Chapter 5

Immunological and Haematological Changes in HIV Infection

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Additional information is available at the end of the chapter

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1. Introduction

HIV is known to be associated with a wide range of immunological and haematological changes.The immunological changes include depletion in CD4+ T cell, cytokine dysregulation and immune dysfunction. The dominant immunologic feature of HIV infection is progressive depletion of the helper T cell (CD4+ T cell), which reverses the normal CD4:CD8 ratio and subsequently lead to immunodeficiency. CD4+ T cells interact with antigen presenting cells (APCs), B cells, cytotoxic T cells (CD8+ T cells) as well as natural killer cells. Thus, infection and depletion of CD4+ T cell population could induce profound immunodeficiency in such patients.

The haematological changes occur mainly due to several factors such as marrow defects and immune cytopenias. It is caused by HIV infection, either directly to the bone marrow, opportunistic infections, development of lymphoma as a secondary neoplasm and side effects of the drugs used for the treatment or drugs used for the complicating infection or lymphoma. In order to further understand the immunological and haematological changes that occur in HIV infection, the chapter begins with the review of immune system as well as normal haematopoiesis. It further highlights the importance of changes associated with clinical symptoms in patients with HIV.

2. The human immune system

The immune system plays an important role to protect the host from infectious agents such as bacteria, viruses, fungi and parasites. In addition, it is also important in the identification and

elimination of tumor cells as well as in response to injury and trauma. Thus, an effective and efficient immune system is essential as a host defense mechanism against infectious diseases and cancer. The immune system can be further subdivided into innate and acquired or adaptive immunity (Table 1).

	Innate immunity	Acquired immunity
Physico-chemical barriers	Skin	Cutaneous and mucosal immune systems
	Mucosal membranes	Antibodies in mucosal secretions
	Lysozyme	
	Stomach acid	
	Commensal bacteria in gut	
Circulating molecules	Complement	Antibodies
Immune Cells	Granulocytes	B lymphocytes
	Monocytes/macrophages	T lymphocytes
	Natural killer cells	
Soluble mediators	Macrophage-derived cytokines	Lymphocyte-derived cytokines

Table 1. Components of the innate and acquired immune systems [1].

2.1. Innate immunity

Innate immunity is the first line of defense mechanism against the invading agents present at birth. The innate immune system includes physico-chemical barriers, circulating molecules, immune cells as well as soluble mediators [1, 2]. As compared to acquired immunity, innate immunity has no memory, poor specificity and has immediate response with lower potency.

2.2. Acquired immunity

The acquired or adaptive immune response is the second line of defense mechanism which offer better protection against re-exposure to the same pathogen [2]. The acquired immune system is further subclassified into humoral mediated immunity which involves antibody production by B lymphocytes and cell mediated immunity comprising CD4+ and CD8+T lymphocytes. Acquired immunity has immunological memory and it is highly specific. The specificity occurs because each lymphocyte carries surface receptors for a single antigen [1]. When compared to innate immunity, it has slower response, however it is much more potent and robust and the response varies among individuals.

Innate and adaptive immune systems as well as complement counteract each other to produce an effective function and mechanism of eliminating the invading agents.

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2.2.1. B lymphocytes

B lymphocytes are known by their ability to produce antibodies (immunoglobulins), which are specific for particular antigen [1]. Antibodies work in several ways to combat invading pathogens. Some pathogens, particularly viruses and some bacteria as well, infect individuals by entering cells. Some of these pathogens escape humoral immunity and later on will be encountered by cell-mediated immunity, which is conferred by T lymphocytes.

2.2.2. T lymphocytes

Mature T lymphocytes express T-cell receptors on their surface. They are able to recognize only those antigens which are associated with the protein termed major histocompatibility complex (MHC): in humans the MHC is known as human leukocyte antigen (HLA) [2] that are presented to them on a cell surface by antigen presenting cells (APCs) [2]. When an APC (e.g. macrophage) encounter an antigen or pathogen, it will engulf, process and present the pathogen to the T cell [2].

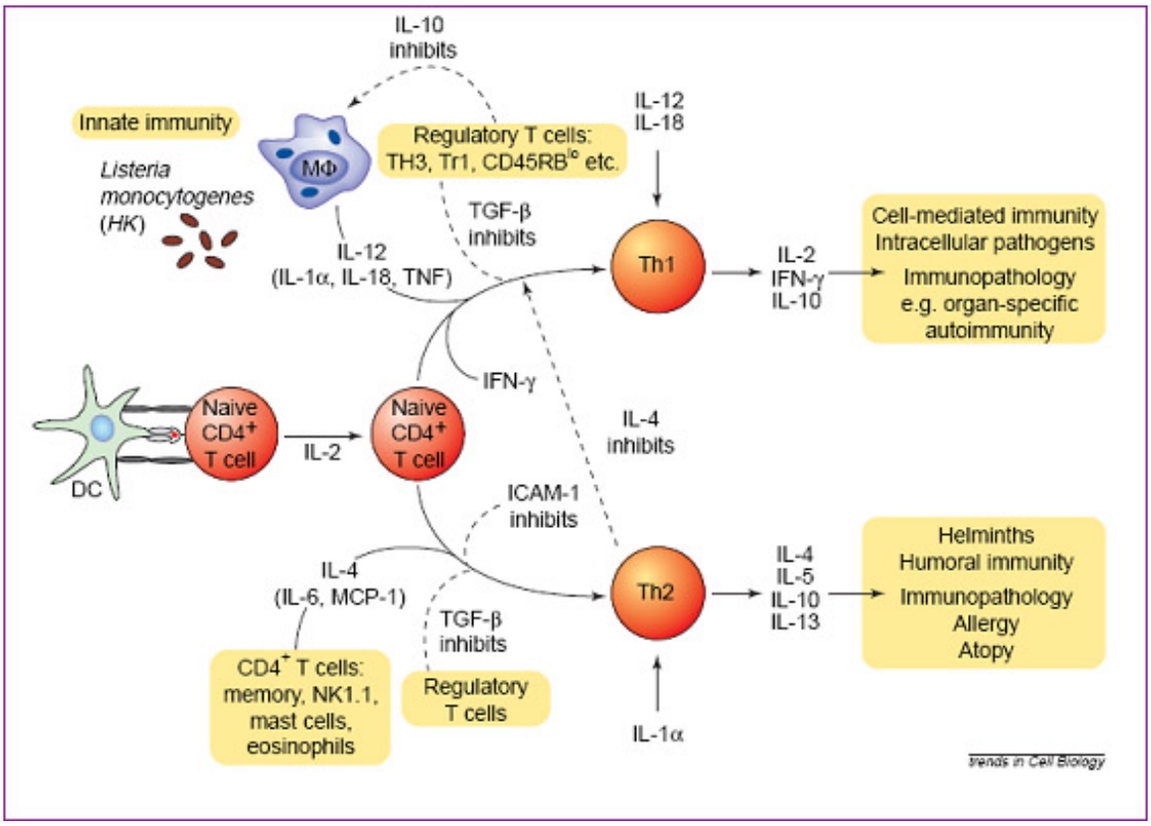
T lymphocytes can be further subdivided into CD4+ T cells (Helper T cells) and CD8+ T cells (Cytotoxic T cells). CD4+ T cells are further subclassified into Th1 (T helper 1) and Th2 (T helper 2). Th1 is important for eliminating intracellular pathogen whereas Th2 is important for immunity against extracellular pathogen [2].

The regulation of the immune response is shown in Figure 1. The Th1 cells are involved in cell-mediated immunity against intracellular pathogens. They produce cytokines such as interleukin-2 (IL-2), interferon-gamma (IFN-gamma) and interleukin-10 (IL-10) [2]. IL-2 promotes proliferation of T lymphocytes [2] while IFN-gamma activates the cells involved in the elimination of pathogens and cells with tumour properties (e.g. monocytes, macrophages, cytotoxic T lymphocytes, natural killer cells) [1]. By contrast, the Th2 cells are mainly involved in humoral immunity and immunity against helminthic infection [2]. They produce cytokines such as IL-4, IL-5, IL-10 and IL-13. It is known that IL-4 stimulates proliferation of B lymphocytes while IL-5 promotes activation of eosinophils [2]. IL-10 has inhibitory effect while IL-13 involves in allergic reaction and helminthic infection [2]. Thus, both Th1 and Th2 cells and their cytokines counteract with each other to regulate the immune response [1].

The communication within the acquired immune system and between innate and acquired immunity involves cell surface proteins (e.g. adhesion molecules) and the soluble molecules which can produce signals from one cell to another, that is called cytokines [1].

2.3. Cytokines

Cytokines are a diverse group of intercellular signaling peptides and glycoproteins with molecular weight between 6000 and 60,000. They are produced by a variety of tissues and cells in response to stimuli [2]. Cytokines have diverse and pleiotropic effects [4]. Their binding to specific receptors on the cell surface leads to changes in growth, development or activity of the target cells. They do not only regulate immune and inflammatory responses but also involve in wound healing, haematopoiesis, angiogenesis as well as other biologic processes [2, 4].



The diagram shows the mechanism of regulation of immune response. Th1 is mainly involved in cell-mediated immunity and offers protection against intracellular pathogens while Th2 is important in humoral immunity, protection against helminthic infection as well as allergic or atopic diseases such as allergic rhinitis, asthma etc.

Figure 1. The regulation of immune response [3]

3. Immunological aspects of HIV infection

3.1. Clinical features of HIV infection

In primary infection of HIV-1, patients may be asymptomatic though sometimes the disease is self-limiting. Within the incubation period of about 6 weeks, patients can present with a mononucleosis-like syndrome, which is characterized by fever, cough, painful swallowing, myalgias, arthralgias, diarrhea as well as maculopapular rash and lymphadenopathy [5]. In most circumstances, the symptoms are usually mild as contrasted to severe cases, where pneumonitis, oropharyngeal and esophageal ulcers may occur. Encephalitis, meningitis, neuropathy, radiculopathy and myelopathy are not common sickneses in HIV infection. Although the true incidence of this syndrome is not precisely known, it may also depend on the degree of exposure to the virus, it may be as high as over 50% in persons who acquire HIV-1 infection [5].

World Health Organization [6] categorized an adult or adolescent (aged > 12 years) as having AIDS in presence of at least two of the major signs in combination with one of the minor signs (Table 2).

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Major signs	Minor signs
· Weight loss of more than 10% bodyweight	· Persistent cough for more than 1 month*
· Chronic diarrhea for more than 1 month	· Generalised pruritic dermatitis
· Prolonged fever for more than 1 month (intermittent or constant)	· History of herpers zoster
	· Oropharyngeal candidiasis
	· Chronic progressive or disseminated herpes · simplex infection
	· Generalised lymphadenopathy
* Persistent cough for more than 1 month should be considered as a minor sign in patients with tuberculosis	

Table 2. Major and minor signs of HIV infection [6].

3.2. The classification of HIV disease

The staging and classification of HIV disease are standard tools for monitoring HIV epidemic and also serve as a guide for clinicians in managing HIV patients. It provides important information for patients and clinicians regarding the staging of HIV disease and clinical management. Currently, two major classification are used: World Health Organization (WHO) Clinical Staging and Disease Classification System and the United State Centers for Disease Control and Prevention (CDC) Classification System.

The WHO Clinical Staging and Disease Classification System (revised in 2007) is usually used in resource-constrained settings without access to CD4 cell count measurements or other diagnostic and laboratory methods [7] The system classifies the HIV disease based on the clinical manifestations of patients. By contrast, the CDC classification system assesses the HIV disease severity by CD4 cell count as well as by the presence of specific HIV-related conditions [7]. This classification system is usually beneficial in the clinical as well as epidemiologic research.

3.2.1. WHO clinical staging of HIV/AIDS and case definition

The clinical staging and case definition of HIV was developed by WHO in 1990 and revised in 2007. It is based on the clinical findings rather than CD4 cell count. This staging system has been used by some countries for managing HIV patients where the CD4 cell count testing is not available [7]. The staging was categorized as 1 to 4 based on clinical severity and progression from primary infection to advanced stage. The adult and adolescents were defined as individuals aged ≥ 15 years. WHO, 2007 [8] classifies the clinical staging of HIV/AIDS based on the clinical conditions or symptoms (Table 3).

Clinical Stages	Clinical Conditions or Symptoms
Primary HIV Infection	· Asymptomatic
	· Acute retroviral syndrome
Clinical Stage 1	· Asymptomatic
	· Persistent generalized lymphadenopathy

Clinical Stage 2	· Moderate unexplained weight loss (<10% of presumed or measured body weight) · Recurrent respiratory infections (sinusitis, tonsillitis, otitis media, and pharyngitis) · Herpes zoster · Angular cheilitis · Recurrent oral ulceration · Papular pruritic eruptions · Seborrheic dermatitis · Fungal nail infections
Clinical Stage 3	· Unexplained severe weight loss ("/>10% of presumed or measured body weight) · Unexplained chronic diarrhea for "/> 1 month · Unexplained persistent fever for "/> 1 month ("/>37.6ºC, intermittent or constant) · Persistent oral candidiasis · Oral hairy leukoplakia · Pulmonary tuberculosis · Severe presumed bacterial infection (e.g., pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteremia) · Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis · Unexplained anaemia (haemoglobin<8 g/dl) · Neutropenia (neutrophils <500 cells/μL) · Chronic thrombocytopenia (platelets < 50, 000 cells/ μL)
Clinical Stage 4	· HIV wasting syndrome, as defined by the CDC (see Table 1, above) · *Pneumocystis* pneumonia · Recurrent severe bacterial pneumonia · Chronic herpes simplex infection (orolabial, genital, or anorectal site for "/>1 month or visceral herpes at any site) · Esophageal candidiasis (or candidiasis of trachea, bronchi, or lungs) · Extrapulmonary tuberculosis · Kaposi sarcoma · Cytomegalovirus infection (retinitis or infection of other organs) · Central nervous system toxoplasmosis · HIV encephalopathy · Cryptococcosis, extrapulmonary (including meningitis) · Disseminated nontuberculosis mycobacteria infection · Progressive multifocal leukoencephalopathy · Candida of the trachea, bronchi, or lungs · Chronic cryptosporidiosis (with diarrhea) · Chronic isosporiasis · Disseminated mycosis (e.g., histoplasmosis, coccidioidomycosis, penicilliosis) · Recurrent nontyphoidal *Salmonella* bacteremia · Lymphoma (cerebral or B-cell non-Hodgkin) · Invasive cervical carcinoma · Atypical disseminated leishmaniasis · Symptomatic HIV-associated nephropathy

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	· Symptomatic HIV-associated cardiomyopathy · Reactivation of American trypanosomiasis (meningoencephalitis or myocarditis)

Table 3. WHO Clinical Staging of HIV/AIDS for Adult and Adolescents [8].

3.2.2. CDC classification system for HIV infection

The CDC classification of HIV/AIDS is based on the level of CD4 cell count and on previously diagnosed HIV-related conditions (Table 4) [7]. For example, if a patient had met the criteria for category B but currently is asymptomatic, the patient would remain in category B. The categorization is shown in Table 5. Patients in categories A3, B3 and C1-C3 are considered to have AIDS.

CD4 Cell Count Categories	CLINICAL CATEGORIES		
	A: Asymptomatic, Acute HIV or PGL	***B: Symptomatic Conditions, not A or C**	****C: AIDS-Indicator Conditions**
1. ≥ 500 cells/μL	A1	B1	C1
2. 200-499 cells/μL	A2	B2	C2
3. < 200 cells/μL	A3	B3	C3

PGL: persistent generalized lymphadenopathy for *B and **C Clinical Categories refer to Table 5

Table 4. CDC Classification System for HIV-Infected Adults and Adolescents [7].

***Category B: Symptomatic Conditions**	Definition: Symptomatic conditions occurring in an HIV-infected adolescent or adult that meet at least one of the following criteria: 1. They are attributed to HIV infection or indicate a defect in cell-mediated immunity 2. They are considered to have a clinical course or management which is complicated by HIV infection 3. Include the following: · Bacillary angiomatosis · Oropharyngeal candidiasis (thrush) · Vulvovaginal candidiasis, persistent or resistant · Pelvic inflammatory disease (PID) · Cervical dysplasia (moderate or severe)/cervical carcinoma in situ · Hairy leukoplakia, oral · Herpes zoster (shingles), involving two or more episodes or at least one dermatome · Idiopathic thrombocytopenic purpura · Constitutional symptoms, such as fever ("/>38.5°C) or diarrhea lasting "/>1 month · Peripheral neuropathy

****Category C:**	· Bacterial pneumonia, recurrent (two or more episodes in 12 months)
AIDS-Indicator Conditions	· Candidiasis of the bronchi, trachea, or lungs
	· Candidiasis, esophageal
	· Cervical carcinoma, invasive, confirmed by biopsy
	· Coccidioidomycosis, disseminated or extrapulmonary
	· Cryptococcosis, extrapulmonary
	· Cryptosporidiosis, chronic intestinal ("/>1 month in duration)
	· Cytomegalovirus disease (other than liver, spleen, or nodes)
	· Encephalopathy, HIV-related
	· Herpes simplex: chronic ulcers ("/>1 month in duration), or bronchitis, pneumonitis, or esophagitis
	· Histoplasmosis, disseminated or extrapulmonary
	· Isosporiasis, chronic intestinal ("/>1-month in duration)
	· Kaposi sarcoma
	· Lymphoma, Burkitt, immunoblastic, or primary central nervous system
	· *Mycobacterium avium* complex (MAC) or *Mycobacterium kansasii*, disseminated or extrapulmonary
	· *Mycobacterium tuberculosis*, pulmonary or extrapulmonary
	· *Mycobacterium*, other species or unidentified species, disseminated or extrapulmonary
	· *Pneumocystis jiroveci* (formerly *carinii*) pneumonia (PCP)
	· Progressive multifocal leukoencephalopathy (PML)
	· *Salmonella* septicemia, recurrent (nontyphoid)
	· Toxoplasmosis of brain
	· Wasting syndrome caused by HIV (involuntary weight loss "/>10% of baseline body weight) associated with either chronic diarrhea (two or more loose stools per day for ≥1 month) or chronic weakness and documented fever for ≥1 month

Table 5. B and **C Clinical Categories of HIV infection [7].

3.3. Immunologic changes in HIV infection

3.3.1. Depletion of CD4+T cells causes immunodeficiency

Untreated HIV-1 infection is associated with a gradual loss of peripheral $CD4^+$ T cells. Loss of $CD4^+$ T cells and systemic immune activation are the major hallmarks of HIV infection [9]. There are two major phases of HIV disease, acute and chronic infection. Acute infection is associated with gradual loss of $CD4^+$ T cells in the mucosal tissue [10] while chronic infection is characterized by immune activation which is associated with massive production of proinflammatory cytokines [11]. This subsequently leads to decrease in peripheral $CD4^+$ T cells and profound immunodeficiency.

The major mechanism of $CD4^+$ T cell depletion in HIV patients is due to apoptosis, in which the number of apoptotic cells exceed the number of HIV-infected cells [12]. Other causes

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of $CD4^+$ T cell depletion include impairment of *de novo* production of T lymphocytes by the thymus [9], induction of syncytium formation, alteration of membrane permeability, mitochondrial dysfunction as well as killing by HIV-specific $CD8^+$ T cells due to immune activation [9].

3.3.2. Loss of function of CD4+ T cells

Functional defects in the immune system of HIV-infected individuals exacerbate the immune deficiency caused by depletion of CD4+ T cells. These functional defects include a decrease in T cell responses to antigens as well as weak humoral immune responses even though total serum Ig levels may be elevated [13]. The defects might be due to the direct effects of HIV on $CD4^+$ T cells through:

1. Binding of gp120 (viral encoded membrane glycoprotein) to newly synthesized intracellular CD4+ T cells that result in the interference of normal protein processing in the endoplasmic reticulum as well as block the surface expression of $CD4^+$ T cells.This makes the cell incapable of responding to antigenic stimulation and,
2. $CD4^+$ T cells which bound to gp120 may not be available to interact with class II major histocompatibilty complex (Class II MHC) molecules on antigen presenting cells (APCs), thus T cell responses to antigens would be inhibited. Alternatively, gp120 binding to $CD4^+$ may deliver signals that downregulate helper T cell function.

In addition, HIV-infected T cells are unable to form tight synapses with APCs, therefore interferes with T cell activation [13]. Failure of the activation process will lead to incapability of the T cells particularly $CD4^+$ T cells to interact with other immune cells and subsequently lead to failure of elimination of the virus [13].

3.3.3. Cytokine dysregulation and coagulopathy in HIV infection

Cytokines play an important role in controlling the homeostasis of the immune system. Cytokine dysregulation is a major immunopathogenic factor in HIV infection [14]. The rise in serum levels of pro-inflammatory and inflammatory cytokines contribute to viral replication and many manifestations of immunodeficiency [15]. Cytokine production can increase viral replication due to its activation role in HIV infected cells [16]. Such cytokines include IL-2, IL-4 and interferon type II (IFN-γ) which primarily are required for expansion aid of antiviral T cells and antibody responses [16].

3.3.3.1. Cytokine dysregulation

Infection with HIV results in dysregulation of the cytokine profile *in vivo* and *in vitro*. During the course of HIV-1 infection secretion of T-helper 1 (Th1) cytokines, such as interleukin (IL)-2 and antiviral interferon (IFN)-gamma important for intracellular infection is generally decreased, whereas production of T-helper 2 (Th2) cytokines required for extracellular infection such as IL-4, IL-10, proinflammatory cytokines (IL-1, IL-6, IL-8) and tumour necrosis factor (TNF)-alpha, is increased [13, 14, 17]. This altered balance of Th1 and Th2 responses may

partially explain the susceptibility of HIV-infected individuals to infection by intracellular microbes. In addition, Th2 cytokines also may inhibit macrophage-mediated killing of microbes [13] and consequently lead to failure of macrophage activation in the killing process of the virus.

Tumour necrosis factor-alfa (TNF)-α, IL-1 and IL-6 which are produced by monocytes and macrophages, play an important role in activation of neutrophils, monocytes and macrophages to initiate bacterial and tumor cell killing, increase adhesion molecule expression on the surface of neutrophils and endothelial cells, stimulate T and B lymphocytes proliferation as well as initiate the production of other proinflammatory cytokines [1, 2]. In addition, these cytokines can cause systemic symptoms such as fever and weight loss as well as influence the production of acute phase protein in the liver [1]. Inflammation is one of the components of innate immune response. Thus, production of appropriate amounts of TNF, IL-1 and IL-6 is important in response to infection [1]. Increased production of these cytokines, particularly TNF-α has been found in acute and inflammatory conditions (e.g., trauma, sepsis, infection, rheumatoid arthritis) [18]. The observed increase in proinflammatory and inflammatory cytokines following cell injury or infection subsequently leads to immune dysfunction.

3.3.3.2. Coagulopathy in HIV patients

Normal levels of protein S, protein C and antithrombin activities are necessary for coagulation process. In HIV patients, protein S, protein C and antithrombin activities decrease with an increase in plasma D-dimer [19, 20]. Binding of viral and bacterial components to Toll-like receptors (TLRs) stimulate the procoagulant tissue factor to initiate the coagulation cascade. This leads to thrombin activation which then cleaves the fibrinogen to fibrin [21]. Plasmin cleaves the fibrin to produce fibrin degradation products. An increase in monocyte tissue factor expression leads to increase in D-dimer in HIV infection [21]. Decrease protein S, protein C and antithrombin activities as well as increase in plasma D-dimer are the predisposing factors which will increase the risk HIV patients to thrombosis.

3.4. Prognostic markers of HIV infection

Prognostic markers are important tools for monitoring the HIV disease progression. Proper monitoring of the disease may reduce the morbidity as well as mortality rate. Some markers have been identified for monitoring the HIV disease progression. The markers is classified as immunologic (CD4+ T cells), virologic (RNA viral load), serologic (serum $\beta_2$microglobulin and neopterin) [22] as well as biomarkers (lipid peroxidation) [23]. Among the markers, CD4+ T cell count and RNA viral load are two most commonly used prognostic markers for clinical progression of HIV infection [24, 25].

3.4.1. Immunologic marker of infection

The CD4+ T cell count is the most important laboratory indicator of immune function in HIV-infected patients. It is also the strongest predictor of subsequent disease progression and survival according to findings from clinical trials and cohort studies [22, 26].

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Measurement of CD4+ T cell count is necessary prior to the initiation of HAART. Since the CD4+ T cell count reflects the status of overall immune function of HIV-infected patients, the measurement is important as a guide for initiation of HAART to HIV patients as well as to start or discontinue the prophylaxis for opportunistic infection (OI). Most of OIs occur in patients with CD4+ T cell counts<200 cells/mm^3 [27], however some patients may have OIs at higher CD4+ T cell counts. For patients who are on therapy, adequate response is defined as an increase in CD4+ T cell counts in the range of 50 to 150 cells/mm^3 during the first year of HAART. Patients who has faster response will show the response within the first 3 months of treatment and subsequent increase in the range of 50 to 100 cells/mm^3 per year until it reaches a steady state [27]. Patients who has undergone the therapy at a low CD4+ T cell counts [28] or at an older age [29] may have a minimal increase in CD4+ T cell counts despite virologic suppression.

3.4.2. Virologic marker of infection

RNA viral load is the best indicator of progression to AIDS and death followed by $CD4^+$ T cell count, serum neopterin levels and serum $\beta_2$microglobulin. It strongly predicts the rate of decrease in $CD4^+$T cell counts and progression to AIDS and eventual death, but the prognosis of HIV is best predicted by combination of both plasma HIV-1 RNA and $CD4^+$ T cells [21].

In addition, it is also a marker of response to HAART. The main goal of HAART is to achieve and maintain durable viral suppression. A patient's pre-HAART viral load level and the magnitude of viral load decline after initiation of HAART provide prognostic information about the probability of disease progression [30]. Thus, the most important use of the viral load is to monitor the effectiveness of therapy after initiation of HAART. The minimal changes in the viral load is considered to be statistically significant (2 standard deviations) when there is a three-fold change (equivalent to a 0.5 $\log_{10}$ copies/mL change) in the viral load [31]. Optimal viral suppression is defined as presence of persistent viral load below the level of detection (HIV RNA <20 to 75 copies/mL, depending on the assay used) [31].

Data from previous studies and clinical trials reported that reduction in viral load following initiation of HAART are associated with reduced risk of progression to AIDS or death [30]. Therefore, RNA viral load measurement is an established surrogate marker for treatment response.

3.4.3. Serologic markers

Beta-2 microglobulin is a low molecular weight protein, which comprises the light chain of class 1 MHC proteins and is noncovalently bound to the heavy chain [2]. It is present on the surface of all nucleated cells. Dissociation during metabolism and degradation leads to its release to all biological fluids. In HIV disease, an increased level of Beta-2 microglobulin in cerebrospinal fluid (CSF) correlates with the disease progression and a decrease level indicates successful therapy [32].

Similarly, neopterin, a marker of immune activation is a low molecular weight compound derived from guanosine triphosphate [5]. It is produced by monocyte/macrophages upon

stimulation with IFN-γ. The production is increased in HIV infection and infection by intracellular organisms such as parasite, autoimmune disease, malignant tumours, allograft rejection, neurological as well as cardiovascular disease [33]. However, it has slightly low predictive value compared to beta-2 microglobulin [5]. Neopterin and beta-2 microglobulin levels were proved to be significant predictors of AIDS risk in HIV-1 seropositive patients. The predictive value of both parameters is equal to $CD4^+$ T cell counts. Therefore, neopterin and beta-2 microglobulin are recommended to be used as an additional marker to predict AIDS risk for HIV-1 seropositive patients and is beneficial particularly in the setting where the CD4+ T cell count measurement is not available [34].

3.4.4. Biomarker

Oxidative stress is a condition in which there is increased amounts of reactive oxygen or nitrogen species. This condition is now recognized to be a prominent feature of many acute and chronic disease and even in normal ageing process. Lipid peroxidation was found to be one of the biomarkers to assess oxidative stress status in human disease including HIV [23]. A study done by Friis-Moller *et al*. [35] have shown that HIV-infected patients have oxidative imbalance early in the disease; low serum and tissue antioxidants and elevation of peroxidation products. Besides, high plasma levels of malondialdehyde (MDA), reduced plasma glutathione (GSH) and decreased superoxide dismutase activities were also found [36].

4. Haematologic aspects of HIV infection

4.1. Normal haemopoiesis

Haemopoiesis is the formation of blood or blood cells in the living body. It involves the production of three (3) major cell lines which are red blood cells, white blood cells and platelets [37]. In humans, this process occurs in the bone marrow. In certain diseases, the process can be altered either directly or indirectly. Patients with HIV infection will have altered haemopoiesis [38], affecting both red and white blood cells and platelet formation.

Haemopoietic stem cells (HSCs) are the earliest cells recognized in the bone marrow (Figure 2). HSCs produce all blood cells [37]. About 5% of the HSCs in the bone marrow are functioning at one time, thus maintaining the haemopoietic system for the lifetime in a human body. Growth factors, also play an important role for production and differentiation of blood cells in the bone marrow. Erythropoietin, a type of hormone which is mainly produced by the kidney and thrombopoietin, which is mainly produced by the liver, are the growth factors that are necessary for production and proliferation of red blood cells and platelets respectively.White blood cells have five (5) major components which include neutrophil, monocyte, eosinophil, basophil and lymphocyte. The lymphocytes are further subdivided into B-lymphocytes and T-lymphocytes, which are important for functional activity.

In most circumstances, HIV infection causes reduction in blood cell formation [40]. These include red blood cell (anaemia), platelet (thrombocytopenia) and white blood cell (leucope-

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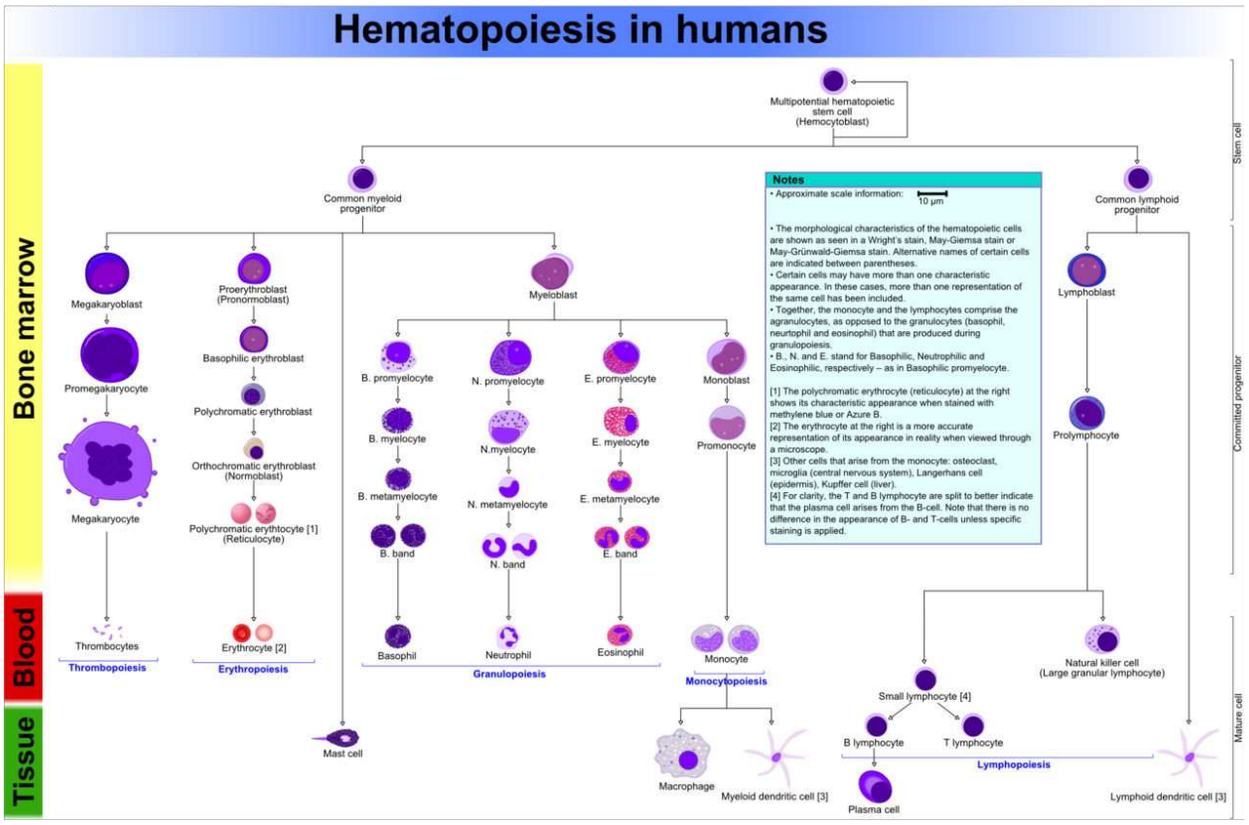

The diagram illustrates the hematopoiesis process that occurs in the bone marrow which gives rise to production of various cell lines from the marrow stem cell.

Figure 2. Production of red and white blood cells and platelets in the bone marrow [39]

nia) or any combination of these lineages (Table 6). The cause of these changes in HIV infection are not fully understood.

White blood cell	Normal Value (x10⁹/l) %		Reduce lineage
1. Neutrophil	2.0 – 7.0	40 – 80	Neutropaenia
2. Monocyte	0.2 – 1.0	2 – 10	Monocytopaenia
3. Eosinophil	0.02 – 0.5	1 – 6	
4. Basophil	0.02 – 0.1	<1 – 2	
5. Lymphocyte	1.0 – 3.0	20 - 40	Lymphopaenia

Table 6. Haematological abnormalities in HIV infection and normal adult reference values.

4.2. Haematological changes in HIV infection

Haematological abnormalities are common complications of HIV infection. These abnormalities increase as the disease advances. On both antiretroviral-treated and untreated individuals, different types of haematological abnormalities are common [41, 42, 43, 44] (Table 6).

Since the impact of HIV infection can be found in the peripheral blood and bone marrow, disorders of the haemopoietic system include anaemia, leucopenia, thrombocytopenia and thrombosis. These could be because of direct effects of the virus on the bone marrow, suppression of bone marrow by secondary infections or neoplasms causing ineffective haematopoiesis, nutritional deficiencies or side effects of the drugs used [44]. The disorders commonly occur throughout the course of HIV infection.

4.2.1. Blood abnormalities

The majority of the HIV cases present haematological abnormalities in the middle or advanced stages of the infection. However, some of the changes such as low haemoglobin and platelets have been reported in the early stages of HIV infection [44].

4.2.1.1. Anaemia

Anaemia refers to decrease in the haemoglobin (Hb) concentration with reference to healthy individuals of the same age group, sex, physiological state and environment (altitude).Normal haemoglobin for men is more than 13 g/dL while for women is 12 g/dL [38]. This can be classified based on the etiology or morphology of the red blood cell. The normal red blood cell shows a normochromic normocytic morphology. Anaemia is one of the commonest abnormalities seen in HIV, occurring in more than 50% of patients [40]. In a study done in HIV patients without myelosuppressive therapies, 8% of asymptomatic HIV-seropositive patients, 20% of those with symptomatic middle-stage HIV disease, and 71% of those with Centers for Disease Control (CDC)-defined AIDS were found to be anaemic [40]. Anaemia can be the earliest haematological manifestation, especially in children with HIV infection. Normochromic normocytic anaemia is the usual feature, but sometimes the HIV patients can present with a hypochromic microcytic anemia [45, 46, 47, 48, 49].

The causes of anemia in HIV patients are multifactorial. Inflammatory cytokines released by lymphocytes such as tumour necrosis factor (TNF), Interleukin-1 (IL-1) and interferon gamma play an important role in the pathogenesis of anaemia. These cytokines have been shown to inhibit red cell production (erythropoiesis) *in vitro* [51]. TNF levels were found to be consistently elevated in HIV infection and this condition is correlated with viral load [52]. Presence of dyserythropoiesis and opportunistic infections have also resulted in functional and morphological abnormalities of red blood cells [41]. This can alter the normal function of red blood cell as oxygen carrier or alter its normal biconcave shape. Other factors that contribute to the development of anaemia include underlying chronic disease, mixed nutritional deficiencies, opportunistic infections and side effects from the treatment [53]. As HIV disease progresses, the prevalence and severity of anaemia also increases [48, 54].

Disseminated Mycobacterium avium complex (MAC) disease may be present in HIV patients. It has been reported that about 76% of patients with this infection have severe anemia [52]. Another isolated red cell disorder, chronic pure red cell aplasia has been reported in HIV patients infected with parvovirus B19 [55]. This indicates that the underlying infection due to immunosuppression can give rise to anaemia.

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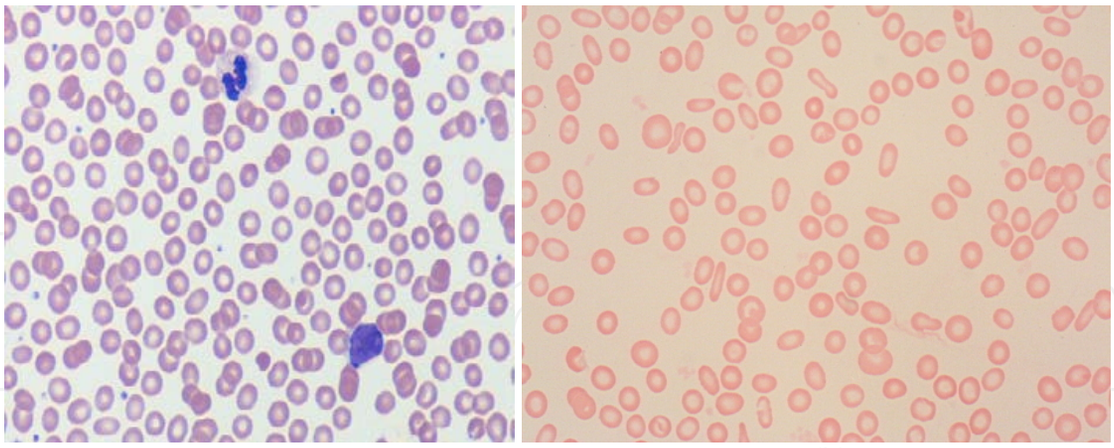

The peripheral blood smear in Figure 3(a) appears normal morphology, this condition is a normochromic, normocytic anemia. The utilization of iron is impaired due to a cytokine-mediated blockage in transfer of iron from the storage pool to the erythroid precursors in the bone marrow. The RBC's in Figure 3(b) appear smaller than the normal morphology and have an increased zone of central pallor. This feature shows a hypochromic (less hemoglobin in each RBC) and microcytic (smaller size of each RBC) anemia.

Figure 3. (a) Normochromic normocytic red cells and (b) Hypochromic microcytic red cells [50]

Nutritional anaemia in HIV patients frequently arises from an inadequate balanced diet intake and malabsorption. Infection and drug toxicity are common causes of gastrointestinal disease. Vitamin B12 deficiency is seen in up to one-third of HIV-positive subjects. Iron and folate deficiency are also common in this type of patients [38]. Bone marrow infiltration by tumour, such as lymphoma, is more common among HIV patients as compared to the normal population.The infiltration can suppress the production of red blood cells which can lead to anaemia. Anaemia is known to occur as an adverse effect of drug therapy for HIV infection or its complications. Myelosuppression can be caused by dose limiting toxicity of zidovudine [56]. Other drugs such as primaquine, dapsone and ganciclovir can lead to anaemia in HIV patients [52]. HIV patients with lymphoma on chemotherapy may present with anaemia due to the myelosuppressive treatment.

4.2.1.2. Thrombocytopaenia

Platelets are produced in the bone marrow. Normal platelet count is between 150 – 400 x 10^9/L. Reduction in the number of platelet count can be due to ineffective and/or reduced production of platelets in the bone marrow or increase destruction/ consumption of platelets in the peripheral blood. In HIV disease, thrombocytopaenia is the second most frequent hematological complication of HIV infection. It is found in 3% to 40% of individuals with HIV infection and could occur at any stage [57]. Presence of thrombocytopaenia is independent of the disease progression.

The mechanism of thrombocytopenia in HIV infection is mainly due to ineffective platelet production and at the same time increased platelet destruction [58]. There is a significant platelet sequestration and destruction in the spleen in HIV-associated thrombocytopenia.

Platelet destruction normally occurs early in the course of the disease. The destruction is often antibody mediated [59]. There are HIV-specific antibodies that have been shown to share a common epitope with antibodies against glycoprotein on the platelet surface (platelet GPIIb/IIIa) [59]. Nonspecific absorption of immune complexes onto platelets also occurs which predisposes the cell to immune thrombocytopaenia. Interestingly, there was a study that correlated the presence of lupus anticoagulant and anticardiolipin antibodies in HIV patients with the presence of thrombocytopaenia [60]. The other common cause of reduced platelet production in HIV patients is direct infection of megakaryocytes by the virus itself [61]. This gives rise to abnormal megakaryocytes morphology in the marrow. Other causes of thrombocytopenia include marrow infiltration by opportunistic infection or lymphoma, presence of complications such as thrombothic thrombocytopaenic purpura, and myelosuppressive effects of drug therapy.

4.2.1.3. Leucopaenia

Leucopaenia is the reduction in total white blood cell (WBC) count. In adults, normal WBC count is between 4.5 to 11.0 x 10^9/L. Leucopaenia is frequently seen in HIV patients and predominantly due to lymphopenia, decrease in the number of lymphocyte count, mainly $CD4^+$ lymphocytes. Leucopaenia generally correlates with the disease progression in HIV patients [62]. Reduction in absolute number of $CD4^+$ T-cells occurs as one of the earliest immunologic abnormalities of HIV infection and is one of the important prognostic indicators of risk of developing opportunistic infections.

Production of granulocytes and monocytes is also reduced, but less well recognized feature as compared to lymphopaenia. Occurance of neutropaenia is hinged on several other factors which are commonly seen in patients with advanced HIV disease. It can also occur due to concurrent infections, immune mediated or therapy related factors. Another cause of neutropaenia might be decreased bone marrow production of granulocytes due to inhibition of granulocyte progenitors. It has been postulated that a glycoprotein present in the marrow of infected patients might have an inhibitory effect [62]. Despite cellularity changes, morphological changes may occur in HIV patients. The changes are mainly due to dysplasia [38]. Peripheral blood smear will show some neutrophil changes such as detached nuclear fragments, abnormal nuclear fragmentation either hypofragmentation or hyperfragmentation, and abnormal nuclear granulation.

4.2.1.4. Haematological changes in HIV infection with correlation to CD4 cell count

In 2012, Parinithia and Kulkarni had done a study among 250 HIV patients to determine the haematological changes that occur in HIV patients as well as to evaluate its correlation with the CD4 cell count. They reported that among the HIV patients studied, anaemia, lymphopenia and thrombocytopenia was found in 210 (84%), 163 (65.2%) and 45 (18%) cases respectively [61]. Majority of the cases (70%) had CD4 cell counts below 200 cells/mm^3, 54 cases (21.6%) had CD4 cell counts between 200 to 499 cells/mm^3 and in 21 cases (8.4%), the CD4 count is more than 500 cells/mm^3. In patients with CD4 cell counts less than 200 cells/mm^3, anaemia, leucopenia, lymphopenia and thrombocytopenia was observed in 91.4% 26.8%, 80% and 21.7%

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cases respectively [61]. This study revealed that there was a significant increase in the number of cases of anaemia and lymphopenia with decreasing CD4 cell counts. Thrombocytopenia was also seen but did not show significant increase with disease progression.

5. Bone marrow associated haematological abnormalities

Bone marrow abnormalities are frequently seen in HIV infected patients.However, these changes do not seem to be specific, but maybe typical for HIV patients. The most common findings are dysplasia affecting one or more cell lineages. Bone marrow examination is not routinely done in HIV infected patients. It is usually performed to evaluate peripheral cytopenias or when systemic infections or malignancies are suspected.

5.1. Cellular abnormalities

The cellularity of the bone marrow can be assessed based on trephine biopsy. It can be normal, reduced or increased, depending on the patients' condition. Normally the bone marrow will show normal or increased cellularity. However, the marrow cellularity does not always correlate with the peripheral blood findings. The commonly observed pancytopenia (reduction in the 3 major cell lineages, which are red cells, white cells and platelets) in the peripheral blood is often associated with an active marrow [45], suggesting dysmyelopoiesis or increased peripheral destruction. Other than cellularity, the morphology and function of the cells can be altered. These include presence of severe nutritional deficiencies in advanced stages of HIV infection, bone marrow suppression by opportunistic infections or neoplasm, underlying chronic and toxic side effects of antiretroviral compounds (or other medications used to treat the complications of HIV disease). Megaloblastic changes where the red cell series are macrocytic, are occasionally seen in the bone marrow aspiration of HIV patients and this may reflect myelodysplastic changes or concurrent effect of treatment [45]. There is possibility of HIV directly infecting the haematopoietic precursor cells and inhibiting their differentiation and maturation [45].

The increased number of plasma cells has been observed in some HIV patients. This may be related to repeated infections that always occur in immunocompromised patients. Haemophagocytosis is frequently seen in a bone marrow examination, especially in patients with CMV and herpes simplex infection. Features of increased macrophage activity may also be seen in tuberculosis [63] and histoplasmosis infection associated with HIV disease [64].

5.2. Opportunistic infections

An opportunistic infection is an infection caused by pathogens, particularly opportunistic pathogens, such as bacterial, viral, fungal or protozoal infections that usually do not cause disease in a healthy host with a healthy immune system. All HIV-infected individuals are in the immunosuppressive state. They are susceptible to a wide array of opportunistic infections and are at higher risk to pathogenic organisms that plague the general population [65]. Infectious agents reported to attack the bone marrow in patients with HIV include Mycobac-

terium avium complex, Mycobacterium tuberculosis, Mycobacterium xenopi and kansasi, Histoplasma, Cryptococcus, Toxoplasma, Cytomegalovirus and Pneumocystis carinii [66]. These infections may cause marrow changes either directly by the the organism itself or indirectly by causing reactive changes.

5.3. HAART and other medication that cause bone marrow changes

Introduction to highly active antiretroviral therapy (HAART) has resulted in a highly significant decline in mortality [67]. However, some of these drugs frequently cause haematologic toxicity. Several studies have shown that zidovudine and dideoxycytidine inhibit erythroid colony forming units (CFU-E) that are needed for erythroid formation and granulocyte macrophage colony forming units that is important for granulocyte formation [68]. Leucopenia can be seen in HIV patients treated with Ganciclovir. Pyrimethamine and sulfadiazine used in the treatment of toxoplasmosis cause leucopenia and thrombocytopenia. Chemotherapeutic agents used in the treatment of malignancies, especially lymphoma result in myelosuppression which is often dose limiting. Alpha interferon used in the treatment of Kaposi sarcoma in HIV patient is frequently associated with haematologic toxicity [69].

5.4. Lymphoma in HIV disease

Lymphoma is a group of diseases caused by malignant lymphocytes that accumulate in lymph nodes [37]. Lymphadenopathy or enlargement of the lymph nodes is the main clinical feature. It can be subdivided into Hodgkin's lymphoma and non-Hodgkin's lymphoma.

Patients with HIV disease have an increased incidence of lymphoma as compared to general population, especially diffuse non-Hodgkin's lymphoma subtype [70]. This is often of high grade lymphoma and mostly of B cell origin [70, 71]. The increased risk of lymphoma appears to be related to many factors, which are mainly related to a variety of genetic lesions, including infection by Epstein-Barr virus (EBV), c-myc gene rearrangement, bcl-6 gene rearrangement, ras gene mutations, and p53 mutations/deletions [72]. The malignant lymphoma, probably arises as a monoclonal outgrowth from a pool of proliferating B lymphocytes, which have been stimulated by the infective agents such as EBV and CMV.These opportunistic infections contribute to the pathogenesis of lymphoma more seen in HIV infection. Lymphoma in HIV patients tends to metastasize to brain or spread extranodal [73]. The relapse rate is high and overall patients will have a poor prognosis. However, with the introduction of HAART treatment, the risk of lymphoma has decreased and the clinical outcome improved [73].

6. Conclusion

The HIV epidemic clearly has broad and significant implications and impact on individuals infected and affected globally. Possibility of HIV patient developing severe HIV-related disease or not, depends on the degree of suppression of the immune system as well as the extensive reduction in the blood count. Therefore, it is important to identify those patients who are at risk of having the disease for proper assessment of infection and/or disease progression

and subsequent monitoring. Prompt and consistent treatment should be given to those who are early diagnosed and side effects or drug toxicity assessment clearly made for effective consideration of drug change or drug discontinuation. Recently, with the introduction of HAART most of the immunological and haematological complications has been reduced, though some patients still develop unpredictable complications.

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