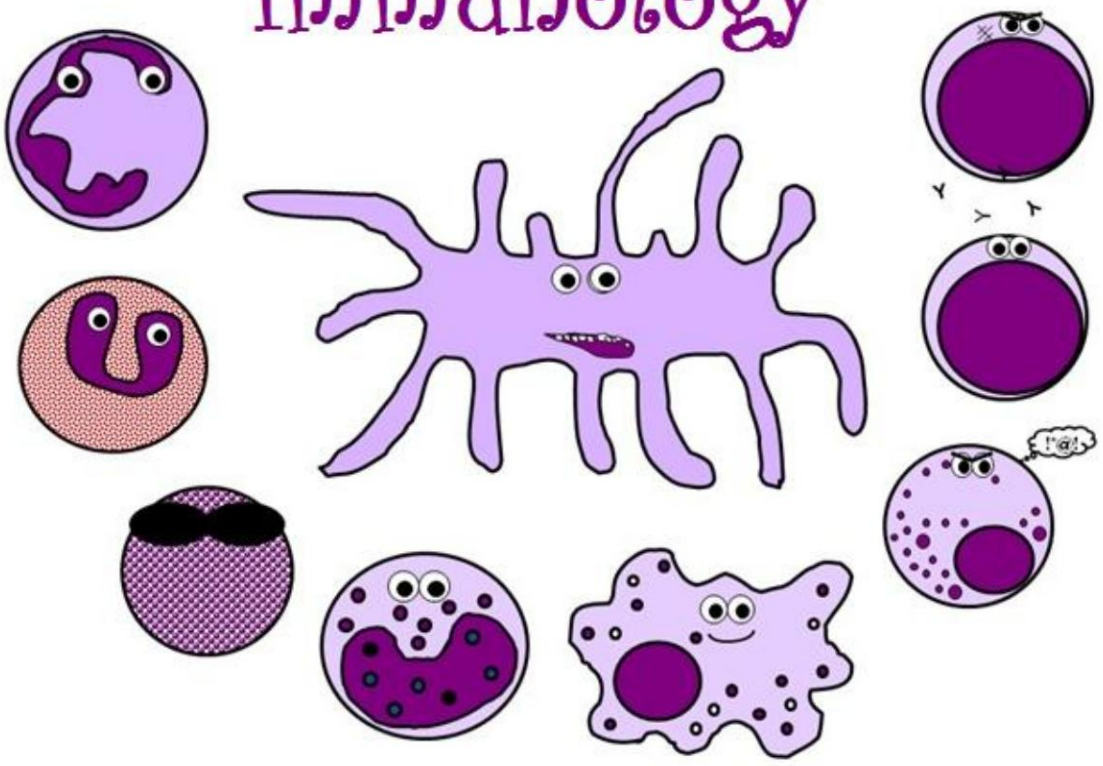
Immunology



Sheet Slides

Number : 23

Subject : AIDS

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AIDS in mother and child

** Lecture outlines:*

- Introduction
- HIV infection and the typical course of the disease
- Routs of transmission
- Case of the Pinkerton Family
- ELISA and western blot
- Opportunistic pathogens and cancers
- Questions

** This sheet was written according to section 2 record (the arrangement of ideas here is different), the slides and the old version of the book.*

Introduction

This will be the last case study for this course, our lecture will be an example for HIV infection from mother to child, and the transmission in this case is caused by contaminated blood transfusion - one of the main routs of transmission at the beginning of the disease, around 1980s. Nowadays there is **screening programs** which prevent getting the disease by this rout.

HIV infection and the typical course of the disease

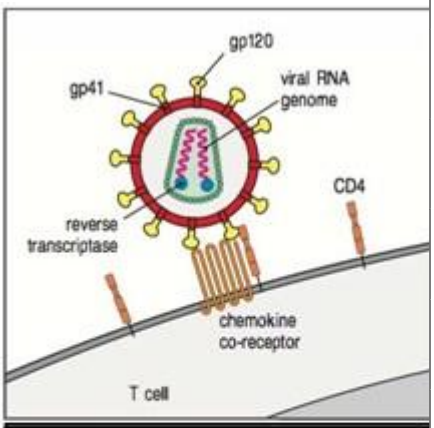
✓ Human Immunodeficiency Virus (HIV)

This virus targets certain cells in the immune system leading to failure of the system→ **immunodeficiency**, thus increasing the risk of infections and rare tumors that usually, the system can fend off.

There are two known types of the virus: **HIV- 1** which causes infection in North and South America and in Europe, and **HIV-2** in West Africa and Southeast Asia. HIV-2 is really rare.

CD4 is the receptor for HIV, so this virus target cells which can express CD4, such as: **T helper cells** (main target of the virus), **macrophages**, **dendritic cells**, **glial cells** in the brain, and **megakaryocytes** (platelets precursors). Note that **CD4 is a very good marker for T helper cells** since other cells will express it at much lower rate & in very low amounts.

HIV requires **both** CD4 and an obligatory chemokine coreceptor to infect cells. One Gp120 will **bind CD4** and another Gp120 will **bind the coreceptor**, then **Gp41** mediate the **fusion** of the enveloped virus with the target cell; allowing viral genome (single stranded RNA) to enter the cell. There, viral enzyme called **reverse transcriptase** will **form DNA** from the single stranded RNA, which will fuse with the host cell genome. When the cell get activated; it will replicate its own genome along with the **viral genome**, the virus will also use the cell machinery to **synthesize its own proteins**, **assembly** of these components occur; the virus exit the cell and attack other CD4 cells.



Gp120 is the main antigen used in ELISA for antibody test against the virus. Notice *chemokine coreceptor* and *CD4*.

There are a lot of different chemokine families, and not all of them can bind to HIV; the two main families that involve HIV binding are: ***CXCR4*** and ***CCR5***.

The exact sequence of amino acid define the family, meaning if we find cysteine , any amino acid , cysteine and 4 Arginine; this motif belong to ***CXCR4*** family and so on.

C : Cysteine
X : Any amino acid
R : Arginine

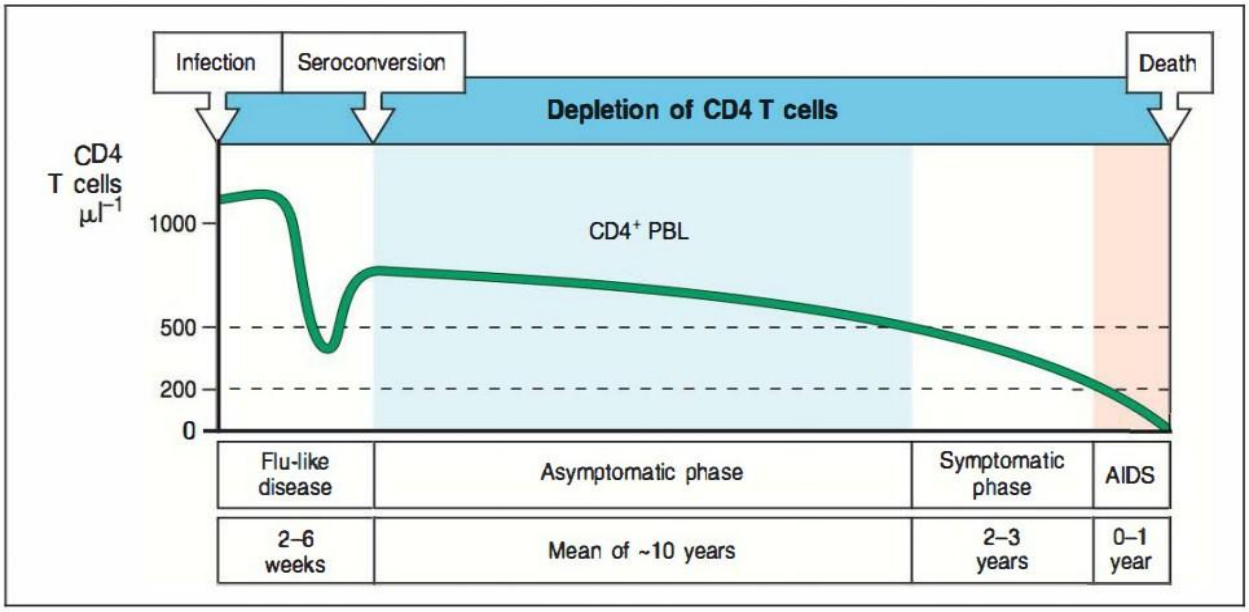
CD4+ count is so important; any drop **below 500** cells/µl constitutes the beginning of **the symptomatic phase**. That's why we do flow cytometry for HIV patients; to know CD4+ count, CD8+ count, the ratio, etc... Because **CD4+ count** is absolutely essential in deciding when are these patients going to go into the **symptomatic phase and the AIDS phase**.

In some population chemokine coreceptors are **resistant** to HIV infection and some elite controllers are **deficient** in CCR5 or CXCR4, so the virus will not bind properly nor enter! (Very very tiny population). This also affects their ability to recruit immune cells.

Elite responders: patients who take a **very long time to become symptomatic** compared to normal people, the advantages of that are:

- They have much stronger initial immune response.
- They secret higher amounts of type 1 interferon.
- Their cytotoxic T cells are very aggressive
- Their MHC molecules are very efficient in presenting viral antigen
- They are much better controllers of HIV infection.

✓ **Typical course HIV infection**



1. ***Acute phase***

At the beginning of the infection, there is a **massive decrease** in CD4 T cell count; because of viral replication, followed by a **slight increase** in their count when the immune system initiate a counterattack and try to control viral replication. **Seroconversion** marks the end of this phase, which last for 2-6 weak with flu-like symptoms.

Seroconversion: time at which antibodies against viral antigens become detectable in the serum.

2. ***Clinical latency***

Asymptomatic /Chronic phase. The virus is latent, it replicates gradually inside the cells and that's marked by the **gradual decrease in CD4 T cell count**, it may last for years or even decades without symptoms, until number of T cell fall below 500 cells/µl. {Note: refer to a small paragraph written in *italic* in **page 10** to make things more clear :)) }

3. ***Symptomatic phase***

Number of T cell is between 500-200 cells/µl, it is called **prodormal stage**; i.e. the stage when **prodormal signs** appear, (early signs and symptoms that indicate the start of the disease, before specific symptoms occur).

4. ***AIDS***

It's the last stage of infection by HIV, number of T cells dropped below 200 cells/µl, it last 0-1 year and followed by ***death***.

Prodromal stage	AIDS
500-200 cell/ µl Symptoms: • Low-grade fever • Night sweats • Candidiasis (thrush) in the mouth. • Swelling of lymph nodes **2-3 years**	**<200** cell/ µl HIV disease → AIDS Symptoms: • Immune system severely compromised • **Weight loss** (important) • Excessive fatigue • Persistent fever • Diarrhea • **Opportunistic infections & rare tumors** **0-1 year**

❁ Routs of transmission

1. Contaminated blood transfusion, the way the mother in our case gets the disease.
2. Contaminated needle→ common between drug addicts.
3. Sexual intercourse.
4. Vertical transmission (mother-to-child transmission), the way the son gets the disease.

❁ Case of the Pinkerton Family

(Read the following quickly, focus on what you think is important)

Benjamin Pinkerton was a captain in the US navy, he got married to Cheiko -a Japanese woman- .They had a healthy girl then after a year, Cheiko got pregnant but her baby died inside her; a **caesarian** was done to remove the dead fetus and because of blood loss during surgery; she had been **given 2 units of blood**. Sadly, these units were contaminated with HIV; so she got infected and pass the disease to her husband and her new child, named Franklin.

It's not necessary for the new child to have HIV, the probability is 1/4 (25 %!)

Franklin **weighted normal** at birth, and he received routine **immunization** with tetanus and diphtheria toxoids and

pertussis bacteria (DPT) as well as oral polio vaccine, he seemed to be thriving :D.
At *6 Months* he became sick and **lost weight**. He developed severe diarrhea, fever, Otitis media and thrush (white spots in the oral cavity indicating Candidal infection).At *7 months* her developed mild difficulty in breathing and by *10 months* he had unsteady gate and lost 10 Kg.

On physical examination he had low grade fever, Candidal thrush, diaper rash and inspiratory rales (crackles) in both lungs.
His WBC count was found to be normal with normal differential and normal immunoglobulins but only **CD4 T cells was very depressed**. He showed no delayed type hypersensitivity (DTH) response to intradermal *Candida* antigens or to PPD (purified protein derivative of tuberculosis).

The fact that CD4 population is killed **selectively**; doesn't mean that we will see significant drop in WBC count, **don't look for leukocytopenia in HIV patient,** because you will not find it!

Normal CD8 cells can be found normal or even higher because there is compensation, but **no benefit of CD8 cell without CD4 cell**, T Helper is the **Maestro** between lymphocytes.

His serum contained **antibodies to IV** by ELISA and western blot. After this discovery, the rest of the family was tested; his **mother** and **father** tested **positive**, his **sister** tested **negative**. (Notice that she was born before the cesarean section done to her mum, i.e. before the contaminated transfusion).

Bronchial washing showed positive *Pneumocystis Carinii* stain, lung biopsy grew *CMV, RSV, Pseudomonas aeruginosa* and duodenal biopsy contained *CMV*.

Pneumocystis Carinii **was treated with Trimethoprim and sulfa.**

He developed hemoptysis and died of respiratory failure.

Meanwhile, the mother (Chieko) had felt run down and complained low grade fever and swollen lymph nodes, she attributed these symptoms to the stress of Franklin illness.

After being tested positive for HIV; she started on AZT (**zidovudine**) therapy, but she developed *Pneumocystis Carinii* when her blood CD4 T cells dropped **below 200** cells/µl, and she died of respiratory failure.

Captain Pinkerton remained **asymptomatic**, most probably because he is in the asymptomatic phase and will develop symptoms later, or he might be an elite controller.

Contaminated blood transfusion was one of the main modes of transmission around 80s – from the beginning of HIV around 1980, to 1987 when this mum had her baby-. Doctors worked very actively on screening bloods; so hopefully, we will not hear about cases like this in our clinical practice –patients receive HIV from blood transfusion-.

❀ ELISA and western blot

(The following is attached from the book and doctor's notes will be in *italic*)

✓ ELISA

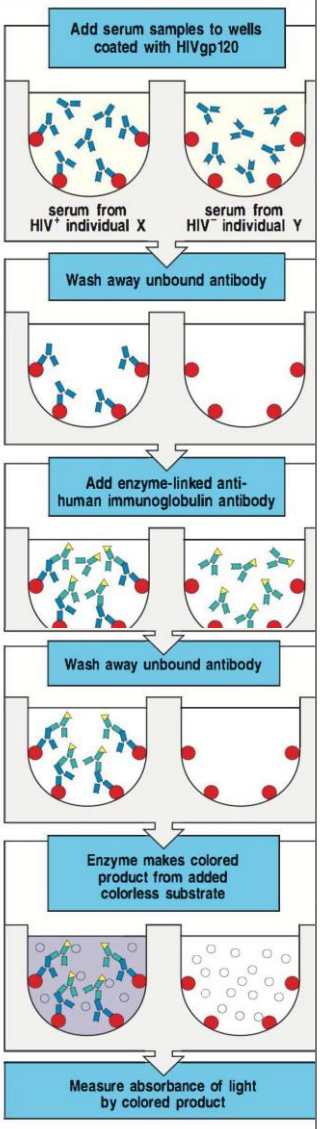


Fig. 10.2 Use of the enzyme-linked immunosorbent assay (ELISA) to detect the presence of antibodies against the HIV coat protein **gp120**.
Purified recombinant gp120 is coated onto the surface of plastic wells to which the protein binds nonspecifically; residual sticky sites on the plastic are blocked by adding irrelevant proteins (not shown).
Serum samples from the individuals being tested are then added to the wells under conditions where nonspecific binding is prevented, so that only binding to gp120 causes antibodies to be retained on the surface. Unbound antibody is removed from all wells by washing, and anti-human immunoglobulin that has been chemically **linked to an enzyme** is added, again under conditions that favor specific binding alone. After further washing, the colorless substrate of the enzyme is added, and colored material is deposited in the wells in which the enzyme-linked anti-human immunoglobulin is found. This assay allows arrays of wells known as microtiter plates to be read in fiberoptic multichannel spectrometers, greatly speeding the assay.

*This is called **indirect ELISA** (which looks for antibody), there is **direct ELISA** (looks for antigen) and **sandwich ELISA** (antibody above which there is antigen above which there is antibody).*

Antibody antigen combo *is used nowadays in antibody testing with window of detection of* ***1 month*** *instead of 3 months as in ELISA.*

Notice that ***secondary antibody*** *binds to the* ***Fc portion*** *of the primary antibody and it's conjugated to* ***enzyme****. (ELISA=Enzyme Linked immunosorbent assay). This enzyme will bind to a substrate and change its color.* ***HRP (horseradish peroxidase)*** *is the most enzyme used in ELISAs and its substrate is* ***TNB*** ***(tetrazolium Nitroblue)*** *that changes its color from colorless to* ***blue,*** *we read this change by spectrophotometry.*

Spectrophotometer gives ***zero*** *when reading something* ***transparent****, the more* ***bluish*** *the solution gets; the* ***higher*** *the number it gives, and according to a* ***standard curve*** *with known concentrations, we can determine the position of our sample on the curve and determine the concentration.*

So ELISA is not only qualitative, it's also quantitative; i.e. we can know the ***concentration*** *of antibodies present.*

- ✓ **Western blot**

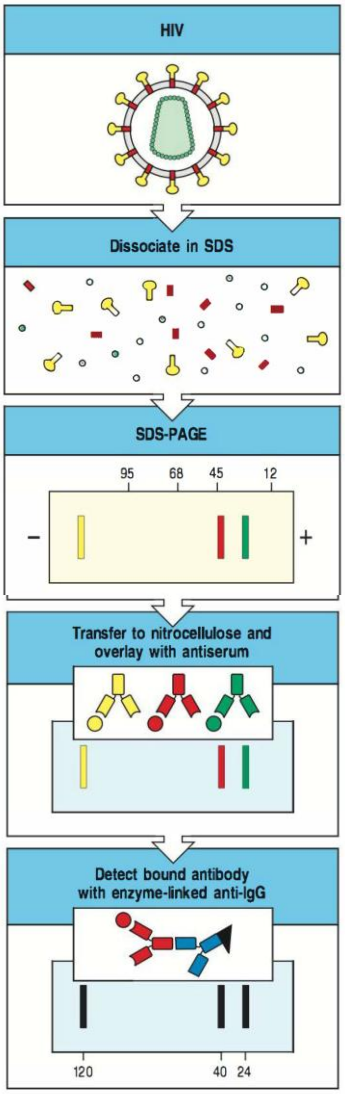


Fig. 1 0.3 Western blotting is used to identify antibodies against the human immunodeficiency virus (HIV) in serum from infected individuals. The virus is dissociated into its constituent proteins by treatment with the detergent SDS, and its proteins are separated by SDS-PAGE. The separated proteins are transferred to a nitrocellulose sheet and reacted with the test serum. Anti-HIV antibodies in the serum bind to the various HIV proteins and are detected by using enzyme-linked anti-human immunoglobulin, which deposits colored material from a colorless substrate. This general methodology will detect any combination of antibody and antigen and is used widely, although the denaturing effect of SDS means that the technique works most reliably with antibodies that recognize the antigen when it is denatured.

The main difference between ELISA and western blot is that ELISA test for antibodies while western blot test for proteins.

Here we don't wait for indirect measure i.e. the antibodies formed against the virus, instead; we take a blood sample and ***detect the virus itself****.*

The idea of the test is that we put a lysis buffer (it lyses the cell and cut its proteins into small fragments) on the blood

sample that we look for a protein in (HIV protein in our case). The cells will burse so all proteins will exit and now we have a pool of proteins.

*We take certain amount of the proteins and put a loading buffer (**blue** in color) that will stain them and give a negative charge to all the samples for preventing charged amino acid from affecting the protein while passing through the gel.*

*Agarose gel with small perforations is used, through which proteins pass according to **molecular weight** in Kilo Dalton. We will have the main **big** proteins **above**, and the **small** proteins will migrate further **below**. (Big protein will face difficulty while passing through the perforation so it will migrate a short distance).*

***Gp120** molecular wight = 120 kD*

***Gp42** molecular weight = 42 kD*

*We pass electric current i.e. electrophoreses, and then we transfer the proteins into a membrane to detect them. We can add antibodies to the membrane (Gp120 antibody, Gp42 antibody…) if **Gp120 is found on the membrane the antibody will bind :D ,** then –just like ELISA- we wash , put secondary antibody , wash , put substrate; if it gives a light we can see it in the dark room using X-ray film, that will be burned on the position of a lighted band i.e. we will see a black band, then we put the film in a machine that will process it, and give us a processed image containing bands, according to their position (their molecular weight) we can know which antigen is present.*

- **Opportunistic Infections and malignancies associated with HIV**

Infections	
Parasites	*Toxoplasma* spp. *Cryptosporidium* spp. *Leishmania* spp. *Microsporidium* spp.
Bacteria	*Mycobacterium tuberculosis* *Mycobacterium avium intracellulare* *Salmonella* spp.
Fungi	*Pneumocystis jirovecii* *Cryptococcus neoformans* *Candida* spp. *Histoplasma capsulatum* *Coccidioides immitis*
Viruses	Herpes simplex Cytomegalovirus Herpes zoster

Malignancies
Kaposi's sarcoma (invasive) Non-Hodgkin's lymphoma, including EBV-positive Burkitt's lymphoma Primary lymphoma of the brain

** Those what actually kill the patient, not the AIDS itself.*

Questions

1. **How did we rule out SCID diagnosis for the boy?**

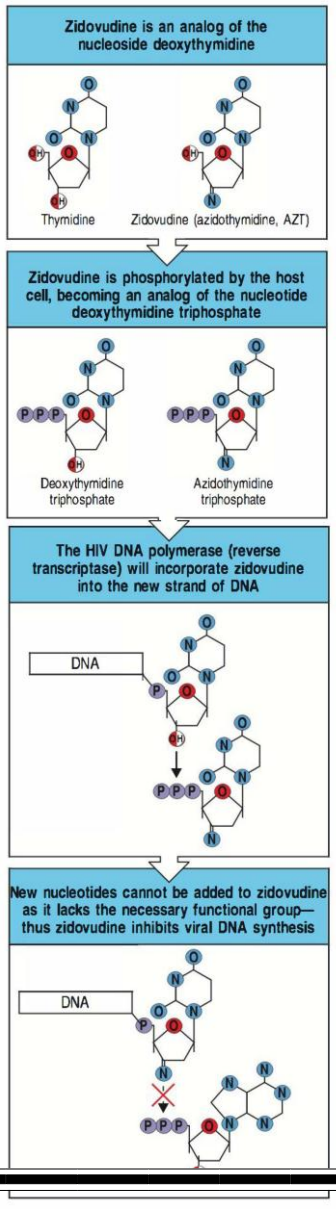
Normal serum Igs levels and low CD4+ count as well as hyper plastic germinal centers in the lymph nodes of AIDS patients.

2. **What is the difference between HIV course in pediatric vs. Adult patients?**

HIV progression is much more rapid in infants.
Infant has immature immune system because they depend on IgG from the mother, their T helper cells have less ability to produce interferon gamma, and their cytotoxic T cells are less aggressive, so they have very little acquired adaptive immunity against infectious microorganisms.

Adult's adaptive immune system, on the other hand, had more time to recognize many pathogens and form memory cells & Igs against it, and that help in preventing recurrent infections. (Recall, Asplenia)

3. **How does AZT (Zidovudine) work?**



It's a nucleotide RT inhibitor (NRTI), and thymidine analogue, the only difference is the presence of **nitrogen in AZT** instead of a **hydroxyl group** which is found **in thymidine** structure; the hydroxyl group is crucial to bind the next nucleotide.
When the drug enter the body it get phosphorylated, this phosphate group is required for binding to the hydroxyl group of the prior nucleotide, nitrogen group in AZT is no longer able to bind to the phosphate groups in the next nucleotide → ***termination of reverse transcription.***
In Highly active retroviral therapy we use combination of drugs, including protein inhibitors, non nucleotide RT inhibitor (NNRTI), integrase inhibitors and nucleotide RT inhibitor (AZT).

4. **Why are CD4+ cells depleted in HIV infection?**

The presence of the virus inside the CD4 T cells is harmful; the virus is replicating and budding out of the cell killing it.
In the same time, **cytotoxic T cells** will kill the infected CD4 T cells; its presence is important to control the virus in the latent phase.

Latent phase *is conflict between the virus and the immune system; the virus is presented by MHC I and the infected cells are killed by cytotoxic T cells, BUT* ***the virus is replicating and infecting new cells & integrate into the genome of new cells****, while T helper cells is secreting cytokines to enhance cytotoxic T cell function until reaching a stage when CD4 T cells are low in number and not capable of giving enough help for* ***cytotoxic T cells*** *which will* ***quit****, and we enter to the symptomatic phase and AIDS phase.*

5. **What is the most important determinant in the progression of HIV?**

CD4 T cell count is important clinically to determine the progression of HIV infection in the patient; did we enter the symptomatic phase, AIDS phase, whether to start treatment or not...
Sometimes we ask for **CD8** & for the ratio **CD4:CD8** (we shall worry if **the ratio is less than 1**).
We don't look for ESR and CRP as they are very general markers.

6. **What causes weight loss in HIV patients?**

TNF-α is secreted in high amounts in HIV infection in an effort from the body to fight the infection, but as a byproduct; TNF-α will cause **anorexia** (فقدان الشهية) & **increased body heat expenditure,** and when those two things happen the patient will lose weight.

Sorry for any unintended mistake :)

Best regards ❀