DIAGNOSTICS UPDATE .COM

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From my Keyboard

I would like to acknowledge the wonderful assistance provided by Diagno-firm's Public Rela-tions Officer Carrie Tilbury in producing this issue of the newsletter and hope the help will always be available.

This year's World AIDS Campaign officially began on the 1st of December 2004 with the theme **"Women, girls, HIV and AIDS"** and the strap line **"Have you heard me today?"** The main focus this year is on how gender inequality in the world causes the spread of the HIV/AIDS epidemic and how to encourage people worldwide to address female vulnerability to HIV. Many women and girls are susceptible to HIV because of the high-risk behavior of others. Young women and girls are more vulnerable to HIV than males with studies showing that they can be 2.5 times more likely to be HIV infected than males. This higher vulnerability is due to inadequate knowledge about AIDS, insufficient access to HIV prevention services, inability to negotiate safer sex and a lack of female controlled HIV prevention methods. Women and girls are prone to sexual violence, which also accelerates the spread of HIV. Close to half of the 37.2 million adults living with HIV are women according to UNAIDS/WHO Report. Sub-Saharan Africa has 13.3 million women between 15-49 years being HIV positive which means 57% of adults living with HIV are women. The UNAIDS report indicates that in every single country in Africa women between the ages of 15-49 constitute over 50% of those infected, with Botswana sitting at 58%. Equality for women will help fight the HIV/AIDS epidemic. Without a change in attitude towards girls and women and those living with HIV/AIDS, the country will be overwhelmed by the epidemic and the government's efforts to fight the epidemic will continuously be undermined. ■

"Have you heard me today? please take action now and lets face the future together"

Munyaradzi Mangwendeza Ed.

Inside this Issue

People Queuing for Free HIV Testing on World AIDS Day in Gaborone

DIAGNOFIRM MEDICAL LABORATORIES

by SiLas Nunu

Recent Events @ Diagnofirm

Another quarter of the year has come. It's been quite a busy time for Diagnofirm as many events have taken place. Our social obligations during this period have been guided by some factors that play enormous roles in our present day life, that is are our religion and our health. We have been involved in AIDS campaigns as the internationally recognized AIDS day dawns upon us and we've also donated to the needy as is encouraged for before, during and after the Holy month of Ramadan and also to bring some Christmas cheer to our fellow community members.

For the commemoration of World AIDS day in Selebe Phikwe Diagnofirm was actively involved. The events of the day were organized and coordinated by ACHAP and MASA and Diagnofirm provided logistical material for voluntary HIV testing, provided T-shirts to commemorate the day and also provided education on HIV infection. All in all over 400 individuals were tested on the day making it a very successful outreach programme. 400 individuals were tested on the day making it a very successful outreach programme. Local MPs, ACHAP and private doctors and several other dignitaries attended the event.

Diagnofirm was also actively involved in the Gaborone World AIDS day commemoration held at Tsholofelo Park. In conjunction with BOMAID, Diagnofirm set up a tent in that offered free HIV testing and counseling. The testing was done by Diagnofirm's very own: Desire Mhlabi, Lesley Rahman and Blessing Kadira. The counseling was done by Diagnofirm nurse Faustina Sililo, together with BOMAID's Mavis and Tebogo. The aim was to make people aware of their status and thus be able to positively move forward with their lives regardless of their status after the counseling. The day proved to be a resounding success as 198 people were tested and counseled.

In this quarter, Diagno-firm also donated some food hampers to destitute orphans at Segoditshane Primary School. The food hampers included essentials like rice, cooking oil, milk as well as nutritious body building foods. These foods were donated at an event held at

the school, which was attended by a Government ward councilor, Gaborone North Education Officer, school officials and Diagnofirm staff and management. This school also happens to be the home of one of Diagnofirm's adopted children David German.

Diagonfirm also donated similar food hampers to 200 Moshupa Community orphans and underpriviledged. The event was attended by Hon. Kgomotso Mogami, Kgosi Kwelabebe, the Moshupa community and Diagnofirm staff. ■

We @ Diagnofirm Medical laboratories wish you a MERRY AND SAFE CHRISTMAS and hope for your continued support in your PROS-PEROUS NEW YEAR.

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Tiger Design and Graphics

Patients' column

HIV MONITORING

HIV monitoring tests are used after a patient has been diagnosed as HIV positive so as to ascertain how the body is affected by the virus, and also to monitor the efficacy of any medication. The two most commonly used tests for HIV monitoring are the T-cell sub-sets enumeration (CD4 count) and the viral load test although some tests like full blood count (FBC), liver function test (LFT) and others are also used. The CD4 test is used to evaluate the immune status of the patient, to monitor HIV progression and helps the doctor decide as to when to commence antiretroviral (ARV) treatment. And the viral load test is used to monitor HIV viral multiplication progression and also to monitor therapy.

T – helper cell (CD4) count

TEST EXPLANATION - This test measures the number of CD4 cells (also known as T-helper cells) in your blood and assesses the status of your immune system. CD4 cells are a type of white blood cell, and they play an important role in one's immune system. They help to identify, attack, and destroy specific bacteria, fungi, and viruses that affect the body. CD4 cells are a major target for HIV, which binds to the surface of CD4 cells, enters them, and either

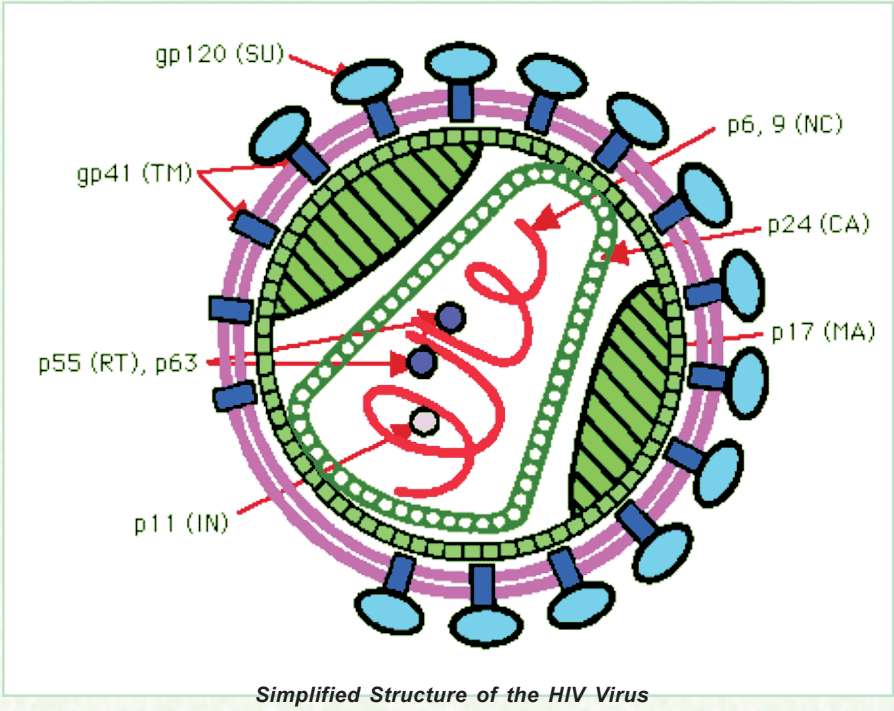
reproduces immediately, killing them in the process, or remains in a resting state, reproducing later. The number of CD4 cells in the blood gradually declines as HIV disease progresses.

TEST USE - The CD4 count is used to assess the strength of your immune system telling your doctor how strong your immune system is, how far HIV disease has advanced (the stage of the disease), and also helps predict the risk of complications and serious infections. The CD4 count is also used to identify possible health problems for which one may be at risk and to determine which medications might be helpful. The CD4 count is most useful when it is compared with the count obtained from an earlier test thus giving a comparative analysis of one's past and present health status.

Once one is diagnosed to be positive for HIV the doctor will request for a CD4 count so as to establish a baseline level. It is then measured at regular intervals e.g monthly or three monthly together with the Viral load test until the doctor feels the need for the commencement of antiretroviral therapy. After starting anti retroviral therapy CD4

Continued on page15

Understanding HIV Testing



Simplified Structure of the HIV Virus

HIV testing methods available:

1- ELISA – Enzyme linked immuno-sorbent assay
2- Western Blot assay
3- IFA
4- P24 antigen detection
5- PCR antigen detection

HIV testing can be done using any of the above tests. The standard HIV blood test does not look for the HIV itself but looks for the antibodies to HIV. Antibodies are made in the human body after it is exposed to HIV. However there is a window period after infection when the antibodies will not show up in the blood stream. Many people will have detectable antibodies in 3-4 weeks. Very rarely, a person can take up to 6 months to produce antibodies.

ELISA

These are very sensitive and inexpensive tests and are almost always used as the first screening tool. They very seldom give false results if the test is carried out outside the window period. They do however in 0.5-2% of cases give false positive reactions because of their great sensitivity. False positive results can occur if someone is tested soon after events that temporarily stimulate the immune system for example viral infections or immunizations. They could also occur because of laboratory errors or because of the test's high sensitivity. **For these reasons, a positive ELISA result must always be confirmed with a Western Blot or IFA test.**

Western blot

This is the test that detects the presence of antibodies which are chemicals produced by he body to fight off infections. Since it is a antibody test it will not be accurate until an HIV infected person seroconverts. Seroconversion describes the process by which the body reacts to the viral infection by trying to defend itself through production of antibodies. This process occurs 2-12 months after infection with HIV. This method is only performed if the ELISA is positive. False positive results are extremely rare since it detects specific antibodies to the HIV and hence s more specific than the ELISA test. Western blot is interpreted as positive if at least 2-3 bands i.e. p24, gp41 or gp 120/160 bands are positive. If only one band is positive, it is described indeterminate. When a test is indeterminate one needs to repeat both the ELISA and the Western Blot after 4-6 weeks until it becomes definitely positive or negative. Like the ELISA the Western blot is also negative during the window period and sometimes in the late phase of HIV infection. It cannot be relied upon for early diagnosis of HIV in the newborn, as it will detect maternal antibodies for as long as 18 months.

Continued on page11

By Carrie Tilbury

Human Immunodeficiency Virus (HIV) Infection

Symptoms

Human Immunodefiency Virus (HIV) progresses in stages. These stages are based on your symptoms and the amount of virus in your blood.

Initial stage

Flulike symptoms often appear within 3 to 6 weeks of initial exposure to virus, although symptoms can develop within just a few days. This first stage is called acute retroviral syndrome. Symptoms of acute retroviral syndrome are often mistaken for symptoms of another viral infection such as influenza, and may include:

- Abdominal cramps, nausea or vomiting
- Diarrhoea
- Enlarged lymph nodes in the neck, armpits and groin
- Fever
- Headache
- Muscle ache and joint pains
- Skin rash
- Sore throat
- Weight loss

What Happens

There are two types of viruses associated with human immunodeficiency virus (HIV)

- HIV – 1 which causes almost all the cases of AIDS worldwide
- HIV -2

How the disease is spread

HIV is spread when blood, semen or vaginal fluids from an infected person enter another person's body, usually through:

- Sexual contact. The virus may enter the body through a tear in the lining of the rectum, vagina,urethra or mouth. Worldwide between 75%-80% of all cases of HIV are transmitted by sexual contact.
- Infected blood . Hiv can be spread when a person
- Shares needles or syringes used for injecting illegal drugs
- Is accidentally stuck with a needle or sharp item contaminated with HIV

Treatment for HIV

The most effective treatment for HIV is highly active antiretroviral therapy (HAART) – a combination of several antiretroviral medications that aims to control the amount of virus in your body. Other treatment includes keeping your immune system strong, taking your medications as prescribed, and monitoring your CD4 (white blood cells) counts to show the multiplication of the virus in your body. If HIV is left untreated, it eventually progresses and can cause opportunistic infections such as TB, cancer and AIDS

Prevention

You can prevent HIV by avoiding behaviours that might result in contact with infected blood, semen or vaginal fluids

- Practice safe sex to prevent HIV. Always use a condom during sexual activity, unless you are in a long-term relationship with one partner who does not have HIV or other sex partners
- Do not have sex, including oral sex, with anyone who is infected with HIV. If you choose to continue to have sex with someone who has HIV, it is important to practice safe sex and to be regularly tested for HIV.
- Reduce your number of sex partners, preferably to one partner
- Ask your sex partner or partners about their sexual history. Find out whether your partner has engaged in high-risk behaviours
- Avoid anal or rough vaginal intercourse. Do not do anything that could tear the skin or moist lining of the genitals, anus, or mouth and cause bleeding
- Avoid alcohol and illicit drugs, which can impair both your judgement and your immune system. People who know and understand safer sex practices do not practice them when they are under the influence of alcohol or drugs
- Avoid sharing intravenous needles, syringes or any sharp article that may be used by others and be contaminated by HIV infected blood eg razors.

If you are infected with HIV or have engaged in sex or needle sharing with someone who could be infected with HIV:

- Tell your sex partner or partners about your behaviour
- Follow safer sex practices
- Do not donate blood, plasma, semen, body organs or tissue
- Do not share personal items such as toothbrushes, razors or sex toys that may be contaminated with blood, semen or vaginal fluids

When To Get Medical Help

Known HIV infection

If you are infected with HIV or caring for someone who is, get medical help immediately if any of the following conditions develop:

- Seizures
- Loss of consciousness
- New weakness in an arm, a leg or one side of the body
- New inability to move a body part (paralysis)
- New change in balance or sensation (numbness, tingling or pain)
- New inability to stand or walk

Go to your clinic if any of the following develop:

- Fever higher than 39.4°C
- Fever higher than 38.3°C for 24hrs
- Shortness of breath
- Cough that produces sputum
- Ongoing diarrhea
- Unusual bleeding, such as from the nose, gums or in the urine.
- Ongoing headache
- Changes in vision
- Rapid, unexplained weight loss
- Night sweats
- Fatigue
- Swelling of lymph nodes in the neck, groin or armpit
- Unusual sores on the skin or in the mouth
- Increased outbreak of coldsores
- Severe numbness of the hands or feet
- Sores, bumps, rashes, blisters or warts that appear on or around the genital or anal areas

Exams and Tests

HIV is diagnosed when antibodies to HIV are detected in the blood. Two primary blood tests used to detect the HIV antibodies are:

- ELISA
- Western Blot assay

It can take up to six months from the time you become infected with HIV for the antibodies to be detected in your blood. This is called the "window period". During the window period, you are contagious and can spread the virus to others. If you think you have been exposed to Hiv but you test negative for it, you should be tested again within 3-6 months.

Continued on page14

By Desire B. Mhlabi

BLEEDING DISORDERS – A GENERAL OVERVIEW

Bleeding disorders are medical problems that lead to poor blood clotting and continuous bleeding. These are also referred to as Coagulopathies, abnormal bleeding or clotting disorders. Blood clotting or coagulation is the process that controls bleeding and basically involves up to 20 different plasma proteins (clotting factors) to form Fibrin thus stopping bleeding

Symptoms

General symptoms of bleeding disorders include excessive bleeding, excessive bruising, easy bleeding, nosebleeds and abnormal menstrual bleeding.

Complications

Joint disease due to bleeding into joints, vision loss due to bleeding into eyes, chronic anemia due to excessive blood loss, neurologic/ psychiatric problems and eventually death.

Cause

Bleeding disorders can be inherited (family history) or acquired due to Vitamin K deficiency, severe liver disease, use of anticoagulant drugs and prolonged use of antibiotics.

We will take a brief look at the individual disorders.

Von Willebrand's disease

It is the hereditary deficiency or abnormality of the Von Willebrand factor in blood. Von Willebrand factor is the clotting protein in blood that

- Binds factor VIII protecting it from being destroyed
- Binds platelets and enables them to function normally in making the platelet plug and clot.

In this disorder blood platelets don't stick to holes in blood vessel walls as a result bleeding doesn't stop as quickly as it should, although it usually stops eventually.

Factor IX Deficiency (HEMOPHILIA B, Christmas disease)

Hemophilia B is a hereditary disorder in which the clotting ability of the blood is impaired and prolonged bleeding results. Small wounds and punctures are usually not a problem but uncontrolled internal bleeding can result in pain, swelling and permanent damage, especially to joints and muscles.

Inheritance Pattern; caused by an inherited sex-linked recessive trait with the defective gene located in the X chromosome. Females are therefore carriers of this trait. Fifty percent of the male offspring of female carriers will have the disease and 50% of their female offspring will be carriers. All female children of a male with hemophilia will be carriers of the trait. One fifth of all cases of hemophilia B occur when there is no family history of the disorder and its thought to develop as a result of a new or spontaneous gene mutation.

Factor VIII Deficiency (HEMOPHILIA A)

Hemophilia A often called classic hemophilia accounts for up to 80% of all hemophilia cases and is a deficiency in clotting factor VIII. Severity of symptoms varies and severe forms become apparent early on. Prolonged bleeding is the hallmark and typically occurs when an infant is circumcised. Mild cases may go unnoticed until later in life when there is excessive bleeding and clotting problems in response to surgery or trauma.

Inheritance Pattern; caused by an inherited sex-linked recessive trait with the defective gene located on the X chromosome. Females are carriers of this trait and the inheritance pattern is basically similar to Hemophilia B already described above.

Symptoms of both Hemophilia A and B include bruising, spontaneous bleeding, bleeding into joints, GIT or UT hemorrhage, blood in urine or stool and prolonged bleeding from cuts , tooth extraction and surgery.

Treatment; infusing the missing clotting factor treats Hemophiliacs. Hemophilia A was typically treated with infusion of cryoprecipitate or desmopressin acetate (DDAVP) which cause release of factor VIII that is stored within the body on the lining of blood vessels. A hepatitis B vaccine is recommended for individuals with hemophilia B because they are at increased risk of developing hepatitis due to exposure to blood products (factor IX concentrate). Hemophiliacs should avoid drugs that can aggravate bleeding like aspirin, heparin and warfarin.

We will now take a brief look at the other coagulation factors, their deficiencies and their overall contribution to the coagulation cascade.

Factor 1 (Fibrinogen) - Is responsible for platelet aggregation, which is the last step in the clotting process to *glue* the clot together.

Deficiency lead to combined bleeding and clotting disorders in which both platelets and clotting are abnormal. Congenital fibrinogen defects include afibrinogenaemia (lack of), hypofibrinog-enaemia (low levels) that are quantitative disorders and dysfibrinog-enaemia, which is a qualitative disorder. These disorders are however very rare.

Factor II (Prothrombin)

This is a Vitamin K dependant co-enzyme that functions in coagulation. Disorders involve congenital hypoprothrombinaemia and the acquired dysprothrombinaemia but are also very rare.

Factor V (Parahemophilia, Labile factor or Proaccelarin)

Accelerates the activity of thrombin hence low levels lead to delay in blood clotting. Disorders associated with factor V are very rare and a good example is Owren's disease.

Factor VII (Stable factor or Proaccelerin)

Deficiencies are extremely rare and the disorder can be inherited or acquired. Acquired disorder can be due to excessive use of drugs like *Coumarin*, which is used to inhibit blood clotting.

Factor X (Stuart Prower Factor)

Similar to factor II and disorders are very rare and usually due to vitamin K deficiency.

Factor XI (Hemophilia C or Plasma thromboplastin antecedent)

It is activated by thrombin and deficiencies lead to mild bleeding, typically provoked by surgery. Also called Rosenthal syndrome. Deficiencies are rare but common in Ashkenazi Jews (of eastern European ancestry).

Factor XII (Hageman factor)

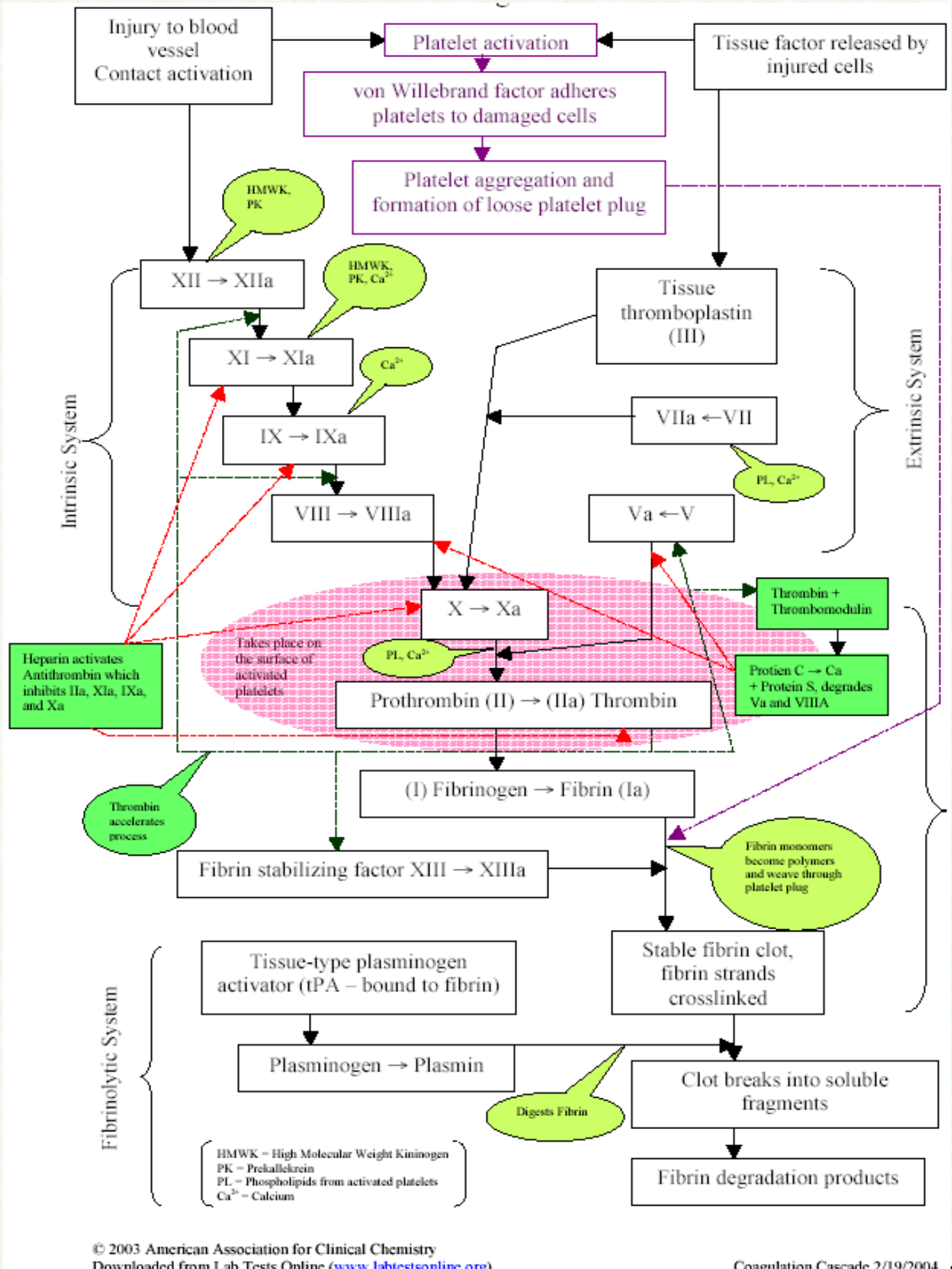
Deficiency does not cause abnormal bleeding.

Laboratory diagnosis of Bleeding Disorders

Prothrombin time (PT), activated partial thromboplastin time (APTT), prothrombin index (PI), its correction international normalized ratio (INR), Thrombin time, bleeding time and clotting time are some of the tests available in the overall diagnosis of bleeding disorders. PT evaluates the adequacy of the extrinsic pathway. It is also increased in bile duct obstruction, Liver cirrhosis, DIC, Hepatitis, vitamin K deficiency, warfarin therapy and deficiencies in factors VII, X, II, V, I. APTT detects deficiencies in the intrinsic pathway. It is also screens for certain conditions that cause abnormal clotting like antiphospholipid antibody syndrome or Lupus anticoagulant syndrome, also monitors heparin therapy. When both of them are prolonged then deficiencies in the common pathway are suspected. Thrombin time measures clotting time of the last step in the coagulation cascade (common pathway) with the conversion of fibrinogen to fibrin by thrombin. PI and INR are mainly used to monitor response to oral anticoagulant therapy

The figure below illustrates the interaction of the various coagulation factors in what is popularly called the Coagulation cascade.

Coagulation Cascade



Coagulation Cascade 2/19/2004

By Prof Bhagat

Aldosterone blockade and cardiovascular disease - A Fateful Alliance

The first novel finding discovered about aldosterone is that it is made locally in various different tissues in the body including the brain, vascular tissue, and the myocardium. In heart failure, myocardial tissue synthesises even more aldosterone. The second novel finding is that mineralocorticoid receptors which are activated by aldosterone are in fact also widespread in the body including the brain, vascular tissue, and the myocardium. This means that aldosterone may act in a paracrine fashion in many tissues—that is, locally made aldosterone may act on local aldosterone receptors to mediate local (mostly adverse) effects. This may be one of many reasons why baseline plasma aldosterone values appear to only poorly predict the efficacy of aldosterone blockade. Other reasons may also explain this (see below and table 2).

However, the main revolution in our new understanding of aldosterone is that it has now been shown to mediate a host of different adverse biological effects in the body, which have only been recognised in the last 10 years. These effects range from vascular endothelial dysfunction to inflammation to widespread tissue injury and repair. These were often first seen in animal models and then found also in man.

In vitro studies showed first that aldosterone reduced nitric oxide produced in response in inflammatory stimuli. In experimental animals in vivo, aldosterone was then found to produce a vascular inflammatory response which is characterised not only by increased expression of cytokines such as osteopontin, but also by tissue injury in several different organs (myocardium, brain, and kidney). In various different animal models, Rocha and colleagues have shown that aldosterone blockade reduces both tissue injury and tissue fibrosis in the myocardium, in the kidney, and in the brain. This tissue protection is seen even when aldosterone blockade is given at a dose too low to alter blood pressure—that is, the tissue protective effect of aldosterone blockade in experimental models is not simply due to its antihypertensive effect. A similar effect may also occur in man (see below) but more data are required before it can be fully accepted that aldosterone blockade specifically reduces tissue injury in man.

The vascular effects of aldosterone may be due to aldosterone increasing free radicals which then inactivate nitric oxide (NO). Such an effect on free radicals could be due to aldosterone increasing NAD(P)H oxidase activity which normally generates superoxide anions. In animal models of atherosclerosis, a similar effect is seen in that the specific aldosterone blocker, eplerenone, decreases NADH/NADPH oxidase dependent free radical production. Extending these observations further is a study showing that spironolactone reduces the p22phox subunit of NADH oxidase. Thus aldosterone may reduce NO bioactivity by increasing NADH oxidase induced free radical production which in turn degrades NO.

Another major effect of aldosterone in experimental animals is to produce tissue fibrosis. Intriguingly, in animal models, aldosterone only produces myocardial fibrosis when it is administered along with a high salt diet. How relevant a high salt diet is to aldosterone induced tissue damage in man is completely unknown, but salt status could be a key determinant of whether aldosterone produces tissue damage in man. Patients with heart failure all tend to have increased total body sodium as well as increased aldosterone concentrations and hence they resemble the animals studied by Weber et al who received both aldosterone and a high salt diet.

The biology of aldosterone has turned out to be amazingly complex and many surprising features have arisen with regard to the new biology of aldosterone. Scientists are trying to shed further light on these complex issues. In order for the clinician to gain some insight into these complex issues, I will give a brief overview of a couple of the intriguing issues currently taxing basic scientists involved in aldosterone research. Each of these basic issues may have clinical implications.

The traditional mineralocorticoid receptor appears not to mediate all the effects of aldosterone. Aldosterone stimulation of the traditional mineralocorticoid receptor in the cytoplasm exerts its biological effect slowly (after 1–2 hours) by way of stimulating transcription, translation, and the expression of new proteins. In addition to this traditional route, aldosterone has been shown to exert some rapid effects which are considered non-genomic because they occur within minutes and within a timescale which could not possibly involve transcription or translation. The intriguing thing about these fast, non-genomic effects of aldosterone are that they are not exerted through the traditional mineralocorticoid receptor and hence drugs such as spironolactone do not prevent them. It is currently unclear which biological effects of aldosterone occur by the traditional mineralocorticoid receptor and which occur by the fast, non-genomic route. Most of the effects described below do appear to occur by the former traditional route.

The second unusual feature about aldosterone biology is that the traditional mineralocorticoid receptor is actually stimulated equally by glucocorticoids (cortisol) and mineralocorticoids (aldosterone). The added twist which makes this intriguing is that plasma cortisol concentrations are generally about 10 times as high as plasma aldosterone concentrations. This means that one would expect the traditional mineralocorticoid receptor to be stimulated more in vivo by cortisol than by aldosterone. What prevents this happening is a bizarre arrangement whereby an enzyme called 11ß hydroxysteroid dehydrogenase (11 ßHSD) sits close to the mineralocorticoid receptor and inactivates cortisol before it can stimulate the mineralocorticoid receptor. This enzyme does not inactivate aldosterone, which means that the mineralocorticoid receptor is in fact mainly stimulated in vivo by aldosterone because this enzyme inactivates cortisol which tries to bind to the mineralocorticoid receptor. Not only is this arrangement bizarre, but it could be of clinical relevance. Some diseases might alter activity of 11 ßHSD and hence alter the normal selectivity of the mineralocorticoid receptor for mineralocorticoids over glycocorticoids. For example, in experimental diabetes, the activity of this enzyme in the kidneys is reduced which could lead to more stimulation of the mineralocorticoid receptor by glucocorticoids. The other possible clinical consequence could be that plasma aldosterone values may not be any indication of the efficacy of aldosterone blockade because the mineralocorticoid receptor can be occupied and activated also by cortisol in certain circumstances (table 2). Indeed the fact that eplerenone reduces blood pressure equally in all types of hypertension (low renin and high renin) may be an example of this very fact (although other explanations are possible) (table 2).

An intense clinical research effort has also revealed a host of new adverse effects produced by aldosterone (table 1). These in general support most of the experimental data described above.

Table 1
The new biology of aldosterone

- Aldosterone synthesis is widespread in the body outside of the adrenal cortex
- Aldosterone receptors are widespread in the body as well

as traditional renal receptors
Aldosterone produces endothelial vascular dysfunction
- Aldosterone produces tissue injury and fibrosis—seen in the myocardium, kidney, and cerebral tissues
- Aldosterone produces baroreceptor dysfunction

Endothelial dysfunction

The endothelium plays a critical role in regulation of vascular tone, platelet aggregation, adhesion of leucocytes, and thrombosis. A growing body of evidence suggests that aldosterone can cause endothelial dysfunction which then makes the vessel "sticky" and prone to microthrombi. Because of this endothelial dysfunction is now recognised as an excellent predictor of future cardiovascular events.

The clearest indication of this is that in normal human volunteers an infusion of aldosterone which does not alter blood pressure does in fact produce endothelial dysfunction. This phenomenon has been called "aldosterone induced vasculopathy".

Before this, the first suggestion that aldosterone induced vasculopathy occurred in man had come from a study we did in chronic heart failure patients which showed that aldosterone blockade with spironolactone increased vascular NO bioactivity. In this study infusion of the NO synthase inhibitor L-NMMA resulted in significantly greater vasoconstriction in spironolactone treated patients compared to those in the placebo group, indicating that basal NO bioactivity had been increased by aldosterone blockade. In the same study, spironolactone was also associated with a significant increase in forearm blood flow in response to acetylcholine, but had no effect on blood flow in response to sodium nitroprusside, an endothelium independent vasodilator. These are exactly the findings one expects to see when a treatment (aldosterone blockade) improves endothelial function by increasing vascular NO bioactivity.

As mentioned above, in animal models, aldosterone induced vasculopathy has an inflammatory element—that is, it is more like an aldosterone induced vasculitis. However, there is as yet no firm evidence of aldosterone being pro-inflammatory in man. Indeed, we have assessed the effect of spironolactone on plasma concentrations of C reactive protein (CRP) (high sensitivity) in heart failure and found that spironolactone did not alter CRP values.

Myocardial fibrosis and cardiac remodeling

Aldosterone also contributes to the progression of heart failure by promoting perivascular and interstitial myocardial fibrosis. This has several different but important consequences. Firstly, it reduces the flexibility of myocardial tissue and could cause "diastolic dysfunction". Secondly, patchy myocardial fibrosis would also be expected to produce electrical inhomogeneity which would be arrhythmogenic. Such arrhythmogenicity may be further potentiated by the potassium and magnesium depletion induced by aldosterone. Illustrative of this is that ventricular ectopy on 24 hour ambulatory ECG falls when aldosterone blockade is administered.

The pro-fibrotic effects of aldosterone have been demonstrated at a cellular level, preclinically, and in several clinical settings. The first proof that aldosterone promotes myocardial fibrosis in man came when it was shown that spironolactone reduced plasma concentration of PIIINP in heart failure.10 PIIINP is procollagen type III amino terminal peptide and is an indirect marker of myocardial collagen turnover in man. A similar effect of spironolactone in reducing PIIINP was also seen in the RALES study.11 Indeed, the antifibrotic effect of spironolactone may partially explain the RALES result, since spironolactone only reduced mortality in RALES in those patients who had an above normal concentration of PIIINP.

The adverse effects of aldosterone on endothelial function could account partially for its pro-fibrotic action. Endothelial dysfunction could lead to microthrombi, tissue injury, and tissue microinfarction, which repairs itself as fibrosis. Whether aldosterone produces fibrosis directly or whether it acts via a vasculopathy induced injury of tissues is an intriguing and as yet unanswered question. A related adverse effect of aldosterone is that it produces adverse left ventricular (LV) remodelling. Spironolactone has been shown clearly to reduce LV dilatation and improve the LV ejection fraction. These effects should improve exercise capacity and reduce deaths caused by progressive heart failure.

Another potentially harmful effect of aldosterone is its ability to blunt the baroreflex response. Perfusing the carotid sinus directly with aldosterone has been shown to reduce maximum baroreceptor discharge, while in dogs chronic administration of aldosterone elevates the threshold for baroreflex activation and decreased peak discharge rate. The first demonstration of such an effect in man came when it was shown that aldosterone inhibits baroreflex sensitivity in normal human volunteers.

The main indication now for aldosterone blockade is heart failure. Like many neurohormones, plasma aldosterone concentrations are increased in heart failure. However, the advent of angiotensin converting enzyme (ACE) inhibitor treatment for congestive heart failure, led initially to the feeling that any adverse effects of aldosterone would be ameliorated by ACE inhibitors. It is, however, now apparent that although ACE inhibitors produce an acute fall in plasma aldosterone, the concentration of aldosterone gradually rises again, and indeed returns to baseline or higher in some patients: this phenomenon has been called "aldosterone escape".

Table 2

Reasons why baseline plasma aldosterone concentrations may not predict the response to aldosterone blockade

- Aldosterone acts locally in a paracrine fashion
- Some effects of aldosterone may occur by the fast, non-genomic route which is not blocked by traditional aldosterone receptor blockers
- Mineralocorticoid receptors can be stimulated by non-mineralocorticoids—for example, glucocorticoids.
- Some diseases decrease 11 ßHSD (11ß hydroxysteroid dehydrogenase) enzyme activity and hence encourage gluco-corticoid stimulation of mineralocorti-coid receptors
- Aldosterone blockade acts as neuro-hormonal blockers when aldosterone concentrations are high but as subtle natriuretic agents when aldosterone concentrations are low

Trial acronyms

EPHESUS: Eplerenone Part Acute Myocardial Infarction Heart Failure Efficacy and Survival Study

HOPE: Heart Outcomes Prevention Evaluation

RALES: Randomized Aldactone Evaluation Study

RESOLVD: Randomized Evaluation of Strategies for Left Ventricular Dysfunction

Aldosterone concentrations > 144 pg/ml were reported to occur in up to 40% of patients with symptomatic CHF14 despite use of an ACE inhibitor. In another study, six weeks of captopril produces only a mean fall of 20% in plasma aldosterone

concentrations. In this latter study, the aldosterone values varied greatly between patients, from 56 pmol/l to 1568 pmol/l, despite captopril treatment.

Even when an ACE inhibitor is given in combination with an angiotensin I receptor antagonist, aldosterone concentrations remain uncontrolled. In the RESOLVD pilot study, patients with CHF who were given both enalapril and candesartan had a significant fall in aldosterone at 17 weeks, but mean aldosterone concentrations had returned to baseline by 43 weeks even with maximum doses of both agents. The precise mechanism by which aldosterone values rise during ACE treatment is unclear. However, it is worth noting that angiotensin II is only one of many secretagogues for aldosterone. Another key secretagogue is potassium. Obviously ACE inhibitors increase potassium, and since potassium is a powerful secretagogue for aldosterone, this may be a major reason for aldosterone escape (fig 1). This means that it is probably impossible to neutralise aldosterone completely by blocking angiotensin II, because blocking angiotensin II is inevitably accompanied by a potassium increase which then increases aldosterone.

Figure 1

Mechanisms of aldosterone escape with angiotensin converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB) treatment. K, potassium

The main demonstration of aldosterone's harmful effects comes from two major prospective trials. In the RALES study, patients with severe CHF (New York Heart Association (NYHF) functional class III–IV) were randomised to receive spironolactone or placebo. The trial was discontinued early because after a mean follow up of 24 months, the relative risk (RR) of death was 0.70 (95% confidence interval (CI) 0.6 to 0.82; $p < 0.001$) among patients receiving spironolactone—that is, a 30% reduction in risk of death with aldosterone blockade. This reduction in mortality was accounted for by both a significant fall in deaths caused by progression of heart failure (RR 0.64, 95% CI 0.51 to 0.80; $p < 0.001$) and to sudden cardiovascular death (RR 0.71, 95% CI 0.54 to 0.95; $p = 0.02$). However, gynaecomastia or breast pain occurred more often in men receiving spironolactone than placebo (10% v 1%; $p < 0.001$) due to the drug's affinity for androgen receptors. The low dose of spironolactone used in the RALES study is said to have no apparent diuretic effect, as judged by a substudy where the sodium retention score was measured.

The reduction in sudden cardiovascular death seen in RALES could be due to many possible mechanisms, ranging from aldosterone worsening endothelial dysfunction and so increasing acute coronary events, to it having arrhythmogenic effects by promoting myo-cardial fibrosis and depleting potassium and magnesium.9 These mechanisms are illustrated in fig 2. The reduction in progressive heart failure deaths was presumably caused by spironolactone improving LV remodelling (see above).

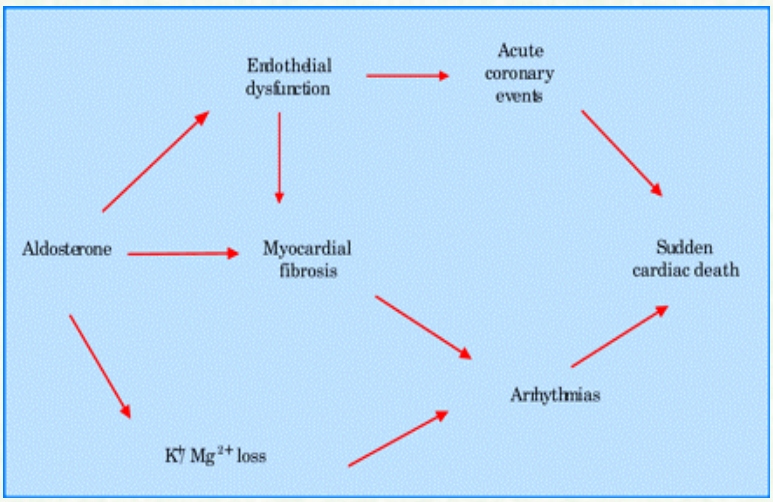


Fig. 1

Figure 2 Mechanisms whereby aldosterone promotes sudden cardiac death

More recently, the EPHESUS trial has evaluated use of the selective aldosterone blocker eplerenone in 6632 patients with acute myocardial infarction complicated by left ventricular dysfunction and heart failure. Severity of CHF was less pronounced than in RALES, with mean left ventricular ejection fraction of 33% compared to 25% in the RALES population. Pharmacotherapy also differed: most notably, 75% of patients received ß blockers versus approximately only 10% of those in RALES. During a mean follow up of 16 months, patients randomised to eplerenone had a 15% reduction in mortality compared to patients on placebo, and risk of hospitalisation for heart failure also fell by 15%. Similar to the RALES study, there was a large fall (21% fall) in sudden cardiac death. This indicates that the myocardial protective effect of aldosterone blockade is maintained even in the presence of optimal treatment and in patients close to the acute phase of myocardial infarction. Incidence of gynaecomastia and impotence did not differ between the eplerenone and placebo groups, due to the much lower affinity of eplerenone for androgen receptors.

The other main indication for aldosterone blockade is hypertension. Several published trials show that the selective aldosterone antagonist, eplerenone, reduces blood pressure in hypertension. The first point of note is that eplerenone appears to reduce blood pressure equally in all different subgroups of hypertension. Eplerenone also reduces blood pressure effectively when given in addition to an ACE inhibitor or angiotensin receptor blocker (ARB) treatment.18 The eplerenone induced reduction in systolic blood pressure was 13.4 mm Hg in those patients on ACE inhibitors and –16 mm Hg in those on ARB treatment, in comparison to –8/9 mm Hg in response to placebo. In a comparison with losartan, eplerenone reduced blood pressure similarly in white patients and to a greater extent than losartan in black

Continued on page15

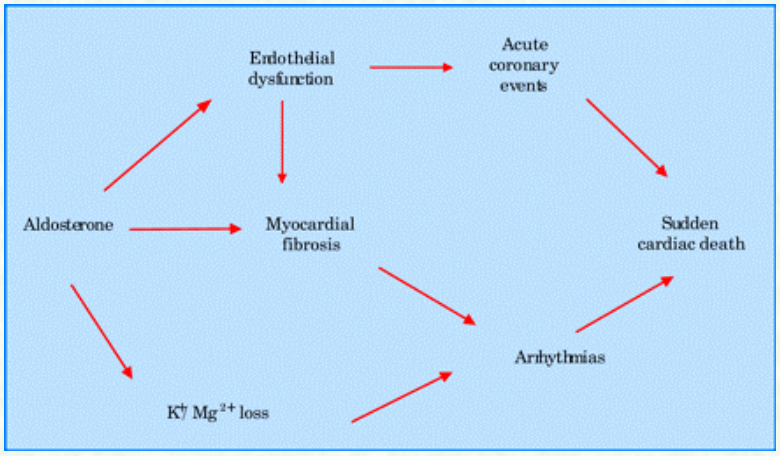


Fig. 2

Diagnofirm in Pictures

Free HIV Testing in Phikwe
Hon. M.P. Karis Kario Looks on.

Mr Chand and Diagnofirm staff members pose for photos with students from Segoditshane primary school.

Mr. I Chand with one of the beneficiaries of the food hamper donation.

Hon. Kgomotso Mogami hands over a diary to Kgosi Kwelakobe while Rita and Moonya look on.

From Left to Right: Mr. I Chand, Bozo, Desire, Peter and Maran.

Diagnofirm staff members with some of the orphans from Moshupa.

Mrs W. Chand hands over a food hamper to one of the students from Segoditshane primary school while Malebogo looks on.

Hon. Kgomotso Mogami with Diagnofirm staff members hand over a food hamper to an orphan from Moshupa.

Tips to prevent medicine errors:

Medicine errors can lead to hospitalizations, injury and death. This fact sheet tells you, the patient, how to prevent medicine errors.

1. **The single most important way you can help to prevent errors is to be an active participant in your own healthcare.**
   That means taking part in every decision about your health care. Research shows that patients who are more involved with their care tend to get better results.

2. **Make sure that all of your doctors know about everything you are taking.**
   This includes prescription and over-the-counter medicines, including dietary supplements such as vitamins and herbs. At least once a year, bring all of your medicines and supplements with you to your doctor.

3. **Make sure your doctor/dentist knows about any allergies and adverse reactions you have had to medicines.**
   This can help you avoid getting a medicine that can harm you.

4. **When your doctor writes you a prescription, make sure you can read it.**
   If you can't read your doctor's handwriting, your pharmacist might not be able to either.

5. **Ask for information about your medicines in terms you can understand – both when your medicines are prescribed and when you receive them.**
   - What is the medicine for?
   - How am I supposed to take it, and for how long?
   - What side effects are likely? What do I do if they occur?
   - Is this medicine safe to take with other medicines or dietary supplements I am taking?
   - What food, drink or activities should I avoid while taking this medicine?

6. **If you have any questions about the directions on your medicine labels, ask.**
   Medicine labels can be hard to

By Bozo and David

URINARY TRACT INFECTIONS(UTI)

The urinary system consists of the kidneys, ureters, bladder and urethra.The kidneys remove excess liquid and wastes from the blood in the form of urine, and maintains a stable balance of salts and other substances in the blood. Narrow tubes called ureters carry urine from the kidneys to the bladder - a triangle-shaped chamber in the lower abdomen.Urine is stored in the bladder and emptied through the urethra.

Normal urine is sterile.An infection occurs when microorganisms, usually bacteria from the digestive tract,cling to the opening of the urethra and begin to multiply.Most infections arise from one type of bacteria,Escherichia coli (E.coli) which normally lives in the colon.Other organisms that may cause UTI include Chlamydia,Klebsiella,Proteus and Staphylococcus aureus. An infection limited to the urethra is called urethritis. From the urethra bacteria can move upwards into the bladder,causing a bladder infection (cystitis). If the infection is not treated promptly, bacteria may then go up the ureters to infect the kidneys (pyelonephritis).

A common source of infection is catheters or tubes placed in the bladder. People with diabetes have a higher risk of a UTI because of changes in their immune system. Pregnant women are also more prone to UTI than other women.

How is UTI diagnosed ?

In the urinalysis test, a mid-stream urine is examined for white and red blood cells and bacteria. Then the bacteria are grown in a culture plate and tested against different antibiotics to see which drug best destroys the bacteria. This last step is called a sensitivity test. Chemistry analysis is also done.

It is recommended that a post treatment culture is carried to assess effectiveness of the treatment. If an infection does not clear up with treatment other tests may be perfomed e.g. X-ray tests.

Proof of a urinary tract infection should yield numbers greater than 100 000/ml otherwise the growth will be reported as insignificant.

The table below shows the sensitivity pattern of E.coli for the period from 01.01.2004 to 31.10.2004. A total number of 2936 samples were processed of which 1220 were from the male patients and 1716 were from female patients.

It can be observed that there is an increase in resistant strains of E.coli as indicated by Chloramphenicol 14%, Augmentin 7% and Piperacillin 9%.

The common causes of increase in resistance can be attributed to the following

1. Unnecessary use of antibiotics by humans.
2. Availability of antibiotics over-the-counter in Botswana.
3. Misuse by health professionals.
4. Patient failure to follow prescribed course of treatment.
5. Wrong dosages of prescriptions by clinicians.

Comparative Antibiogram Sensitivity Report - ECOLI

	Resistant	*Susceptible*
AN: Amikacim	1%	99 %
AUG:Augmentin	7%	93 %
C: Chloramphenicol	14%	86%
CAZ:Ceftazidime	3%	97 %
CEFEPIME	1%	99 %
CEFPROZIL	4%	96 %
CFC:Cefaclor	4%	96 %
CIP: Ciprofloxacin	4%	96 %
CRO:Ceftriaxone	1%	99 %
CXM: Cefuroxime	3%	97 %
F/M: Nitrofuratoin	3%	97 %
GAT : Gatifloxacin	2%	98 %
GM: Gentamicin	1%	99 %
INN : Levofloxacin	4%	96 %
NOR: Norfloxacin	4%	96 %
PIP: Piperacillin	9%	90 %

Continued from page 2

Understanding HIV Testing

HIV Testing Selebi Phikwe

INDIRECT IMMUNO - FLUORESCENCE ASSAY

The IFA can be used instead of the Western Blot to confirm ELISA results. Like the Western Blot it uses a blood sample and is faster than the Western Blot and more expensive. Although HIV antibody tests are the most appropriate for identifying infection, alternate technologies can contribute to an accurate diagnosis, assist in monitoring the response to therapy and an be used to effectively predict disease outcome. Viral isolation through viral culture, nucleic acid tests to detect viral RNA and tests to detect p24 antigen can be used to demonstrate virus or viral components in blood, thereby verifying infection, are some of the tests that can be used.

The p24 antigen assay measures the viral capsid (core) p24 protein in blood that is detectable earlier than HIV antibody during the acute infection. It occurs early after infection due to the initial burst of virus replication and is associated with high levels of viremia during which the individual is highly infectious. When antibodies to HIV become detectable, however, p24 antigen is often no longer detectable due to antigen-antibody complexing in blood. When detected, p24 antigen is highly specific for infection. Testing for p24 can be of value in (1) detecting early HIV infection (2) screening blood for transfusion (3) diagnosis of infection in the newborn. (4) Monitoring antiviral therapy. One disadvantage is that antibodies remain detectable throughout infection whereas p24 antigen characteristically appears early and late during infection.

PCR (Polymerised chain reaction) antigen detection

Two types are available i.e. PCR for DNA of provirus present in infected host cells and PCR for HIV RNA from plasma which detects very low levels of virus. Both are very useful for early diagnosis of HIV infection in the newborn. It is positive early during the window period and during the late phase of HIV infection when the ELISA is likely to be negative. Results are available in 24-48 hours. Quantitative PCR can be done to define the viral load in copies per millilitre of plasma. This is useful before starting therapy and monitoring response following anti-retroviral therapy. The only problem with PCR is its high cost and need for sophisticated laboratory equipment. Qualitative PCR determines the presence of the virus in blood.

CD4+ cell counts

CD4+ T cell counts, CD4+ T cell % and CD4/CD8 ratio are immunological tests done to determine the immune status. CD4+ cells are a type of white blood cells. Gradual decline in CD4+ T cell counts suggests disease progression. CD4 + % is more constant whereas absolute CD4 count is age dependant. It helps to prognosticate, monitor disease progression and helps to determine response and relapse following anti-retroviral therapy. A CD4+ cell count taken at the time of HIV diagnosis serves as the baseline which future CD4+ cell counts will be compared. CD4+ cell counts are useful for (1) evaluating the risk of developing opportunistic infection (2) deciding when to start treatment to prevent opportunistic infection (3) determining when to start anti-retroviral therapy. CD 4 + cell counts are monitored every 3-6 months depending on health status, previous CD4 + cell counts and whether one is taking HAART (Highly active anti-retroviral therapy)

Viral Load

This involves the measurement of HIV RNA in blood. Presence of the viral RNA indicates that the virus is actively replicating. It is normally first done when a person has tested positive for exposure to HIV based on an antibody test. Viral loads done during treatment can also detect viral breakthrough or increases in viral load that occur after a previous undetectable test result.

Drug Resistance testing.

When exposed to a variety of drugs HIV has the ability to change or mutate making it resistant to several drugs. Drug resistance testing is used to give the physician an idea of which medicines may work and which should be avoided. Two types of resistance testing are available i.e. genotypic testing and phenotypic testing. Genotypic testing looks at the virus that is present in the person's blood and sees what mutations exist. Certain drugs are known to cause certain genetic mutations. From knowing the genetic mutations present, doctors can deduce which drugs the virus may be resistant to. Phenotypic testing takes the virus and exposes it to different concentrations of drug to determine that drug's effectiveness. This is the method used early on in the development of new HIV drugs. Genotypic testing is fast and inexpensive and more widely available to patients than phenotypic testing which is not readily available in most laboratories. With resistance testing early therapies can be given according to which drugs will be effective against the already mutated strain of HIV. Drug resistance testing is therefore essential for quality HIV care.

Apart from the obvious HIV tests mentioned here, general chemistry tests like liver function tests, urea and creatinine as well as screens for infectious diseases are also very important. These are vital in monitoring the body's general health so that there can be timely interventions in case of opportunistic infections or other unforeseen diseases. ■

Tips to prevent medicine errors:

understand. For example, ask if "four doses daily" means taking a dose every 6 hours around the clock or just during regular waking hours.

7. **Ask your pharmacist for the best device to measure your liquid medicine. Also, ask questions if you're not sure how to use it.**
   Research shows that many people do not understand the right way to measure liquid medicines. For example, many use household teaspoons, which often do not hold a true teaspoon (5ml) of liquid.

8. **Ask for written information about the side effects your medicine could cause.**
   If you know what might happen, you will be better prepared if it does – or, if something unexpected happens instead. That way, you can report the problem right away and get help before it gets worse.

9. **Never share your medicine with other people.**
   Your medicine, given to someone else may have an adverse reaction or may even cause a fatal allergic response. The science of pharmacology is best left to doctors and pharmacists who are trained in drug inter reactions

10. **Always finish your prescribed antibiotics, do not stop because you are feeling better!**

Talkmore Maruta

HAEMATOLOGIC MANIFESTATIONS OF HIV INFECTION

The estimated worldwide prevalence of Human Immunodeficient Virus (HIV) infection topped 52.5million in June 2003, a mere 20years after the etiological agent was shown to be a sexually transmissible virus with a predilection for the CD4 T lymphocytes (8). A record 20million people have died from the condition in one generation making it the most devastating and persistent epidemic in recorded history (8).

Patients infected with the Human Immunodeficiency Virus (HIV) experience wide range of Hematological abnormalities. Some of the most commonly described abnormalities include impaired haematopoiesis, immune mediated cytopaenias, and altered coagulation mechanisms (1) These abnormalities can either be directly as a result of the HIV infection itself, indirectly as a result of HIV related opportunistic infections, malignancies or as a result of treatment i.e. drug toxicity. The above-described three abnormalities are commonly seen or encountered in the laboratory or in clinical practice as Anemia, Neutropaenia, Lymphopaenia and Thrombocytopaenia (2). In this issue we will focus mainly on anaemia in HIV infection more so because it imparts a range of dimensions of quality of life, most commonly fatigue.

In simple terms anaemia occurs when the Oxygen carrying pigment, Haemoglobin (Hb), contained in red blood cells is below normal. This comes as a consequence of the imbalance between red blood cell production in the bone marrow and their destruction in the spleen. In the laboratory the definition of anaemia uses Haemoglobin levels and Haematocrit (Hct) as the standards. Hb is normally in the range 16+/-2g/dl and 12+/-2gd/l in men and women respectively (1). Hct is a measure of that part of the whole blood that is made up of red blood cells i.e. percentage of the red blood cells in relation to total volume of blood. This normally ranges from 47+/-5% and 42+/-5% respectively (1). A look at your laboratory report which comes with its associated normal ranges will indicate whether you have anaemia or not. However, the severity of the anaemia or the degree of manifestation of symptoms will depend on how low your Hb is. World Health Organisation (WHO) came up with toxicity grades i.e. Anaemia grades as an indication of severity of your anaemia as shown below.

Table 1: WHO Anaemia Grades

Grade	Hb range (g /dL)
1	9.5 - 10.5
2	8.0 - 9.4
3	6.5 - 7.9
4	< 6.5

** Note: Upper limit may depend on laboratory (Adapted from AIDS review 2002; 4:13-20)*

WHAT ARE THE SYMPTOMS OF ANAEMIA

Generally, the symptoms of anaemia depends on how low your Hb is i.e. severity grade as shown in Table 1 above. A combination of symptoms, that may also be seen in other disease conditions, may indicate anaemia. It is therefore very important that you take a blood test in order to ascertain any suspicions. The symptoms of anaemia include

1. Fatigue - the most common dimension of mild anaemia (1)
2. Breathing difficulties on exercise
3. Dizziness
4. Palpitations
5. Angina (chest tightness on exercise)

However, it has to be noted that these general symptoms depends on various factors. These include severity grade (from table1), rapidity of onset, age, and physiological status of the patient (2). It all amounts to the fact that it is very important that you regularly do a blood test.

ANAEMIA - MORBIDITY AND MORTALITY IN HIV.

Several studies have been done and it has been established that anaemia is a very common finding in HIV infection, particularly in those in advanced disease. One study on HIV patients had the following findings (3)

Anaemia has been found to be an independent risk factor for disease progression and even death in patients with HIV infection (2). Three studies have been done, the Concord trial (4), the EuroSida (5), and the Chene G (6) trial and all showed that even a decrease of 1g/dl of Hb is associated with a significant increased risk of death. These associations appear to be consistent across all populations and in pre-HAART and HAART eras. Similarly the Multistate Adult and Adolescent Spectrum of HIV Disease Surveillance Project done in 9 US cities highlighted some information that is of particular important to us Laboratory Scientists and clinicians. In the 32 867 patients enrolled, Anaemia was associated with an increased risk of death and treatment of anaemia by Recombinant Human Erythropoietin and transfusion to get a 1g/dl rise in Hb or to improve a less than 10g/dl to greater than 10g/dl Hb improved survival. These findings remained constant even after correction for CD4 count. The same study also found out that of the patients whose baseline CD4 was less than 200 cells/mm3 and were anaemic had their risk of death 148% higher than the non anaemic ones. Those who managed to recover from the anaemia survived on average 18 months longer than those who could not recover from the anaemia. The EuroSida cohort (5) came out with some interesting revelations. It compared the strengths of CD4 count, HIV RNA (viral load) levels and Hb as predictors of the risk of death in HIV patients. For every 1g/dl decrease in Hb there was a 57% increase in the hazard of death. Patients whose CD4 was reduced by 50% had a 51% increased risk of death hand those whose viral load increased by 1 log had 37% increased risk of death. In other words, anaemia was a stronger predictor of risk than either of these well-established disease markers.

Such revelations could be very helpful in the management of HIV infection in poor communities who cannot afford the much more expensive tests as compared to a simple Full Blood Count (FBC). The above figures may look scary but as noticed, recovery from anaemia greatly improves your life as a HIV patient. It is therefore of paramount important that if any of the above described general symptoms are being experienced you

Group	% Anaemia
As symptomatic HIV - Pos	8
Symptomatic Middle - Stage HIV Disease	20
CDC Defined HIV	71

visit your local health facility that a blood test can be done at the Laboratory. Laboratory Scientists will easily pick up this silent but deadly condition found in HIV patients and the earlier you have it found out the better.

HOW DOES HIV CAUSE INFECTION

The pathogenesis of HIV infection is still far from being fully understood (7). Several researches and trials have been done but still it cannot be explained by direct effect of the virus on the haematopoietic system. However, people have come up with several possible etiologies supported by investigated scientific evidence. The main causes of anaemia in the HIV setting include (2)

1. Anaemia of chronic disease - This is usually in the form of erythropoietin deficiency (an enzyme from the kidneys that stimulate the bone marrow to synthesize red blood cells).

2. Bone Marrow infection - Mainly by parvovirus B19, Cytomegalovirus, Mycobacterium avium and cryptococcus neoformans.

3.Neoplasms - In simple terms this is abnormal growth of tissue and in the HIV setting it is mostly lymphomas.

4. Malnutrition and malarsoption

5. Myelosuppresive drugs e.g. AZT

6. Myelofibrosis

Infection by a bacterium called Mycobacterium avium is diagnosed in up to 18% of HIV patients in advanced HIV infection (CD4 less than 200cells/mm3)(1). In advanced infection, this bacterium causes a high-grade bacteremia (presents of bacteria in blood), which disseminates to various places such as the bone marrow; the place red blood cells are manufactured. Consequently the red blood cells production capacity of the bone marrow is reduced and anaemia is more pronounced than other cytopaenias (decrease in other cell lines like neutrophils lymphocytes etc). Infection by parvovirus B19 is not very common but it has to be noted that this has been recognised as a causative agent of anaemia in immunocompromised patients for a long time now (10). Furthermore, parvovirus DNA has been isolated from serum of HIV positive individuals with severe anaemia. Hence it has been associated with anaemia in HIV infection. Fortunately this type of anaemia has been successfully treated with infusions of immunoglubulin (400mg/kg/day for 5-10days)(10).

A number of medications used in HIV setting may contribute to anaemia. This can either be caused directly by the drug in question or its metabolites (degradation products). One of the most commonly used myelosuppressive drug in HIV infection is AZT and this has been known to contribute significantly to incidence of anaemia. Examination of the bone marrow of patients using AZT has shown pure red cell aplasia (failure to develop), erythroid maturation arrest (stopping the maturation of red blood cells) and megaloblastic erythropoiesis (synthesis of enlarged red cells)(1). AZT has been found to inhibit Hb synthesis by specifically inhibiting globin gene expression in erythroid progenitors (red blood cells precursors) therefore leading to markedly reduced red blood cell growth. (1) Furthermore, the by-products of AZT e.g. AMT have been shown to be toxic to the bone marrow cells particularly red blood cell precursors (1). This AMT has the capacity to encoperate itself into the genetic material (Nuclear DNA) of these early cells and has a down regulatory effect on the erythropoietin receptors of the bone marrow. Hence in the presents of erythropoietin, the bone marrow is not able to respond to it by increasing red blood cells production. . AZT has also been shown to have a toxic effect on mitochondrion (organelles inside cells where production of energy takes place).

However, the good news is that in the Highly Activated Anti Retroviral Treatment (HAART) era, anaemia in HIV has been shown to improve. Recent surveys have shown that erythropoietin deficient anaemias or other resistant anaemias common before have decreased in this HAART era. This has been attributed to several factors i.e. the good effects of HAART. There are some blood factors called cytokines especially interlukin-1, TNF, and interferons, that have been known to impair the functioning of erythropoietin the hormone that stimulate the synthesis of red blood cells by reducing the number of red blood cells precursors in the bone marrow. Now HAART generally diminishes these cytokines, hence its not surprising that such type of anaemia is less frequent in the HAART era (1). Additionally, HAART has greatly reduced the incidence of opportunistic infections, neoplasms and infection related nutrient malabsorption; the contribution of these as causatives of anaemia has also been reduced. But for this to work, it is highly recommended that as a patient you adhere to the instructions as to how, when and how often you should take your medications. Otherwise the beneficial effects will not be realised

So this is it, anaemia in HIV, and its impact on our lives. But this, as seen, can in a great way be improved mainly by you the patient - adherence to instructions on treatment as given by the clinicians and visiting health centres at prescribed times in order that a blood test be done to check the degree of severity of anaemia. Early intervention can improve or even save your life. In the next issue we will discuss other haematologic effects of HIV namely thrombocytopaenia, neutropaenia and lymphopaenia (decreased platelets, neutrophils and lymphocytes respectively).

Play safe. There is no yellow card when it comes to HIV. **CONDOMISE**

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The importance of adherence

The aim of this document is to help us fight against the adverse effects of HIV in our communities and families. A lot of information has already been inseminated into our lives concerning this condition. We all know how it is contracted and the ways of preventing it. Fortunately, because of science now there are drugs to help fight the effects of HIV in the infected individuals. However, sad to say, we still lose some people or some people seem not to benefit from these drugs as expected. This is not the Doctors' fault or anybody else's but of those infected.

Rule number one when it comes to Antiretrovirals is that infected individuals must never be put on ARV treatment until they are prepared to commit themselves to long-term therapy. They must be prepared to take treatment for life as prescribed and at the same time everyday. Failure to commit themselves to this results in failure in adherence hence giving rise to resistance and ultimately failure of treatment. This brings in complications in the sense that the drug regiment will have to changed. In-fact poor adherence is the number one cause of drug resistance. Adherence refers to the extent to which the patient continues the agreed-upon mode of treatment under limited supervision when faced with conflicting demands, as distinguished from compliance or maintenance.

Viruses in the system are always multiplying each day. They do their multiplication in the cells and infect new cells when released. This process goes on and on. The ARVs are present in the plasma where the released viruses would be. They then kill the viruses before they infect new cells and at the same time cut down on viral multiplication. It is important to note that killing of viruses only occurs at certain drug concentration levels called Inhibitory Concentrations (IC50).

When an individual take their medication, at that time the drug concentration levels in their blood rise and after some time begin to drop. The drug concentration levels drop gradually over a period of time. To avoid drug concentration levels far below the one which inhibit viral proliferation, one has to take their medication at their stipulated times. Dosing schedules are designed to maximize drug levels in the blood.

When drug level drop below the levels at which they kill viruses (IC50), the virus will make copies of itself and even make changes (mutations). These mutations can help the virus survive, even in the presence of ARVs. This is called resistance. When the HIV becomes resistant to the drug one would be taking, that drug will stop working leading to an increase in viral load and decrease in CD4s. When CD4s decrease then this will lead to deterioration of one's immune system. Resistance to one drug can sometimes also cause resistance to other drugs one will not have taken (cross-resistance). This can affect one's treatment options for the future by reducing the number of drugs that will work effectively against the virus. To prevent resistance it is therefore very important that one adheres closely to their medication schedule. Recent studies have shown that 95% adherence may be required to receive the most benefits from ARVs.

Having said all the above, one must realize that lack of adherence a greater percentage of the time is not deliberate. There are a number of factors that cause poor adherence and these are some of the following;

1. A busy lifestyle
2. Cultural beliefs
3. Current or past problems with side effects
4. Barriers in accessing treatment
5. Not having disclosed one's HIV status to work colleagues, friends, lovers, or family
6. Alcoholism or other active substances
7. Depression
8. Other life stresses like parenting and childcare

Despite all the above causes of non-adherence, one has to realize that adherence is hard work and takes a lot of commitment. Even though it may be embarrassing, it's important to tell the doctor about the number of times one will have missed a dose or did not take it correctly. The doctor may then suggest a change in your dosing schedule or drug regimen that makes it easier. While all of the HIV drugs can cause side effects, not everyone will experience them. If one experiences a side effect, do not just stop taking pills but follow the recommendations given by the doctor. Only when the problem persists should they speak to their doctor about other solutions including switching drugs.

There are other tips that we can consider to help improve adherence;

1. Believe that the medications will help fight the virus and stay well. If one does not think so, they won't bother taking the medication.
2. Make use of cellphone reminder programs to take note of dosing times if one has busy schedules.
3. If one does not want others to see them taking pills they must quietly slip to a secluded area. If that does not work then they can say the medication is for some other health problem.
4. Use daily activities like waking up or going to bed to remind you of taking the pills.
5. Ask the pharmacist or your doctor to explain exactly how to take the pills and give you written instructions.

The need for adherence can never be over emphasized if we want to reduce the effect of HIV in our system. As we adhere we suppress the multiplication of the virus and keep our immune system improving. Even when one is getting better they must never fail in adhering to their dosage schedule. ■

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Aid for Africa AFA HIV/AIDS workshop 31/07/04 – 01/08/04

Continued from page3

HIV - What Increases Your Risk

- **You are a man who has sex with men**
- **Have multiple sex partners**
- **Inject illegal drugs**
- **Have had a recent episode of a sexually transmitted disease**

Babies born to mothers infected with HIV also have an incrased risk of developing the infection.

The chance that you will become infected with HIV during sexual contact depends on how often you have sex with a partner who has HIV and the likelihood that the virus will be spread during each instance. The likelihood is influenced by:

- **Sexual practices, such as unprotected sex. Consistent use of condoms is the only way to prevent getting or spreading HIV during sexual contact**
- **Whether you have sores on your genitals or in your rectum. These may be caused by rough intercourse or sexually transmitted diseases.**
- **Whether you have open sores in your mouth, especially if you have oral sex with a partner who has HIV**
- **The stage of infection of your partner who has HIV. HIV may be spread more easily in the early stages, when the first flulike symptoms of HIV (acute retroviral syndrome) are present, and again later, when symptoms of HIV related illness are present.** ■

By Silas Nunu & Desire Mhabi

continued from Page 2

HIV Monitoring

count testing is then carried out every 3 – 4 months so as to assess the body's response to treatment.

SIGNIFICANCE OF TEST RESULT

Normal CD4 counts in adults range from 500 to 1,500 cells per cubic millimetre of blood. In general, the CD4 count goes down as HIV disease progresses. Any single CD4 count value may differ from the last one even though your health status has not changed. One should not place too much importance on any one result. What is more important than any single value is the pattern of CD4 counts over time.

If one's CD4 count declines over several months, the doctor may recommend beginning or changing antiretroviral treatment and/or starting preventive treatment for opportunistic infections like Pneumocystis carinii pneumonia (PCP). One's CD4 count should increase or stabilize in response to effective combination of antiretroviral therapy. According to public health guidelines, preventive therapy should be started when an HIV positive person who has no symptoms registers a CD4 count under 350. The recommended count in Botswana though is 200 and less. The Centres for Disease Control and Prevention considers HIV-infected persons who have CD4 counts below 200 to have AIDS, regardless of whether they are sick or well.

Viral load

TEST EXPLANATION - A viral load test estimates how much HIV is circulating in the blood system. The amount of HIV in one's blood is called a viral load. If the doctor knows one's viral load, they can establish what health risk is present according to the amount of HIV virus present. The viral load test helps the doctor decide the time for commencement of antiretroviral treatment.

On infection with HIV, the virus attacks the CD4 bearing white cells of the body. Once the virus has invaded the CD4 cells it will use them to multiply and then destroy the cells to infect others. The viral load test is thus ordered when a patient is first diagnosed with HIV. The test result functions as a baseline measurement that shows how actively the virus is reproducing and whether treatment is immediately necessary. According to research evidence it has been shown that maintaining a viral load as low as possible for a sustained period reduces complications associated with HIV and will prolong life.

TEST USE – In conjunction with the CD4 count test the viral load test is used to: monitor status of HIV disease; guide the course of therapy; and also to predict the course of the infection. For a patient who has been diagnosed with HIV infection but is not on treatment yet, the doctor will use the viral load result to establish whether the HIV circulating within the body is changing and if it is, by how much it is changing. For patients already on treatment the doctor will use the viral load result to assess the efficacy of the treatment on the virus and the body. The result will also be used to predict any resistances in the HIV that may arise.

SIGNIFICANCE OF TEST RESULT

The result is reported as the number of copies of the virus per millilitre of blood. Alternatively the test result can be reported as the logarythm of the copies per millilitre result. In the US, Public health guidelines state that treatment should be considered for asymptomatic HIV-infected people who have viral loads higher than 55,000 copies. A high viral load shows that the virus is actively multiplying and the disease will progress fast. The counts can go as high 15 million copies per millilitre (log 7.17).

Low counts indicate that HIV is not actively reproducing and that the risk of disease progression is low. Sometimes, depending on the sensitivity of the machine used to test, the result maybe "undetectable". For some machines this signifies a viral load of less than 200 or 50 copies/mL, but in our instance at Diagnofirm, this signifies viral copies of less than 25 per mL.C hange in viral load is also a very important measurement. A rising count indicates an infection that is getting worse, while a falling count indicates improvement and suppression of the HIV infection. Normally the increase or decrease in viral load is analysed as the logarithm of the copies/mL count. A change of greater than log 0.6 is considered significant. This will eliminate the possibilities of other elements causing the elevated or declined viral load.

Factors that affect HIV Viral Load and CD4 count

Factors that may lead to increased viral load include

1) Progressive disease
2) Failing ARV therapy
3) Active concomitant infections like active TB, pneumococcal pneumonia and influenza
4) Immunizations such as influenza and Pneumovax also falsely increase the viral load.

Failure to reduce viral load after commencement of ARV therapy suggests non-adherence, resistance, or inadequate

Dr. Bosch for ACHAP with Nicholas World AIDS Day Phikwe

therapy. Factors that may lead to an increased viral load are, active infection such as TB and flu, pneumococcal pneumonia, and immunizations such as influenza.
Factors that influence CD4 cell counts include

1) Analytical variation
2) Seasonal and diurnal variations
3) Some intercurrent illnesses
4) Corticosteroids therapy.

Substantial analytical variations which account for the wide range in normal values reflect the fact that the CD4 cell count is a product of three variables, namely white blood cell count, percentage lymphocytes and the percent CD4 cells. Diurnal changes have been seen to have lowest levels at 12:30pm and peak values at 8:30 pm hence the need for patients to **adhere to routine and convenient but consistent testing times**. Modest decreases in the CD4 cell count have been noted with some acute infections and with major surgery. Corticosteroid administration may have a profound effect (reduction) probably due to a redistribution of leukocytes between the peripheral circulation and the marrow, spleen and lymph nodes. Co-infection with Human T cell lymphocytic virus (HTLV-1) may result in a deceptively high CD4 cell count despite immune suppression. Both patients and clinicians need to abide by recommendations for timing test to ensure correct and accurate results on one testing episode. This in turn will reduce unnecessary repetitions of tests saving the laboratory money and the patient time and inconvenience. ■

Continued from page 8

Aldosterone blockade and cardiovascular disease - A Fateful Alliance

patients. The latter result is not too surprising since ARBs are generally less effective antihypertensives in blacks. Before this result, one might have thought that blacks would respond poorly to eplerenone because blacks tend to have low renin hypertension (which is why they respond poorly to angiotensin blockade). However, this was not seen here which emphasises that for some reason (see below), eplerenone appears to reduce blood pressure equally in all types of hypertension (young, old, black, white, low renin, high renin).

The second main feature about eplerenone in hypertension is that early data do indeed suggest the drug has a target organ protection effect. White and colleagues found in isolated systolic hypertension that eplerenone and amlodipine reduced the systolic blood pressure equally, but that eplerenone outperformed amlodipine in terms of reducing microalbuminuria. An effect of reducing microalbuminuria was also seen by Epstein and colleagues in diabetic hypertensives. Indeed, eplerenone added to enalapril reduced microalbuminuria more than either alone, although potassium needed to be carefully monitored when eplerenone was given together with an ACE inhibitor in diabetics with microalbuminuria. The third example of target organ protection with eplerenone was a study of hypertensives with LV hypertrophy where eplerenone and enalapril both reduced LV mass but more so when they were combined.

In all the above hypertension studies, eplerenone was generally well tolerated. Only in diabetics with microalbuminuria given eplerenone and an ACE inhibitor was there concern about the concentrations of potassium attained. Despite this, potassium and creatinine do need to be monitored after eplerenone in all patients as increases in both can occur in some patients.

The question why eplerenone reduces blood pressure equally in all types of hypertension is an intriguing one. One possibility is that in high renin patients eplerenone reduces blood pressure by acting as a neurohormonal antagonist, while in low renin patients a subtle natriuretic effect of eplerenone is the cause of the blood pressure reduction.

Interestingly hypertension experts tend to be divided as to whether the first antihypertensive drug to be used should be a thiazide or an ACE inhibitor. Eplerenone may act as a bit of both—a diuretic and a neurohormonal antagonist. This does not, however, mean that eplerenone will become a first line antihypertensive in the near future. It does mean, however, that it will be a useful add-on antihypertensive drug, producing a fairly predictable added hypotensive effect in all patients. In addition, it appears to especially protect target organs and it may be particularly beneficial to add to angiotensin inhibiting/blocking treatment in those patients with target organ damage in the form of LV hypertrophy or diabetic microalbuminuria.

Although heart failure and hypertension are the main clinical indications for aldosterone blockade, several other options ought to be explored in the future (table 3). There are good experimental data that aldosterone blockade reduces tissue injury in the myocardium, the kidney, and the brain. There are promising signs from clinical studies that aldosterone blockade may protect target organs, especially left ventricular hypertrophy and microalbuminuria. This suggests the possibility that aldosterone blockade may slow down the deterioration in renal function seen during progressive renal disease. This may occur over and above traditional ACE inhibition in this setting. The other major possibility is that aldosterone blockade will prevent future cardiovascular events in the type of vascular patients seen in the HOPE and EUROPA studies. Again such an effect may occur on top of traditional ACE inhibitor treatment.

The adverse effects of eplerenone and spironolactone may result in an increase in serum potassium concentrations. In clinical trials, the adverse effect profile of eplerenone given along or in combination with other antihypertensive medications was not significantly different from that of placebo, with the exception of this increased risk of hyperkalaemia. In EPHESUS, the incidence of serious hyperkalaemia, defined as a serum potassium concentration 6 mEq/l, was 5.5% in the eplerenone group and 3.9% in the placebo group ($p = 0.002$). In EPHESUS, this was counterbalanced by eplerenone reducing the incidence of hypokalaemia (13% to 8%).

The increased incidence of hyperkalaemia with eplerenone is similar to that seen with spironolactone. In RALES, the incidence of serious hyperkalaemia, defined as a serum potassium concentration > 5.5 mEq/l, increased with increasing dosages of spironolactone, from 5% with 12.5 mg/day to 13% with 25 mg/day, 20% with 50 mg/day, and 24% with 75 mg/day. As expected, the rate of sex hormone related adverse events has been much lower with eplerenone than with spironolactone. The incidence of gynaecomastia or breast pain was significantly greater in men receiving spironolactone compared with those receiving placebo (10% v 1%, respectively; $p < 0.001$). With eplerenone, on the other hand, sex hormone related events have been reported to be no greater than that seen with placebo.

The US Food and Drug Administration (FDA) have licensed eplerenone for use in hypertension but coincidental diabetic microalbuminuria was recommended by them to be a specific area for concern. This was because the incidence of hyperkalaemia was higher in this subgroup. To some extent, this was an odd decision by the FDA because eplerenone was particularly efficacious in this subgroup in terms of reducing microalbuminuria. However in this subgroup, the greater efficacy of eplerenone was indeed matched by a greater incidence of the adverse effect of hyperkalaemia. This is a good example of how in clinical therapeutics, benefit and risk sometimes go together. It might mean that in the future, a careful monitoring schedule could be devised so that in this subgroup of patients, the greater efficacy of eplerenone could be harnessed while also identifying those individuals who develop hyperkalaemia so that treatment in those individuals could be terminated.

A range of newly recognised harmful effects of aldosterone may contribute to its detrimental effect on cardiovascular events, in particular on sudden death. Apart from the well recognised effect of potassium and magnesium depletion, studies have shown that aldosterone promotes endothelial dysfunction and cardiac fibrosis, actions which would be expected to play an important part in promoting cardiac events, cardiac arrhythmias, and cardiac death.

We now have convincing clinical evidence that these adverse effects of aldosterone translate into increased mortality in patients with CHF. Two large scale prospective studies have reported a pronounced and significant benefit from use of an aldosterone blocker in terms of overall mortality, cardiovascular mortality (particularly sudden death), and hospitalisation. In one trial, these improvements were seen even when patients were already taking an ACE inhibitor and a ß blocker in addition to aldosterone blockade. Optimal treatment for patients with congestive heart failure should now routinely include an aldosterone blocker since two major trials clearly documented a highly significant reduction in total mortality by doing so. The only remaining question is how early in the heart failure disease process should we initiate aldosterone blockade.

Aldosterone blockade also reduces blood pressure in virtually all patients with hypertension and it is likely that eplerenone will become a very useful second or third line antihypertensive drug. Aldosterone blockade seems especially able to protect target organs (left ventricular hypertrophy and microalbuminuria) and may prove ultimately to be particularly useful (in addition to ACE inhibitors) in patients who already have these forms of hypertensive target organ damage.