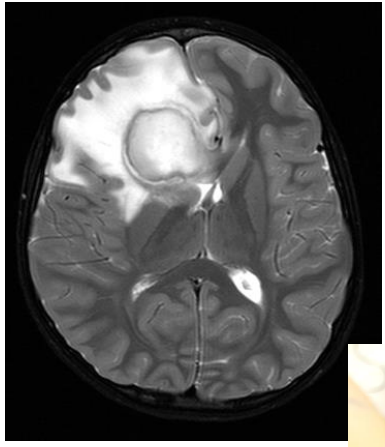
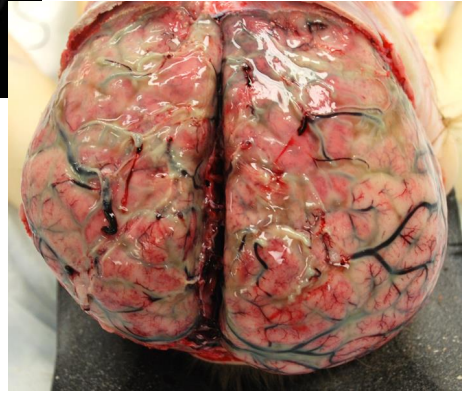
# Microbiology of the central nervous system





The majority of CNS infection are medical emergencies (high mortality rate) and must be quickly identified and treated. They are emergencies because if not treated quickly the injured neurons can not be regenerated. Therefore, we want to prevent/lessen sequelae (condition that occur as consequences of previous disease) and death.

Anas Abu-Humaidan
M.D. Ph.D.

Lecture 1

# Infections of the central nervous system (CNS)

- The central nervous system is ordinarily **sterile** and has no normal microbiota.

- Bacteria, viruses and other microbes can gain access to the CNS, damage tissue, and importantly, **induce an immune response** that is often **detrimental** to the host.

So most of the damage seen isn't from the damage caused by the microbe itself, but the immune response that tries to get rid of the pathogen. This leads to neurological emergencies such as meningitis, encephalitis, etc.

- Classically , the CNS is described as displaying **immune privilege**, as it shows **attenuated responses** to challenge by alloantigen.

(It has less WBCs than other organs). HOWEVER, an immune response is still present.

- However, the **CNS does show local inflammation in response to infection**. Although pathogen access to the brain parenchyma and retina is generally restricted by **physiological** and **immunological** barriers, certain pathogens may breach these barriers.

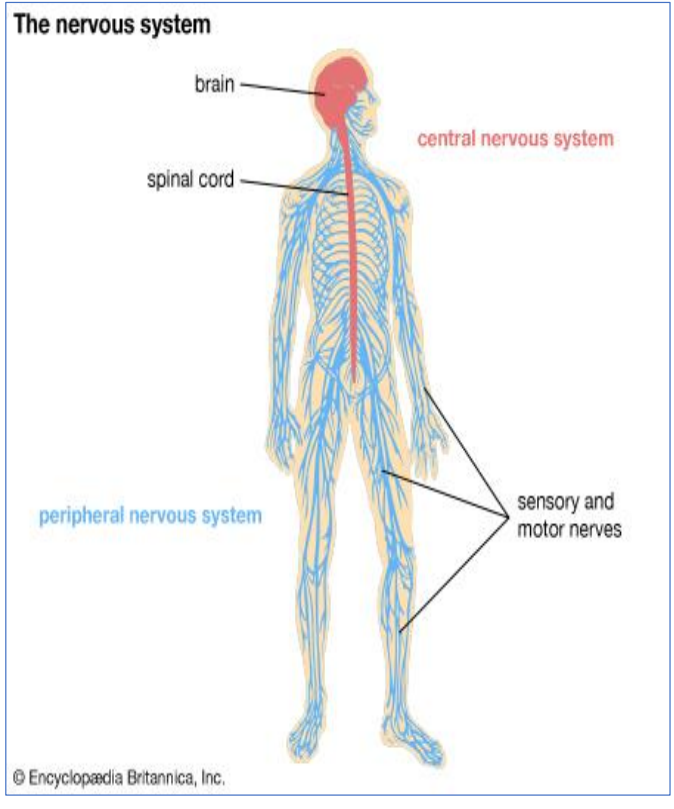
The CNS is well protected physically. The skull and vertebrae for example provide some, but some pathogens may still enter.

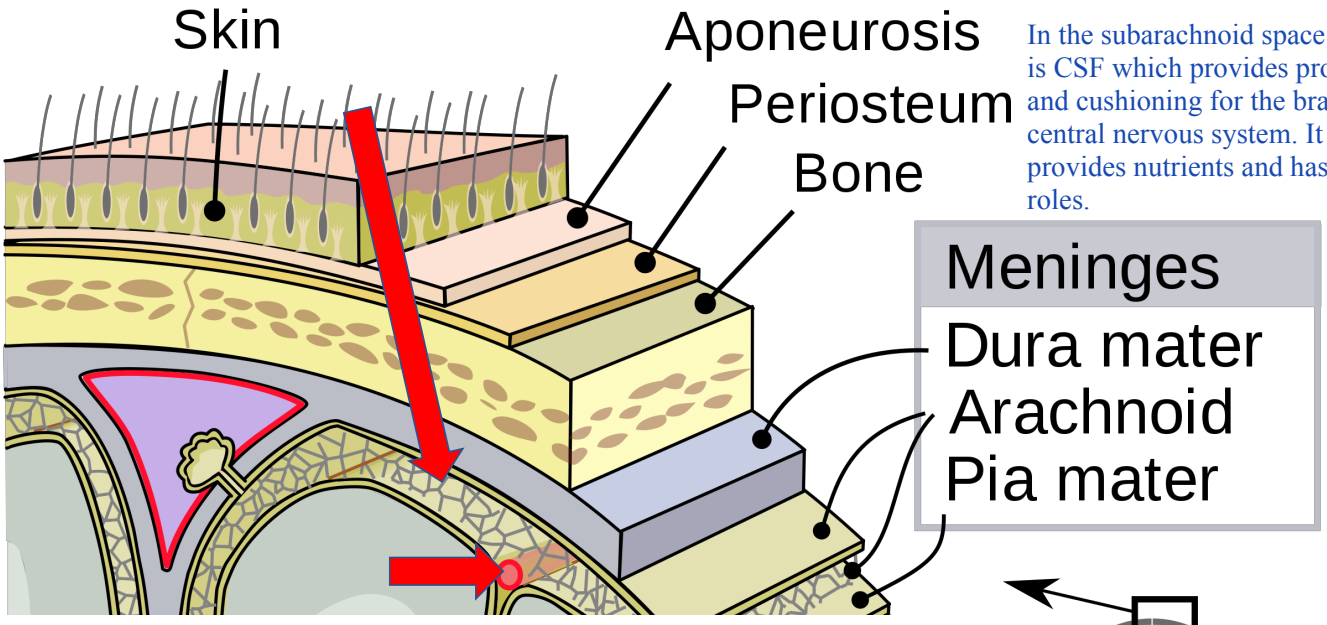
- In the CNS, such pathogens may either cause **devastating inflammation** or benefit from immune privilege in the CNS, where they are **largely protected** from the peripheral immune system.

# Infections of the central nervous system (CNS)

- Distinct clinical syndromes include;

  - **Acute bacterial meningitis**,
  - **Viral meningitis**,
  - **Chronic meningitis**
  - **Encephalitis**
  - **Focal infections**

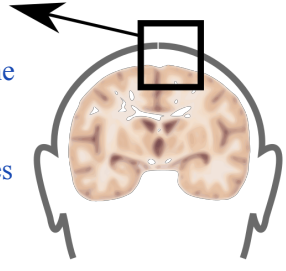


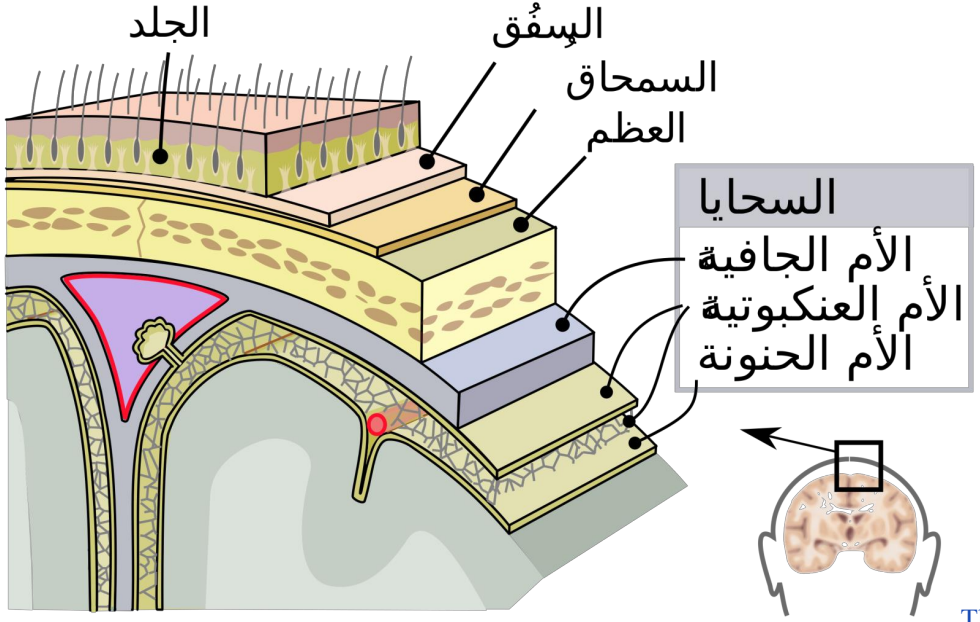


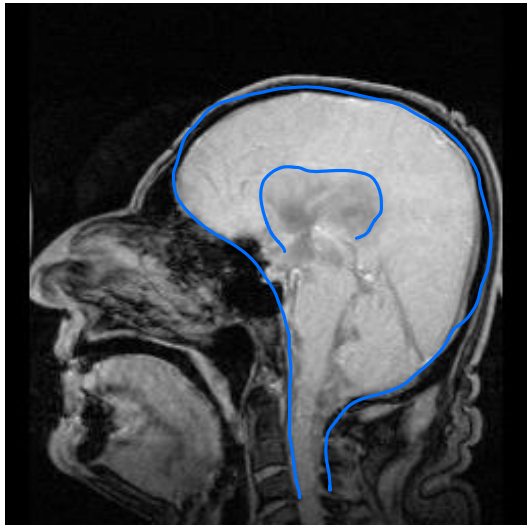

In the subarachnoid space there is CSF which provides protection and cushioning for the brain and central nervous system. It also provides nutrients and has other roles.

The question is with all these coverings of the brain (skin, bone) how will the pathogen enter the meninges (causing meningitis) or brain (causing encephalitis)?

1. Trauma or Surgery (bacteria can enter this way)
2. Hematogenous spread (see red arrow pointing at blood vessel in CSF. The bacteria bypasses the BBB and enters the subarachnoid space.)
3. In some cases, with viruses, they can enter the CNS through neurons.
4. From chronic infection in nearby areas. For examples, chronic otitis media or sinus infections in an individual may make them susceptible to meningitis.

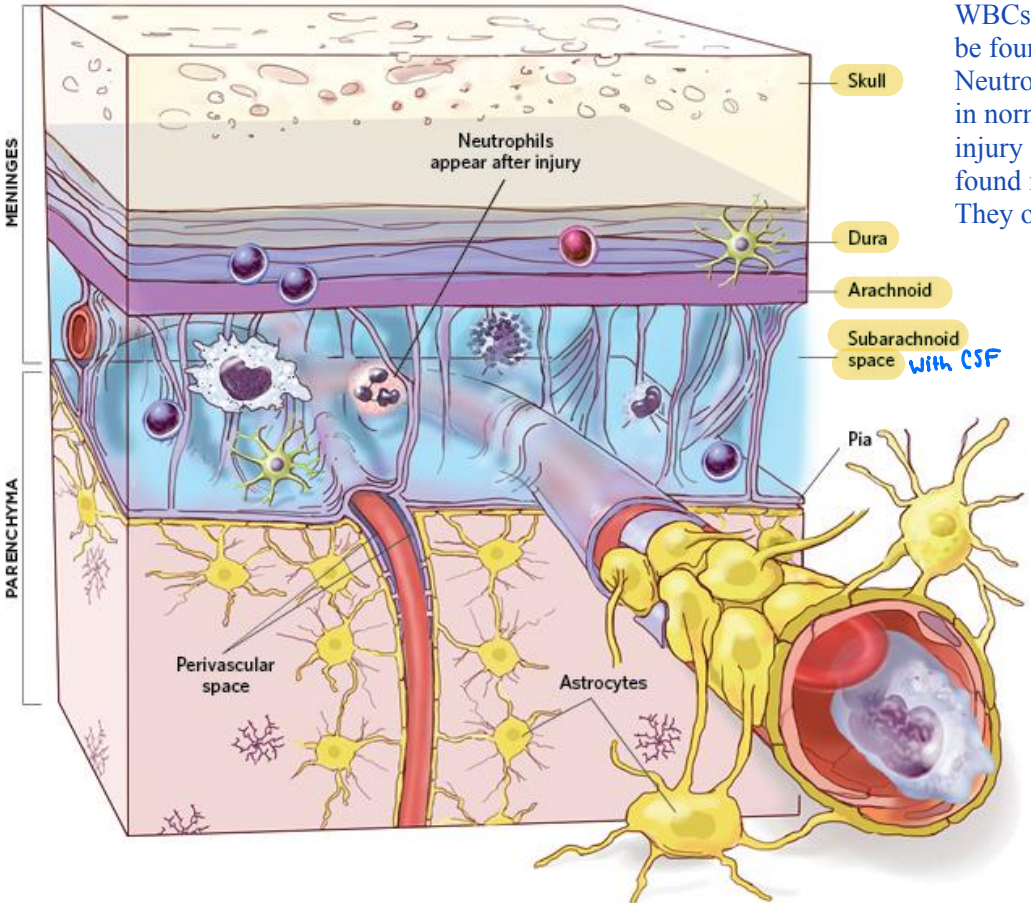


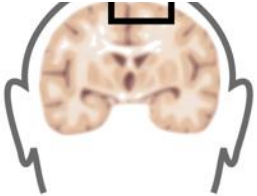


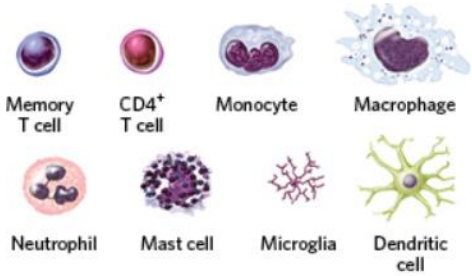



The professor highlighted those areas for where CSF is present. The CSF is continuously being produced and resorbed (150 mL total present at a time) and circulates around the brain and spinal cord. Infection in the subarachnoid space can lead to an issue with resorption, so the CSF accumulates, which can increase intracranial pressure and lead to symptoms.

WBCs, such as Lymphocytes and monocytes, can be found in the CSF but in much lower quantities. Neutrophils are most likely not present in the CSF in normal conditions. They only arrive following injury (remember neutrophils aren't normally found in tissues, they're circulating in the blood. They only enter tissue in response to injury).





- The **immune system** is **a critical part of a functioning central nervous system (CNS)**, even in the absence of injury. But most immune cells are largely relegated to the cerebral spinal fluid (**CSF**), the brain's **meninges**, and the epithelium of the **choroid plexus**. When the CNS experiences a major insult, however, immune cells join **microglia** in the **parenchyma**.

- The brain is rich in **resident macrophages**, called **microglia**, which become activated in response to tissue damage or infections in the brain. The threshold for their activation, however, may be higher than that of macrophages in other tissues.

Microglia are tissue resident macrophages and are the only immune cells present in the brain parenchyma. The rest of the immune cells aren't allowed in the parenchyma because the cytokines they secrete would damage the neurons. But it is needed for the immune system to be present in the parenchyma, and not just because of pathogens. It is also because the immune system plays a role in homeostasis. So the microglial cells are needed to phagocytose dead glial cells. They are also needed to help change the connections of the brain (as the brain continuously cuts connection of neurons and makes new ones: synaptic pruning).Certain cells in the brain also produce complement components, which are essential for the function previously mentioned. This includes C1q.

What is meningitis ?

In medicine, when one talks of meningitis they are usually referring to infectious meningitis. It would present with more immune cells and inflammatory mediators in the CSF.

- Meningitis, an inflammation of the leptomeninges and subarachnoid space, is a **neurologic emergency**.

High mortality rate

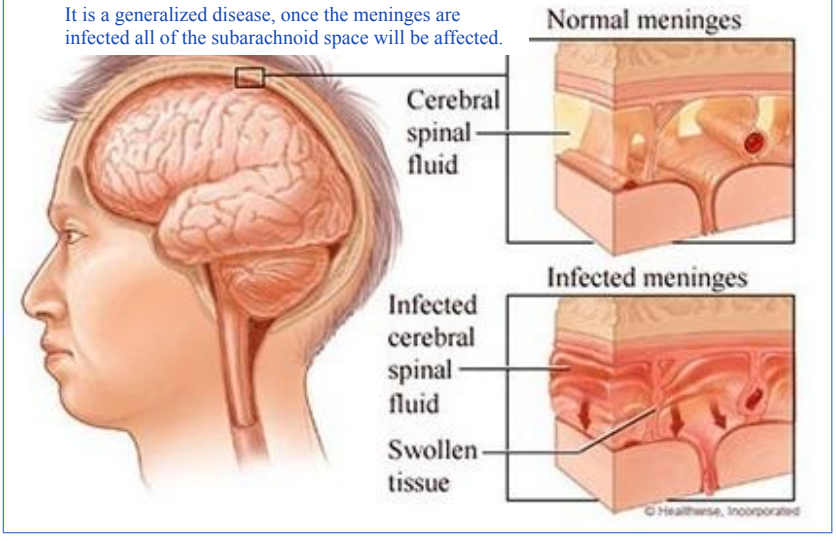
Inflamed CSF becomes turbid, the tissue becomes swollen due to inflammatory mediators

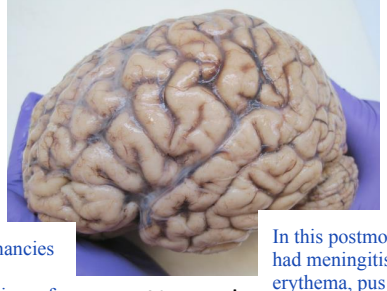
- **Early recognition**, efficient decision making, and **rapid institution of therapy** can be life saving.

- Meningitis commonly has **Infectious causes** (bacterial, viral, fungal and parasitic), but can also be non-infectious (drugs, malignancies, autoimmune diseases).

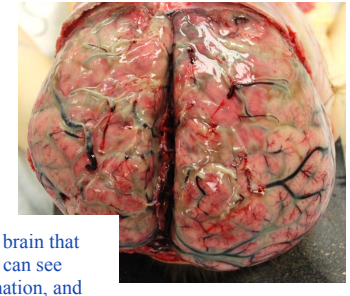
However, very rarely it may have non-infectious causes such as some NSAIDs or malignancies that metastasize into the spinal cord and irritate the meninges to produce inflammatory cytokines. AID such as SLE or vasculitis may create antibodies that can harm the epithelium of the BBB and irritate the meninges to cause meningitiS.

It is a generalized disease, once the meninges are infected all of the subarachnoid space will be affected.



Normal



In this postmortem brain that had meningitis, we can see erythema, pus formation, and perhaps coagulation of some blood vessels.

Meningitis

What is bacterial meningitis ?

- Bacterial meningitis is an acute purulent infection within the subarachnoid space and is the **most common form of suppurative CNS infection.**

- A few bacterial species are often involved in meningitis, they vary by **age** and **predisposing conditions**.

See next page for explanation on the causes.

- Bacterial meningitis mostly presents as a fulminant illness progressing within **hours**.

Table 19.2 Causes of bacterial meningitis

| Age/condition | Common organisms |
|---|---|
| 0–4 weeks | GBS, *E. coli*, *L. monocytogenes*, *K. pneumoniae*, *Enterococcus* spp., *Salmonella* spp. |
| 4–12 weeks | GBS, *E. coli*, *L. monocytogenes*, *K. pneumoniae*, *H. influenzae*, *S. pneumoniae*, *N. meningitidis* |
| 3 months to 18 years | *H. influenzae*, *N. meningitidis*, *S. pneumoniae* |
| 18–50 years | *N. meningitidis*, *S. pneumoniae*, *S. suis* |
| >50 years | *S. pneumoniae*, *N. meningitidis*, *L. monocytogenes*, aerobic Gram-negative bacilli, *S. suis* |
| Immunocompromised | *S. pneumoniae*, *N. meningitidis*, *L. monocytogenes*, aerobic Gram-negative bacilli (e.g. *E. coli*, *Klebsiella* spp., *Salmonella* spp., *S. marcescens*, *P. aeruginosa*) |
| Basal skull fracture | *S. pneumoniae*, *H. influenzae*, GAS |
| Head trauma, post-neurosurgery | *S. aureus*, *S. epidermidis*, aerobic Gram-negative bacilli |
| CSF shunt | *S. aureus*, *S. epidermidis*, *P. acnes*, aerobic Gram-negative bacilli |

For the previous slide:

- Up to the first three months, the baby can have meningitis due to bacteria present in the birth canal.
- Group B streptococcus (GBS - strep agalactiae) (gram positive cocci) can colonize the birth canal. In certain cases of the world they screen for GBS and treat the women for it so that there is no risk for the baby.
- E. Coli (gram negative rod) can normally colonize the birth canal but can also come from the GIT (anus and perineal area) during birth as it is in close proximity.
- Listeria Monocytogenes (gram positive bacilli) is a non-spore forming rod. It is a facultative intracellular bacterium. This bacteria infects the very young, very old, and immune compromised.
- For people who had head trauma, or post neurosurgery, or with CSF shunts (that extend into the skin) we expect pathogens of the skin such as S. Aureus and S. Epidermis is.
- In the age group of 18-50, there are two main pathogens: N. Meningitidis (gram negative diplococci) and S. Pneumoniae (gram positive diplococci). They also remain the most important causes in older age groups too.
- The previous two, along with H. Influenzae, are encapsulated organisms and can survive in the blood.
- H. Influenzae infections has decreased greatly due to vaccination of H. Infleuenza type B.
- Infections are now due to non-typable H. Influenzae but they tend to remain localized and do not spread in the blood and meninges.
- Therefore, if H. Influenzae meningitis is seen in a child, then you can suspect the child is immune compromised or has not taken the vaccine.

For the explanation below the professor explained it for both pathogens but the next two slides the figures focus on N. Meningitidis while the one after focuses on S. Pneumoniae

For the next slide:

Let us focus on the two most important causes N. Meningitidis and S. Pneumoniae. Both of them can normally colonize the nasopharynx. In some cases, they can penetrate the epithelial layers, survive in the blood stream due to their capsule (protects them from phagocytosis), cross in the BBB and enter the subarachnoid space where they start to replicate. Then, the cells of the immune system in the CSF, although their quantity is low, (or endothelial cells which have sensors (TLRs) for LPS or peptidoglycan {PGN}), will secrete pro-inflammatory ctyokines (IL-1, TNF) to draw in immune cells. Those immune cells produce pro-inflammatory cytokines and ROS that will damage the tissue leading to meningitis. So it is the immune system making the situation worse in its efforts to clear the bacteria that leads to meningitis.

How do bacteria get to the meninges?

- Attachment and **colonization of the nasopharyngeal epithelium** is followed by crossing the mucosa and **entering the blood**.
- The bacteria then **crosses the blood brain barrier** and gain access to the cerebrospinal fluid, which is **lacking in cellular and humoral immunity**.
- The pathogen replicates in the CSF and an immune response is initiated against it.
- The **immune response** to the pathogen and its products (e.g. LPS, PGN) further **damages** the surrounding tissue.

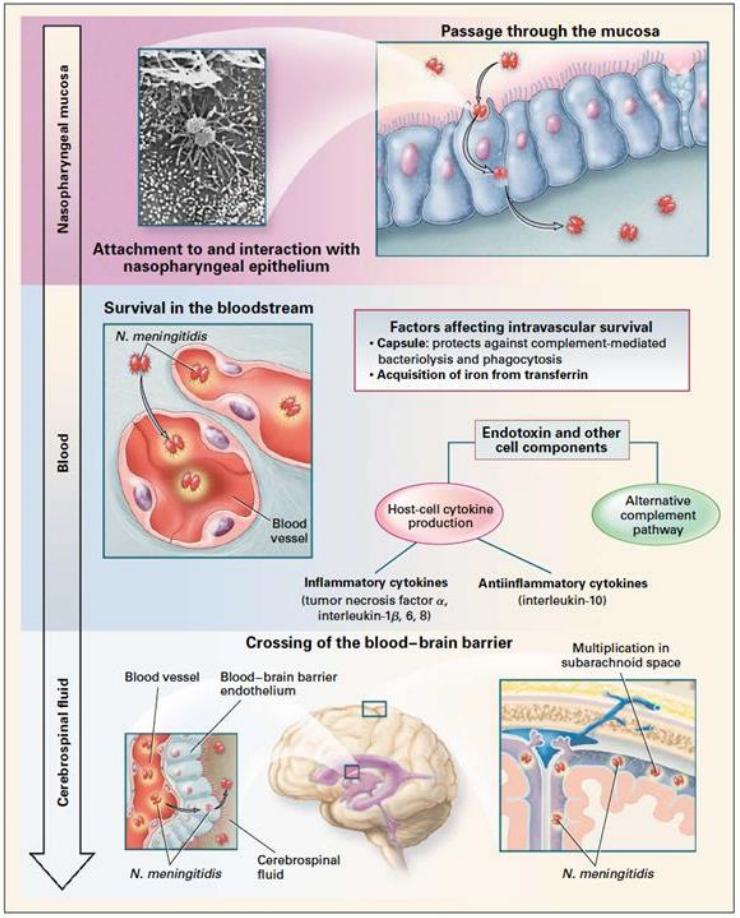


Image to the right: Within the nasopharynx the bacteria can adhere to the nasopharynx, including in healthy individuals and enter the blood.

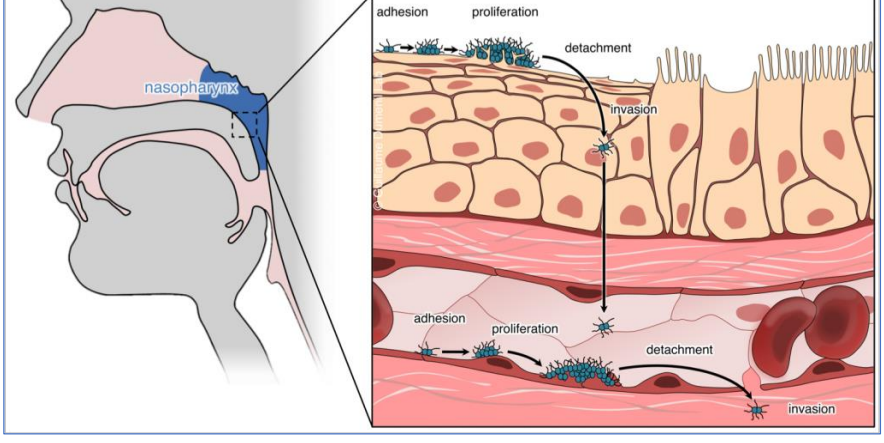
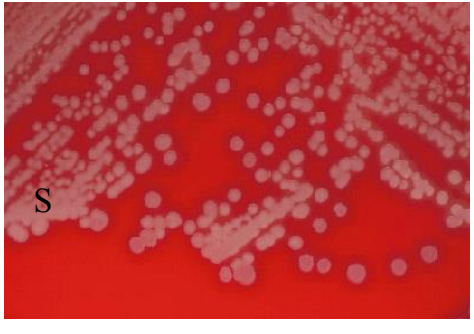
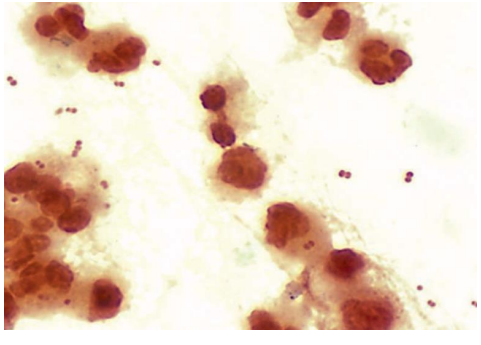



Image below: While in the blood N. Meningitidis can cause meningococcemia that can be seen as skin lesions. In various areas of the body skin rashes appear in which case bleeding under the skin occurs. The lesions can also coalesce and form bullae. Seeing this can help us diagnose meningitis caused by N. Meningitidis.




N. meningitidis colonies on blood agar plate



N. meningitidis gram stain

You can see the gram negative diplococci

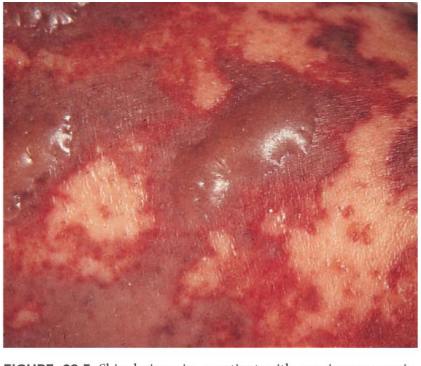
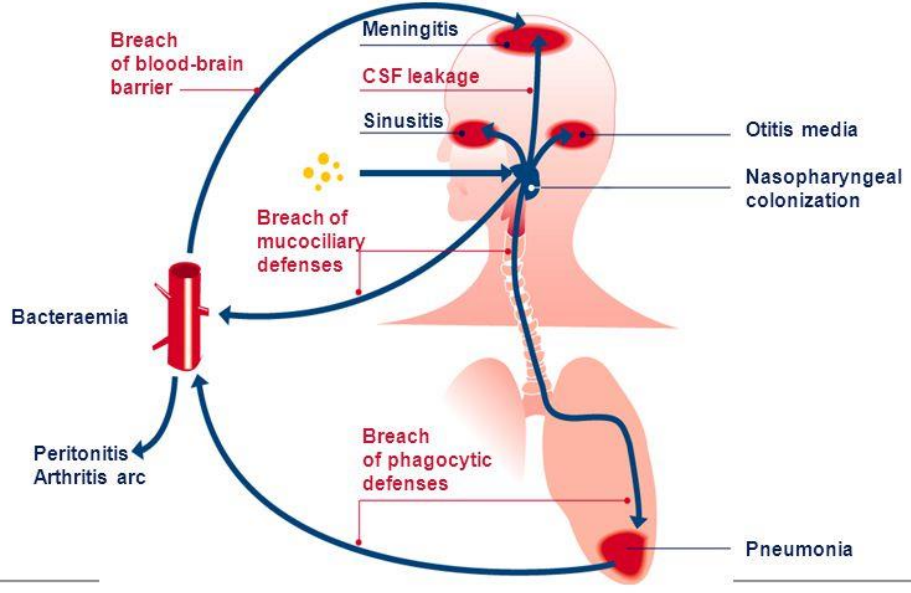


**FIGURE 23-5** Skin lesions in a patient with meningococcemia. Note that the petechial lesions have coalesced and formed hemorrhagic bullae.

# *S. Pneumoniae:* Pathogenesis

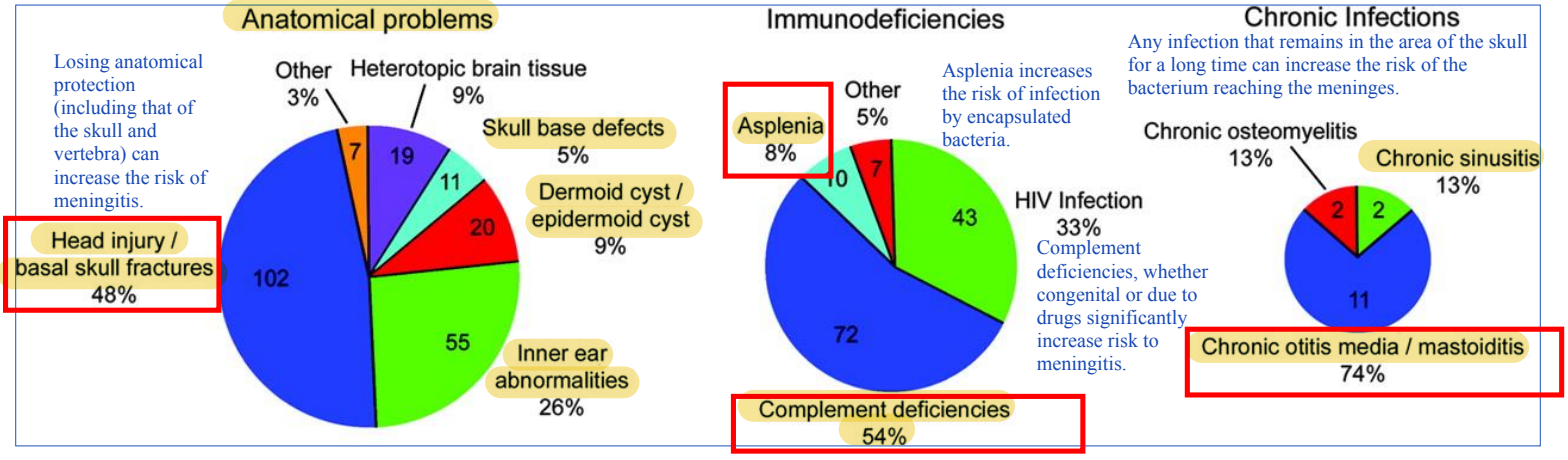



Adapted from Tuomanen & Weiser. In: *Bacterial Pathogenesis: A Molecular Approach*. 1994

S. Pneumoniae also colonizers the nasopharynx. In some cases it may infect the ear or sinuses. Chronic infection may lead to the bacterium traveling to the meninges. Or, those that colonize the nasopharynx may enter the blood, survive due to its capsule, breach the BBB, and cause meningitis.

How common is bacterial meningitis?

Healthy individuals will very rarely get meningitis. There are usually predisposing factors present (as shown in the figure).




- Meningitis is **rare** in general, but incidence varies by region (2-40 per 100,000). For example Sub-Saharan Africa, also referred to as the **meningitis belt**, is known for epidemics of meningococcal meningitis, with incidence rates of 101 cases per 100,000 population.

- With the introduction of ***H. influenzae* type b conjugate vaccines** and **pneumococcal conjugate vaccine**, the incidence of meningitis from these causes decreased significantly.

- Certain Factors can **increase the risk of meningitis** (listed above)

Some patients take complement inhibitors, like one that inhibits C5. This makes them 1000-2000x more at risk of infection by N. Meningitidis.

Vaccine for N. Meningitidis.

Annual Hajj pilgrimages and smaller Umra pilgrimages have historically played a key role in the regional (and to some extent global) spread of meningococcal disease, and have influenced vaccination policies in the region. The mass travel and overcrowded conditions associated with these pilgrimages can facilitate the rapid spread of *N. meningitidis* amongst pilgrims and Saudi nationals.

The Hajj pilgrimage is a key factor influencing outbreaks and transmission, and the use of vaccines has minimized the effects on the home countries of the pilgrims and has decreased global dissemination of disease. Wider use of available polyvalent meningococcal conjugate vaccines may provide broader protection against the range of serogroups causing disease or posing a threat in the region.

Neisseria meningitidis is consistently reported to be one of the leading causes of bacterial meningitis in the Middle East and North Africa (MENA) region.

How do meningitis patients present?

High-grade fever and headache are due to the release of cytokines. Meningism are signs that indicate meningeal irritation. Of importance is neck stiffness/rigidity because to move the neck requires movement of the meninges and to stretch an inflamed meninges leads to pain. They may also have photophobia (sensitivity to light).

Clinical triad

- Classical features include **fever**, **headache**, **meningism** (neck stiffness, photophobia, positive Kernig's sign and Brudzinski's sign).

Meningeal irritation can be tested for using clinical signs: Kernig's sign and Brudzinski's sign.

- **Cerebral dysfunction** (confusion and/ or reduced conscious level) can be present if the brain parenchyma is involved in the inflammatory reaction. (**meningoencephalitis**).

So it spread from the meninges into the parenchyma. The patient could come in comatose (which is a bad sign).

- **Seizures** can occur in neonatal and adult meningitis patients and varies by the etiological agent.

By how they disrupt the brain circuits.

- Accompanying symptoms is often present, such as **petechial rash** in meningococcal septicaemia. Or **rhinorrhoea** suggesting basal skull fracture.

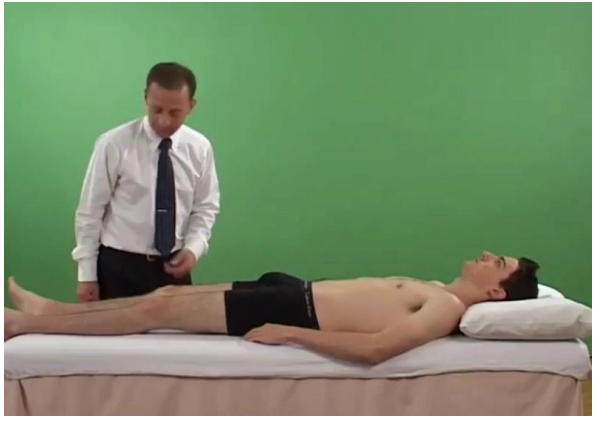
This means fluid leaking from the nose and it has CSF. In this case, the infectious organism comes from the oropharynx. Such as s. Pneumoniae or H. Influenzae or N. Meningitidis. Head traumas in other areas are caused by staph aureus and epidermidis.

- **Increased intracranial pressure** secondary to meningitis can have ocular symptoms like optic disc swelling (**papilledema**) and cranial nerve palsies

Due to increased pressure on the nerves.

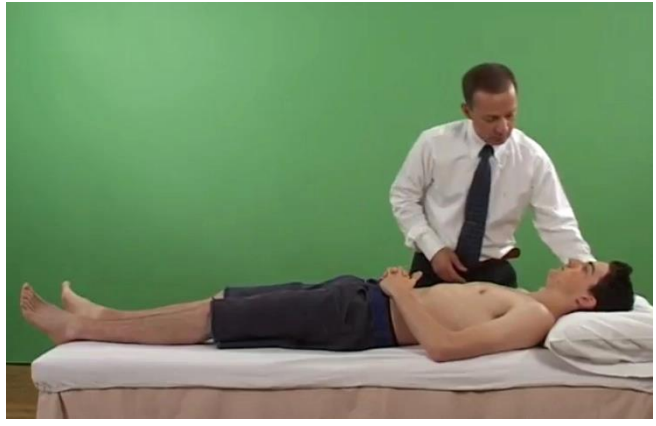
Increased ICP is usually present in meningitis cases that last for quite some time.

How do meningitis patients present?



## Kernig's Sign

The clinician flexes the hip and then extends the knee. This stretches the meninges, The test is positive if there is generalized spinal pain and resistance to the test or involuntary flexion of the opposite hip. A positive test indicates meningitis (but not definite). But no pain does not exclude meningitis. The figure (these are videos) also says leg pain and localized low back pain alone may indicate radiculopathy.



## Brudzinski's sign

The clincian actively flexes the neck of the patient. The test is positive if the. Patient involuntarily flexes their hips and knees in an attempt to lessen traction forces to the meninges. A positive test may signify meningeal irritation or meningitis). As with Kernig's sign, this does not confirm or exclude the diagnosis.

Remember! **Neonates** may present with **non- specific symptoms**, e.g. temperature instability, listlessness, poor feeding, irritability, vomiting, diarrhoea, jaundice, respiratory distress.

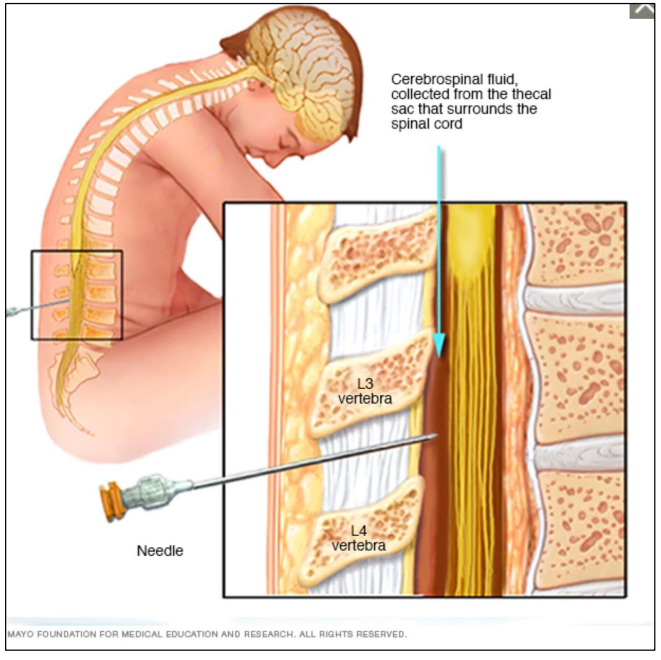
Baby may also always be crying and doesn't know how to sit still. Neonates are difficult to diagnose.

How to confirm a diagnosis of bacterial meningitis?

- **CSF examination** and **culture** are important.
- There is about 125mL of CSF at any one time, and about 500 mL is generated every day. CSF acts as a cushion or buffer, providing basic mechanical and immunological protection to the brain inside the skull.
- If possible, three tubes (1 ml each) of CSF should be collected for **microbiology**, **chemistry**, and **cytology**.

The microbiologist will be looking at the gram stain and culture. The chemistry lab will look at protein and glucose levels and cytology will look for WBCs and determine their type and quantity.

- Blood should be collected when a spinal tap is contraindicated, or bacteremia suspected.




Before we administer antibiotics, we must do a lumbar puncture (because it may have negative results after Abx). Once the lumbar puncture is done antibiotic therapy can be immediately done.

| | Normal | Bacterial | Viral | Fungal/TB |
|---|---|---|---|---|
| **Pressure (cmH20)** | 5-20 | > 30 | Normal or mildly increased | |
| **Appearance** | Normal | Turbid | Clear | Fibrin web |
| **Protein (g/L)** | 0.18-0.45 | > 1 | < 1 | 0.1-0.5 |
| **Glucose (mmol/L)** | 2.5-3.5 | <2.2 | Normal | 1.6-2.5 |
| **Gram stain** | Normal | 60-90% Positive | Normal | |
| **Glucose - CSF:Serum Ratio** | 0.6 | < 0.4 | > 0.6 | < 0.4 |
| **WCC** | < 3 | > 500 | < 1000 | 100-500 |
| **Other** | | 90% PMN | Monocytes<br>10% have >90% PMN<br>30% have >50% PMN | Monocytes |

In bacterial infections:

- The pressure of the CSF increases.
- Turbid appearance
- Protein levels quite elevated (antibodies, cytokines, enzymes, broken down bacteria etc.)
- Glucose levels are rather decreased due to increased metabolism by the cells or the bacteria uses the glucose.
- Gram stain commonly positive.
- Much higher WCC (the majority of which are PMNs, while in viral infection there are more monocytes/lymphocytes).

Let's say the micro lab said the culture shows gram negative dipolococci that are fastidious and grow on special agar: N. Meningitidis.

| TEST | BACTERIAL | VIRAL | FUNGAL | TB |
|---|---|---|---|---|
| Pressure(70-180mm H2O) | + | Normal | Variable | Variable |
| WBC(0-5 cells) | >1,000 | <100 | Variable | Variable |
| Cells | PMNs | Lymphocytes | Lymphocytes | Lymphocytes |
| Protein(<40mg/dL) | ++ | + | + | +++ |
| Glucose(40-70mg/dL) | - - - | Normal | - | - |

How to manage suspected bacterial meningitis?

A third generation cephalosporin is usually given (ex: cefotaxime) along with vancomycin or ampicillin

Empirical means we dont know the specific organism.

- **Prompt empirical antibiotic therapy should be initiated** before results of the CSF examination and culture.
- Adjunctive therapy with corticosteroids (**dexamethasone**) to lessen the inflammatory response is sometimes warranted.
- **Reduction** of raised intracranial pressure if present.
- **Chemoprophylaxis** should be given within 24h to **household contacts** (any person with contact to respiratory or oral secretions)

Table 19.3 Empirical antibiotic therapy

| Age/condition | Empiric therapy |
|---|---|
| Age 0–4 weeks | Ampicillin + cefotaxime or aminoglycoside |
| Age 4–12 weeks | Ampicillin + cefotaxime or ceftriaxone |
| Age 3 months to 18 years | Cefotaxime or ceftriaxone |
| Age 18–50 years | Ceftriaxone or cefotaxime ± vancomycin |
| Age >50 years | Ceftriaxone or cefotaxime + ampicillin |
| Immunocompromised | Vancomycin + ampicillin + ceftazidime or meropenem |
| Health care-associated meningitis | Vancomycin + ceftazidime or meropenem |
| Basal skull fracture | Cefotaxime or ceftriaxone |
| Head trauma/ neurosurgery | Vancomycin + ceftazidime |
| CSF shunt | Vancomycin + ceftazidime |
| β-lactam allergy | Vancomycin + moxifloxacin ± co-trimoxazole (if *Listeria* suspected) |

Table 19.4 Specific antibiotic therapy

| Organism | Antimicrobial therapy |
|---|---|
| *S. pneumoniae* | Penicillin MIC <0.06 micrograms/mL: benzylpenicillin<br>Penicillin MIC ≥0.12 and <1 microgram/mL: ceftriaxone<br>Penicillin MIC ≥1 microgram/mL: ceftriaxone plus vancomycin |
| *N. meningitidis* | Penicillin MIC <0.1 microgram/mL: benzylpenicillin or ampicillin<br>Penicillin MIC 0.1–1 microgram/mL: ceftriaxone |
| *L. monocytogenes* | Ampicillin or benzylpenicillin |
| GBS | Ampicillin or benzylpenicillin |
| *E. coli* | Ceftriaxone or cefotaxime |
| *P. aeruginosa* | Ceftazidime or meropenem |
| *H. influenzae* | β-lactamase-negative: ampicillin<br>β-lactamase-positive: ceftriaxone |
| *S. aureus* | Meticillin-susceptible: flucloxacillin<br>Meticillin-resistant: vancomycin |
| *Enterococcus* spp. | Ampicillin-susceptible: ampicillin + gentamicin<br>Ampicillin-resistant: vancomycin + gentamicin<br>Ampicillin- and vancomycin-resistant: linezolid |

What is the outcome of bacterial meningitis?

- **Mortality is high** even with promt antibiotic therapy**,** and varies with etiological agent (e.g. 5% for N. meningitidis, 20% for S. pneumoniae )

Comorbid conditions: Old, diabetes, immune compromised, cancer

- **Delay in treatment** and **comorbid conditions** affect survival and sequalea.

If it has already reached the brain, it will have lifelong effects (or higher chance of death)

- Decrease level of consciousness on admission, onset of seizures within 24 h of admission, signs of increased ICP all increase mortality.

indicate brain is already affected

- **Neurological sequelae** occur in a **substantial amount** of patients following bacterial meningitis. Most frequently reported sequelae are **focal neurological deficits**, **hearing loss**, **cognitive impairment** and **epilepsy**.

Some children infected as kids can grow up with cognitive impairment, epilepsy, or hearing loss.

A specific area of the brain with a specific function has been infected. Depending on where it may lead to specific paralysis.

## Clinical Case 19-2 Group B Streptococcal Disease in a Neonate

The following is a description of late-onset group B streptococcal disease in a neonate (Hammersen et al: *Eur J Pediatr* 126:189–197, 1977). An infant male weighing 3400 grams was delivered spontaneously at term. Physical examinations of the infant were normal during the first week of life; however, the child started feeding irregularly during the second week. On day 13, the baby was admitted to the hospital with generalized seizures. A small amount of cloudy cerebrospinal fluid was collected by lumbar puncture, and *Streptococcus agalactiae* serotype III was isolated from culture. Despite prompt initiation of therapy, the baby developed hydrocephalus, necessitating implantation of an atrioventricular shunt. The infant was discharged at age 3.5 months with retardation of psychomotor development. This patient illustrates neonatal meningitis caused by the most commonly implicated serotype of group B streptococci in late-onset disease and the complications associated with this infection.

## Case Study and Questions

A 35-year-old man was hospitalized because of headache, fever, and confusion. He had received a kidney transplant 7 months earlier, after which he had been given immunosuppressive drugs to prevent organ rejection. CSF was collected, which revealed a white blood cell count of 36 cells/mm$^3$, with 96% polymorphonuclear leukocytes, a glucose concentration of 40 mg/dl, and a protein concentration of 172 mg/dl. A Gram stain preparation of CSF was negative for organisms, but gram-positive coccobacilli grew in cultures of the blood and CSF. Gram-positive coccobacilli in immune suppressed patient: most likely cause is L. monocytogenes

1. *What is the most likely cause of this patient's meningitis?*
2. *What are the potential sources of this organism?* Cold cuts and other sources
3. *What virulence factors are associated with this organism?*
4. *How would this disease be treated? Which antibiotics are effective in vitro? Which antibiotics are ineffective?*

## Further reading:

- Oxford handbook of infectious diseases and microbiology-

Part4: Clinical syndroms
Chapter 19: Neurological infections

- Harrison's Infectious Diseases 3rd Edition

SECTION III Infections in organ systems
Chapter 36