be able to perform immunocytochemistry for S100, CD1a or Langerin, or electron microscopy for Birbeck granules.

Resources and Suggested Reading

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