Please supplement and learn theory on the basis of the lecture, Mim's book and supplementary materials before class!!!

INTRODUCTION

PRINCIPLE: UNDER NATURAL CONDITIONS BLOOD IS STERILE

IMPORTANT TERMS:

Nosocomial infection .....
Bacteremia .....
Viremia .....
Fungemia .....
Sepsis .....
SIRS .....
MODS .....
ARDS .....
DIC .....
CRBI .....
BSI .....

FEVER of UNKNOWN ORIGIN - definition

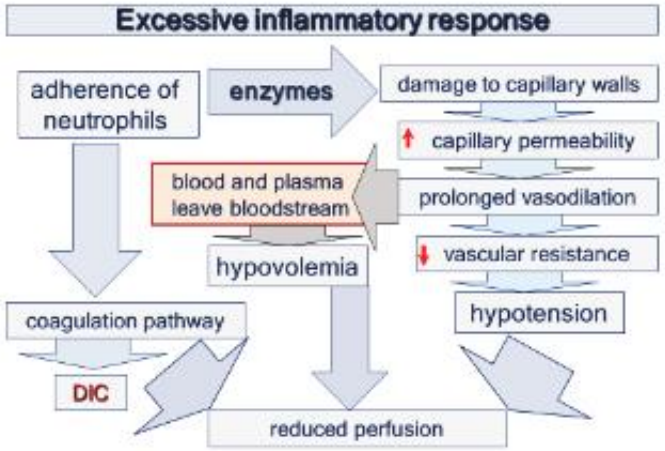
TYPES of BACTEREMIA

•Examples:	•Examples:	•Examples:

PATHOMECHANISM OF SEPSIS

Main bacterial antigens inducing septic shocks include:

a) .....
b) .....



TYPES of BLOODSTREAM INFECTIONS

1. .....
Examples:

2. .....
Examples:

Most common factors predisposing to blood infections include:

a) .....
b) .....
c) .....
d) .....

ETIOLOGY

Any bacterium can get to the bloodstream producing bacteremia. However, not all bacteria can grow bloodstream infections or sepsis.

Most commonly, blood infections are caused by bacteria-producing specific systemic infections, e.g., meningitis, pneumonia, soft tissue infections, endocarditis, UTI, etc.

Most common etiologic agents of blood infections include:

GP COCCI	GN COCCI	GN RODS
•	•	• .....
	•	• .....

GP BACILLI	FUNGI	Miscellaneous
•	•	• .....
•	•	• .....

ENDOCARDITIS	INFUSION FLIUDS	CATHETER-RELATED BLOOD INFECTIONS (CRBI)
•	•	• .....
•	•	• .....

HACEK (develop the abbreviation) :

H .....

A .....

C .....

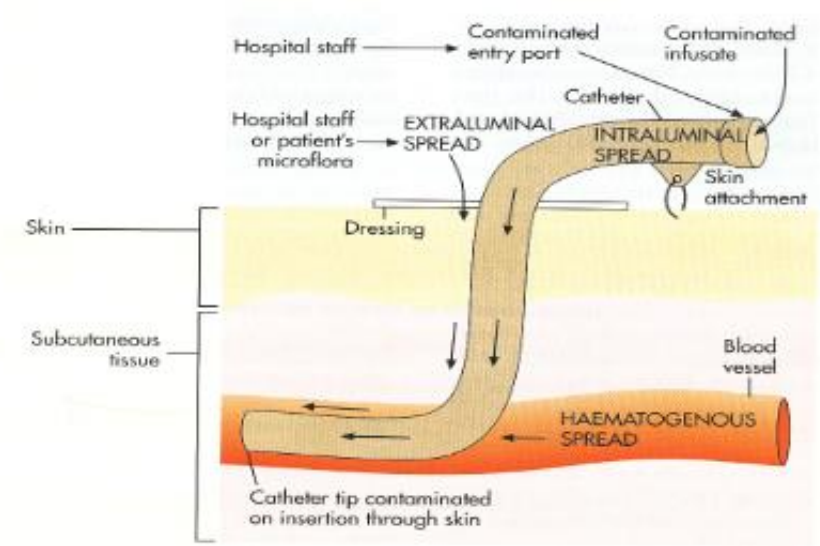
E .....

K .....

Reservoir of HACEK:

.....

CATHETER-RELATED BLOOD INFECTIONS



PRIORITY (ALERT) NOSOCOMIAL PATHOGENS

A list of priority pathogens you will find in the supplementary materials

Priority 1. CRITICAL:

.....

.....

Priority 2. HIGH:

.....

.....

Priority 3. MEDIUM:

.....

.....

Priority (alert) pathogen definition

Bacteria are urgently needed for new antibiotics because of their high resistance to antimicrobials. Their presence in a hospital environment should alert hospital staff

The most common resistance mechanisms to antimicrobials of nosocomial pathogens include:

Staphylococci: MRSA, MRSE, VISA, GISA

Enterococci: HLAR, VRE, GRE

Enteric bacteria and non-fermentative rods: ESBL, MBL, KPC, resistance to aminoglycosides

PART III. TREATMENT *depends on the etiology*

Examples of empirical treatment

CRBI	Glycopeptides (teicoplanin, vancomycin), fluoroquinolones (ciprofloxacin), β-lactams: penicillin, flucloxacillin, carbapenems, aztreonam; macrolides (clarithromycin), clindamycin
Infective endocarditis	β-lactams (penicillin, amoxicillin, flucloxacillin, ceftriaxone, meropenem); gentamycin, glycopeptides
Sepsis treatment depends on the primary site of infection	
a) CAP	Cephalosporin (III, IV)+aminoglycoside
b) HAP	Cefepime or imipenem + aminoglycoside
c) Abdominal infections (hospitalized patients)	Imipenem + aminoglycoside or piperacillin/tazobactam + aminoglycoside
d) Nosocomial skin and soft tissue infection	Vancomycin + imipenem or piperacillin/tazobactam Vancomycin + cefepime
e) Nosocomial urinary tract infection	Vancomycin + cefepime Ciprofloxacin + aminoglycoside
d) Nosocomial CNS infection	Meropenem + vancomycin Vancomycin + cephalosporin (III gen.) or meropenem

IMPORTANT !!!

Immediate antibiotic administration is crucial - within one hour of suspected diagnosis **„golden hour"- the survival rate is 80%**

DIAGNOSIS of BLOOD INFECTIONS

Patient's sample: blood samples obtained by aseptic venipuncture (phlebotomy=drawing of blood) from peripheral veins that are immediately transferred into bottles containing a particular transport-culture medium (**hemomedium**):
for adults, one bottle contains 90 ml of medium, and 10 ml of blood should be transferred to the bottle
pediatric bottle contains 9 ml of medium, and 1-2 ml of blood should be transferred to the bottle
so the blood sample is diluted at 1:10 in the transport-culture medium
In systemic infections co-existing with a blood infection, other patient samples (e.g., CSF, swabs, sputum) should be collected and sent to the lab together with blood samples.

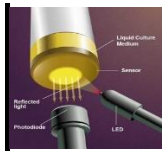
EXAMPLE of SYSTEM for BLOOD CULTURE

BACTEC System
(automated radiometric blood culture system)

Bottles for BACTEC System

The Bactec System detects the growth of bacteria using radiolabeled carbon (^{14}C) in the broth medium. When the bacterium in the blood culture used ^{14}C–labeled substrate, $^{14}CO_2$ is produced. The system monitor $^{14}CO_2$ production and sends a signal when it exceeds the threshold level. It means that the bottle is positive.

1. Positive and negative culture



The bottles have pH-sensitive membranes in the bottom of the bottles. Microbial growth causes a release of CO_2, which changes the pH in the sensor and color from gray to yellow. The color change is measured by reflected light. The level of CO_2 is measured colorimetrically.

Due to increased risk of contamination from colonized bacteria, blood cultures should not be drawn through indwelling intravenous or intra-arterial catheter

Blood sampling guidelines:

2. **Blood sampling guidelines:**
If a blood sample is poorly collected, the results may be inaccurate and misleading to the clinician, and the patient may have to undergo the inconvenience of repeat testing. The three major issues resulting from errors in the collection are hemolysis, contamination, and inaccurate labeling.

Major issues
Hemolysis - a collection of the blood from catheters, use of a needle of too small a gauge (23 or under), underfilling a tube so that the ratio of anticoagulant to blood is greater than 1:9, mixing a tube too vigorously, failing to let alcohol or disinfectant dry
Contamination: not aseptic blood collection.
The rate of isolation of microorganisms from blood is directly related to the volume of blood collected. Therefore, it is recommended that a blood culture set (1 set=two bottles) consist of a total of 30 mL (for adults) and less than 10 mL for pediatrics collected from two separate venipuncture sites.

PRINCIPLES:
a) The bottles making up a set should be collected simultaneously.
b) It is critical that skin be disinfected carefully before venipuncture to prevent contamination of the specimen, which could result in inappropriate antimicrobial therapy. Disinfect the entry site with a 70% alcohol swab for 30 seconds and allow it to dry completely (30 seconds).
Note: alcohol is preferable to povidone-iodine because blood contaminated with povidone-iodine may falsely increase potassium, phosphorus, or uric acid levels in laboratory test results. DO NOT touch the cleaned site.
c) Disinfect the caps of bottles with hemomedium before transferring the blood sample.
d) Transfer blood samples (in an appropriate volume) into bottles and mix bottles carefully. Fill the aerobic blood culture bottle first !!!!
e) If blood is taken from the same venipuncture for other analyses, the blood culture bottles should be inoculated first to avoid contamination !!! However, it is preferable to take blood for culture separately.
f) Label bottles. Do not cover with label the barcodes on the bottles.
g) Blood samples in bottles should be transported at 37°C.

Important:
a) When CRBI is suspected, apart from blood samples, the catheter must be removed, and its tip (ca. 5 cm long) should be cut off, put into the sterile container, and sent to the lab with blood samples.
b) Blood should be drawn as soon as possible after a fever spike (except for endocarditis)

c) Blood samples within bottles should be loaded to continuous monitoring the blood culture system as soon as possible and within a maximum of 4 hours!

Number of sets (**1 set = one aerobic bottle & one anaerobic bottle**) to collect for culture:
acute febrile episode: 1 set within 10 minutes (before antibiotic therapy or just before the next dose of antibiotic)
non-acute disease: 1 set within 24 h (before antibiotic treatment or just before the next dose of antibiotic)
endocarditis acute: 2 sets within 1 to 2 hours (before antibiotic therapy or just before the next dose of antibiotic)
endocarditis subacute: 2 sets within 24 hours – if negative at 24 h, obtain 2 or 3 more sets

1. Take the bottle off from the incubator and mix carefully but not vigorously and then disinfect carefully the cap

2. Insert syringe into cap and take some medium

3. Drop 3-4 drops of the medium from syringe onto culture media: blood agar and MacConkey agar
Then using loop, spread the material onto the whole surface of a plate. Label culture media and put them into incubation for 24 hours

4. Next day, inspect media for bacterial growth, the number of bacterial species etc. Perform necessary diagnostic assays e.g. microscopic slide, biochemical tests, and antimicrobial susceptibility testing

CLASS TASKS

Task 1. Your patient is a young man hospitalized for FUO, and a blood sample from the patient was collected and sent to the laboratory. Plate a blood sample from the patient onto blood agar and MacConkey agar plates. First, wear gloves, and disinfect the bottle's cap with blood sample using alcohol. Next, using a syringe, take some medium sterilely and drop 3-4 drops onto both agar media. Then, spread the material on the agar surface according to the streak plate technique using a loop. Dispose of the syringe's needle into a designated container; dispose syringe and gloves into the can. Label both plates with your initials and incubate them at 37° C for 24 hours.
Next week:

2. Estimate bacterial colonies on both culture media (their color, surface, size, presence of hemolysis) and record results:
blood agar ----------
MacConkey agar ----------
How growing bacteria may be identified as the species?

Which groups of antimicrobials may be used to treat the infection caused by the isolated microorganism?

What mechanisms of resistance isolated species may present?
---------- -------

Read and record antimicrobial susceptibility testing results:
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Task 2. Your patient is a 60-year-old woman with suspected CRBI, and this patient's blood was collected and sent to the laboratory. Plate a blood sample from the patient onto blood agar and MacConkey agar plates. First, wear gloves, and disinfect the bottle cap with the blood sample using alcohol. Next, using a syringe, take some medium sterile and drop 3-4 drops onto both agar media. Then, spread the material on the surface of agar according to the streak plate technique using a loop. Dispose syringe's needle into a designated container; dispose of the syringe and gloves in the can. Label both plates with your initials and incubate them at 37° C for 24 hours.
Next week:
2. Estimate bacterial colonies on both culture media (their color, surface, size, presence of hemolysis) and record results:
blood agar ----------
MacConkey agar ----------
How growing bacteria may be identified as the species?

Which groups of antimicrobials may be used to treat the infection caused by the isolated microorganism?

What mechanisms of resistance isolated species may present?

Read and record antimicrobial susceptibility testing results:

CLINICAL CASES

Case 1.

A 64-year-old man presents with symptoms of endocarditis. His past medical history is significant for rheumatic heart disease and a dental procedure a few weeks before admission. He currently shows no symptoms of endocarditis on physical examination, although endocarditis is suspected. What is the most likely organism for this patient?

High-dose parenteral therapy with bactericidal antibiotics continued for weeks is a general rule in treating infective endocarditis. Explain why:

Case 2.

Late on Friday evening, Mary, a 35-year-old woman, is taken to the emergency department following consultation with her GP by phone. That afternoon she had become feverish, nauseous, and generally weak. Later, she experienced two episodes of rigor. A week before the presentation, she complained of dysuria and frequency and was prescribed oral ampicillin for presumed cystitis. On examination, Marry looks flushed and has a temperature of 40°C and a tachycardia of 120/min. General physical examination is normal apart from acute tenderness in the costovertebral or renal angle. There is protein and blood in a urine sample. What diagnosis should be suspect in Mary's case? What can the microbiological investigation be done to confirm the diagnosis? Should she be hospitalized?

How many blood culture sets should be taken?

On admission to the hospital, blood cultures and midstream urine samples (MSU) are taken, and Mary is prescribed a first-generation cephalosporin, cephradine, administered orally. A lactose-positive, oxidase-negative coliform is isolated from the MSU, in which numerous pus cells are seen, and GN rods are seen in blood cultures. Mary's antibiotic therapy is changed as she remains symptomatic. What is the likely identity of the organism in the urine and blood?

Comment on the choice of antibiotics and route of administration in treating Mary's condition.

Case 3.

Bill, a 65-years man, presented to the hospital with vomiting, right-sided abdominal pain, and fever. At laparotomy, a mass was detected in the ascending colon, with bowel perforation and generalized peritonitis. The tumor was resected, and a right hemicolectomy was performed. Histological examination revealed a poorly differentiated adenocarcinoma. His postoperative course was characterized by fever, leucocytosis (white cell count 24x109/l), and a wound infection caused by S. aureus and Bacteroides fragilis. Despite a 10-day course of intravenous cefotaxime, flucloxacillin, and metronidazole, he remains pyrexial with a temperature of 39-40°C and is generally unwell two weeks after surgery. He cannot tolerate oral feeds, is being fed parenterally via a central line, and has a urinary catheter. What steps should be taken to identify the source and etiology of possible infection?

The initial results of Bill's investigations are as follows: blood cultures are sterile after 48 hours, a scanty growth of an Enterococcus is isolated from the wound swab, a catheter urine specimen is sterile; Candida albicans is isolated from a pharyngeal swab, and respiratory commensals only are grown from sputum. Other examinations (X-ray and CT scan) and negative. Topical nystatin is prescribed for the oral candidiasis, and the antibiotics are discontinued. Was it a good decision to stop the antibiotics?

Is the oral candidiasis likely to be the cause of his persistent pyrexia?

Three days later, it is noticed that the site of the central line is inflamed, and pus is present. A sample of this is sent to the lab for culture, and repeat blood cultures are taken. The following day the lab calls to say that yeasts are present in one of the blood culture bottles. Is the presence of yeasts in only one set of blood cultures likely to represent contamination?

What should be done next to confirm this diagnosis?

How is systemic Candida infection usually diagnosed?

What are the options for the treatment of systemic Candida infection?

Issues to be discussed during class

1. Why does the appropriate endocarditis treatment last for weeks, and why are relapses common?

2. Why people with a heart defect require prophylactic antibiotics during invasive procedures?

3. Why in the case of bloodstream infection should be taken 2 or 3 samples at different times?

4.What can we suspect in the case of negative blood culture in a patient with infective endocarditis? What is the diagnostics?

5. If only one of several blood cultures shows a growth of Bacillus spp., Corynebacterium spp., and Propionibacterium acnes what does it mean?

SELF-ASSESMENT

1. Explain what vegetation means and how it is formed?
2. Name infectious diseases associated with the presence of bacteria in the blood?
3. What is the most common source of extravascular bloodstream infection
4. How many blood samples are taken in endocarditis?
5. Give examples of most common sources of nosocomial and community-acquired sepsis
6. Types of bacteremia are.....? Give examples of diseases associated with types of bacteremia
7. Give examples of common etiologic agents of bacteremia
8. Name bacteria most commonly associated with post-transfusion bacteremia
9. Name alarming pathogens commonly associated with blood infections and indicate why they are considered alert pathogens?
10. What are options of treatment of sepsis? Give examples depending on the disease
11. Name most common microorganisms causing infective endocarditis.
12. What are most common etiologic agents of catheter-related blood infections?
13. How infections with typical bacteria are diagnosed in bacteriologic lab?
14. How we can diagnose infections caused by fungi? And viruses? And atypical bacteria?

15. How catheter-related blood infections are diagnosed? What samples should be send to the lab? What are important conditions that must be done?
16. How many blood samples are taken in different blood infections?
17. Describe procedure of taking blood samples from patients? How these samples should be transported?
18. Develop abbreviations: DIC, SIRS, MOSF, ARDS
19. Give examples of most common sources of nosocomial and community-acquired sepsis.

SUPPLEMENTARY MATERIALS

WHO priority pathogens list:

Priority 1: CRITICAL

- *Acinetobacter baumannii*, carbapenem-resistant
- *Pseudomonas aeruginosa*, carbapenem-resistant
- *Enterobacteriaceae*, carbapenem-resistant, ESBL-producing

Priority 2: HIGH

- *Enterococcus faecium*, vancomycin-resistant
- *Staphylococcus aureus*, methicillin-resistant, vancomycin-intermediate and resistant
- *Helicobacter pylori*, clarithromycin-resistant
- *Campylobacter* spp., fluoroquinolone-resistant
- *Salmonellae*, fluoroquinolone-resistant
- *Neisseria gonorrhoeae*, cephalosporin-resistant, fluoroquinolone-resistant

Priority 3: MEDIUM

- *Streptococcus pneumoniae*, penicillin-non-susceptible
- *Haemophilus influenzae*, ampicillin-resistant
- *Shigella* spp., fluoroquinolone-resistant

Pathomechanism of septic shock

Gram-Positive Cell Wall Components: Lipoteichoic Acids and Peptidoglycan Cell Wall Fragments

1) The lysis of gram-positive bacteria causes peptidoglycan monomers (the building blocks of peptidoglycan and lipoteichoic acids) to be released from the gram-positive cell wall. However, remember that similar effect LPS of Gram-negative bacteria may induce.

2) These peptidoglycan fragments and lipoteichoic acids, in turn, bind to toll-like receptors (TLRs) such as TLR-2 and TLR-6 that are specific for these cell wall components and are found on the surface of body defense cells called macrophages. (These gram-positive cell wall components can also bind first to binding proteins circulating in the blood that subsequently carry them to CD14 molecules on the macrophages.)

3) Binding of the cell wall components to the TLRs of the macrophages triggers them to release various defense regulatory chemicals called cytokines, including tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1), inflammatory chemokines such as IL-8, and platelet-activating factor (PAF). The cytokines then bind to cytokine receptors on target cells and initiate an inflammatory response. They also activate both the complement pathways and the coagulation pathway in a manner similar to endotoxin (LPS) from the gram-negative cell wall.

4) The binding of peptidoglycan fragments or lipoteichoic acids to their TLRs on the surfaces of phagocytic white blood cells called neutrophils causes them to release proteases and toxic oxygen radicals for extracellular killing. Chemokines such as interleukin-8 (IL-8) also stimulate extracellular killing. In addition, cytokines stimulate the synthesis of a vasodilator called nitric oxide.

During minor local infections with few bacteria present, low levels of peptidoglycan monomers and lipoteichoic acids are released leading to moderate cytokine production by defense cells such as monocytes, macrophages, and dendritic cells and, in general, promoting body defense by stimulating inflammation and moderate fever, breaking down energy reserves to supply energy for defense, activating the complement pathway and the coagulation pathway, and generally stimulating immune responses. Also as a result of these cytokines, circulating phagocytic white blood cells such as neutrophils and monocytes stick to the walls of capillaries, squeeze out and enter the tissue, a process termed diapedesis. The phagocytic white blood cells such as neutrophils then kill the invading microbes with their proteases and toxic oxygen radicals.

With the production of large amounts of proinflammatory cytokines, neutrophils adhere to capillary walls in massive amounts. Chemokines cause neutrophils to release proteases and toxic oxygen radicals, the same chemicals they use to kill microbes, but these toxic chemicals are now being dumped onto the vascular endothelial cells to which the neutrophils have adhered during diapedesis. This results in damage to the capillary walls and leakage of blood.

However, during severe systemic infections with large numbers of bacteria present, high levels of gram-positive cell wall components are released resulting in excessive cytokine production by the defense cells and this can harm the body. In addition, neutrophils start releasing their proteases and toxic oxygen radicals that kill not only the bacteria, but the surrounding tissue as well. Harmful effects include high fever, hypotension, tissue destruction, wasting, **acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC)**, and damage to the vascular endothelium. This can result in shock, **multiple system organ failure (MOSF)**, and often death. Keep in mind that a primary function of the circulatory system is perfusion, the delivery of nutrients and oxygen via arterial blood to a capillary bed in tissue. This, in turn, delivers nutrients for cellular metabolism and oxygen for energy production via aerobic respiration to all of the cells of the body. Sepsis is an infection that leads to a systemic inflammatory response resulting in physiologic changes occurring at the capillary endothelial level. This systemic inflammatory response is referred to as **Systemic Inflammatory Response Syndrome or SIRS**.

Systemic Inflammatory Response Syndrome (SIRS) resulting in septic shock. During a severe systemic infection, an excessive inflammatory response triggered by overproduction of inflammatory cytokines such as TNF-alpha, IL-1, IL-6, IL-8, and PAF in response to PAMPs often occurs. The release of inflammatory cytokines eventually leads to vasodilation of blood vessels. Vasodilation is a reversible opening of the junctional zones between endothelial cells of the blood vessels and results in increased blood vessel permeability. Normally, this fights the infection by enabling plasma, the liquid portion of the blood, to enter the surrounding tissue. The plasma contains defense chemicals such as antibody molecules, complement proteins, lysozyme, and beta defensins. Increased capillary permeability also enables white blood cells to adhere to the inner capillary wall, squeeze out of the blood vessels, and enter the tissue to fight infection, a process called diapedesis. Excessive productions of cytokines during a systemic infection results in the following events:

1. During diapedesis, phagocytic WBCs called neutrophils adhere to capillary walls in massive amounts. Chemokines such as IL-8 activate extracellular killing by neutrophils, causing them to release proteases and toxic oxygen radicals while still in the capillaries. These are the same toxic chemicals neutrophils use to kill microbes, but now they are dumped onto the vascular endothelial cells to which the neutrophils have adhered. These events result in damage to the capillary walls and leakage of blood into surrounding tissue.
2. Prolonged vasodilation and the resulting increased capillary permeability causes plasma to leave the bloodstream and enter the tissue. Prolonged vasodilation also leads to decreased vascular resistance within blood vessels that, in turn, contributes to a drop in blood pressure (hypotension). This contributes to hypoperfusion.
3. At high levels of TNF, vascular smooth muscle tone and myocardial contractility are inhibited. This results in a marked drop in blood pressure (hypotension). Cytokine-induced overproduction of nitric oxide (NO) by cardiac muscle cells and vascular smooth muscle cells can also lead to heart failure.

4. Neutrophil-induced damage to the capillaries, as well as prolonged vasodilation, results in blood and plasma leaving the bloodstream and entering the tissue. This can lead to a decreased volume of circulating blood (hypovolemia). This contributes to hypoperfusion.
5. Activation of the blood coagulation pathway and concurrent down-regulation of anticoagulation mechanisms cause clots called microthrombi to form within the blood vessels throughout the body. This is called disseminated intravascular coagulation (DIC). These microthrombi block the capillaries and interfere with perfusion. Activation of neutrophils also leads to their accumulation and plugging of the vasculature.
6. The increased capillary permeability as a result of vasodilation in the lungs, as well as neutrophil-induced injury to capillaries in the alveoli results in acute inflammation, pulmonary edema, and loss of gas exchange in the lungs. This condition is called acute respiratory distress syndrome (ARDS). As a result, the blood does not become oxygenated.
7. Reduced perfusion and capillary damage in the liver results in impaired liver function and a failure to maintain normal blood glucose levels. Overuse of glucose by muscle and a failure of the liver to replace glucose can lead to a drop in blood glucose level below what is needed to sustain life.
8. Reduced perfusion can also leads to kidney and bowel injury.
9. The combination of hypotension, hypovolemia, DIC, ARDS, and the resulting hypoperfusion leads to acidosis. Without oxygen, cells switch to fermentation and produce lactic acid that lowers the pH of the blood. A blood pH range between 6.8 and 7.8 is needed for normal cellular metabolic activities in humans. Changes in the pH of arterial blood extracellular fluid outside this range lead to irreversible cell damage.
10. Collectively, this cascade of: hypotension, hypovolemia, and DIC that result in marked hypoperfusion; ARDS that prevents oxygenation of the blood; drop in blood glucose level from liver dysfunction; acidosis that results in cell death; and cardiac failure leads to: end-organ ischemia (ischemia is a restriction in blood supply that results in damage or dysfunction of tissues or organs); multiple system organ failure (MSOF); and death.

Another example of damage from gram-positive cell wall components is gram-positive bacterial meningitis. The same inflammatory events lead to identical effects in the brain and the decreased delivery of oxygen and glucose to the cells of the brain results in damage and death of brain tissue. One such example is the *Streptococcus pneumoniae*. When *S. pneumoniae* enters the alveoli of the lungs and is lysed by antibiotics or body defenses, glycopeptide cell wall fragments and teichoic acids bind to receptors on endothelial cells, the alveolar epithelium, and leukocytes causing the release of TNF-alpha, Il-1, and chemokines. This leads to increased vascular permeability that enables serous fluids, red blood cells, and leukocytes to enter the air spaces of the lung where gas exchange occurs. This prevents normal gas exchange and the person drowns on his or her own serous fluids. From the lungs, *S. pneumoniae* often invades the blood, crosses the blood-brain barrier, and enters the meninges. Gram-positive bacteria such as *Staphylococcus* and *Enterococcus*, along with the normal flora gram-negative bacteria mentioned above, are among the most common causes of nosocomial infections. The three most common gram-positive bacteria causing nosocomial infections are *Staphylococcus aureus*, coagulase-negative staphylococci, and *Enterococcus* species.