VITAMIN A STATUS OF HIV-INFECTED ADULTS IN SOUTH AFRICA

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DECLARATION

I, Marianne Visser, hereby declare that the work on which this dissertation is based is my original work, and that neither the whole work nor any part of it has been, or is being, submitted for another degree in this or any other university.

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ABSTRACT

Introduction: Several studies in developed countries such as the USA have reported low serum or plasma vitamin A levels in adults with HIV disease. Limited data suggests that HIV-infected adults from developing countries show even lower vitamin A levels. Factors that contribute to a low vitamin A status include a poor intake, malabsorption and repeated episodes of infections resulting in a decreased hepatic mobilisation of vitamin A during the acute phase response, an accelerated utilisation of vitamin A or increased urinary losses of vitamin A. **Aim:** To determine the vitamin A status of HIV-infected adults without major active opportunistic infections with WHO clinical stages 1 to 4 HIV-infection. **Methods:** One hundred and thirty two HIV-positive patients were included in a cross-sectional study at the outpatient clinic at Groote Schuur Hospital. Exclusion criteria included current use of multivitamin or vitamin A supplements, pregnancy, pyrexia (> 38 ° C) and patients who had received TB treatment for less than 12 weeks. We obtained data on demographic characteristics, weight and height, CD4 lymphocyte levels, CD4:CD8 ratio, full blood count and plasma levels of retinol, retinol-binding protein, zinc and CRP. **Results:** The sample consisted of 51, 48 and 33 patients with WHO Stage 1/2, 3 and 4 HIV-infection, respectively. The proportion of patients with borderline vitamin A levels (< 30 μg/dl) for male and female subjects increased linearly across clinical stage categories. Thirty nine percent (20/51) of patients with early disease, 48% (23/48) with Stage 3 HIV-disease and 79% (26/33) of patients with

AIDS showed a borderline vitamin A status ($p < 0.001$). Plasma retinol status was associated with CD4 lymphocyte levels (r=0.27; 95% CI: 0.1-0.43) and the CD4:CD8 ratio (r=0.33; 95% CI: 0.1;0.42). Only one subject demonstrated CRP levels > 100 mg/l. Seventy seven percent (39/51) of patients with early disease had CRP levels < 10 mg/l, compared to 52% (25/48) and 58% (19/33) of patients with stage 3 and 4 HIV disease. CRP levels were divided according to 3 categories: < 10 mg/l, 10-40 mg/l and > 40 mg/l. The median retinol level of patients with CRP levels > 40 mg/l (n=7) was 16.8 μg/l versus 27.3 μg/l and 30.2 μg/l in the other two categories ($p < 0.05$). A similar relationship between CRP and plasma zinc levels was observed, although not significant ($p < 0.1$). Multivariate analysis revealed that a borderline retinol status was independently associated with a 3-fold increase (95%CI: 2-5.6) in the risk of having stage 4 disease or AIDS after adjusting for CD4 lymphocyte count or the CD4:CD8 ratio, haemoglobin, plasma zinc and body weight. **Conclusions:** Patients with advanced disease are more likely to have a borderline vitamin A status in the absence of opportunistic infections. The majority of patients with symptomatic disease had mildly raised CRP levels, possibly reflecting HIV-viral activity. CRP levels were associated with low retinol levels only in a small number of subjects, possibly indicating the presence of underlying infection, despite the clinical review of our data. Although our data indicates an independent relationship between retinol levels and advanced disease, the cross-sectional design precludes causal inferences about this association.

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1 Introduction

Factors affecting survival in patients with the Acquired Immunodeficiency Syndrome (AIDS) was recently reviewed by Mocroft et al. (1). In their response to the review, Baum et al. noted the author's omission of the role of micronutrient status in disease progression and survival. Baum et al concluded: "Although nutrient deficiencies are unlikely to be the principal cause of immune dysregulation in HIV-1 disease, these data indicate that they may be important cofactors and play a contributory role to influence the survival of HIV/AIDS patients" (2).

A number of specific micronutrient deficiencies in plasma or serum including vitamins A, B6, B12, E, copper, zinc and selenium have been reported in Human Immunodeficiency virus (HIV) infection and in AIDS (3,4,5,6,7).

Deficiencies of these micronutrients, as well as more global forms of protein-energy depletion, clearly influence various components of cell-mediated immunity such as T-lymphocyte proliferation, lymphocyte responses to mitogens and natural killer activity, that are also affected by HIV-infection (8).

Among the micronutrients, vitamin A and its metabolites are particularly important for T and B lymphocyte function, cellular differentiation and haematopoiesis (9,10,11). Vitamin A deficiency in HIV-disease has been associated with lower CD_4 lymphocyte counts and with an increased mortality in HIV-disease

(12,13,14). Factors that may result in a deficient vitamin A status in HIV infection include a decreased dietary intake, malabsorption due to diarrhoea and repeated episodes of infections resulting in a decreased mobilisation of hepatic reserves of vitamin A during the acute phase response, increased utilisation of vitamin A by target tissues or increased urinary losses of vitamin A during episodes of intercurrent infections (11,15,16). A wide range of reported vitamin A deficiency prevalence in HIV disease may reflect the inclusion of individuals at various stages of disease, those who have active opportunistic infections versus those who do not, or those taking vitamin supplements versus those who do not (6,12,13,14,17,18).

In conclusion, several studies have looked at the vitamin A status in adults with HIV disease in developed countries such as the USA. Little published data exists for developing countries. The prevalence of vitamin A deficiency in the HIV-positive population and in adult HIV-positive patients attending health facilities in South Africa is unknown. The ideal study design to investigate risk factors such as a low serum vitamin A status with the development of AIDS as endpoint or outcome measure, would be a prospective cohort study. Due to time constraints this was not feasible in terms of this dissertation,and therefore a cross-sectional study design was adopted.

References

1. Mocroft A; Johnson MA and Phillips. Factors affecting survival in patients with the acquired immunodeficiency syndrome. AIDS 1996; 10(10):1057-65.

2. Baum MK and Shor-Posner G. Nutritional status and survival in HIV-1 disease. AIDS 1997; 11(5):689-90.

3. Dworkin BM; Rosenthal WS; Wormser GP; Weiss L. Selenium deficiency in the acquired immunodeficiency syndrome. J Parenter Enteral Nutr 1986; 10:405-7.

4. Falutz J; Tsoukas C; Gold P. Zinc as a cofactor in human immunodeficiency virus-induced immunosuppression. JAMA 1988; 259: 2850-2851.

5. Baum MK; Mantero-Atienza e; Shor-Posner G ; Fletchner MA; Morgan R; Eisdorfer C et al. Association of Vitamin B_6 status with Parameters of Immune function in Early HIV-1 Infection. J Acquir Immune Def Syndr 1991; 4:1122-32.

6. Beach RS; Mantero-Atienza E; Shor-Posner G et al. Specific nutrient & abnormalities in asymptomatic HIV-1 infection. AIDS 1992; 6(7): 701-708.

7. Baum MK; Cassetti L; Bonvehi P, Shor-Posner G; Lu Y and Sauberlich H. Inadequate dietary intake and altered nutrition status in early HIV-1 infection. Nutr 1994; 10(1):16-20.

8. Chandra RK. 1990 McCollum Award Lecture. Nutrition and immunity: lessons from the past and new insights into the future. Am J Clin Nutr 1991; 53: 1087-1101.

9. Nuget J and Clark S. Retinoids, differentiation and disease. Ciba Foundation Symposium Sept 1984, London, Pitman Publishing 1985.

10. Hussey GD; Labadarios D. Vitamin A - beyond vision. CME 1992; 10(8): 1383-1391.

11. Semba RD. Vitamin A, Immunity and Infection. CID 1994:19:489-99.

12. Semba RD; Graham NMH; Caiaffa WT et al. Increased Mortality Associated With Vitamin A Deficiency During Human Immunodeficiency Virus Type 1 Infection. Arch Intern Med 1993; 153 : 2149- 2154.

13. Baum MK; Shor-Posner G; Lu Y et al. Micronutrients and HIV-1 disease progression. AIDS 1995; 9(9): 1051-6.

14. Semba RD; Caiffa WT; Graham NMH et al. Vitamin A deficiency and Wasting as Predictors of Mortality in Human Immunodeficiency Virus-Infected Injection Drug Users. J Infect Dis 1995; 171:1196-202.

15. Campos FACS; Flores H and Underwood B. Effect of an infection on vitamin A status of children as measured by the relative dose response (RDR). Am J Clin Nutr 1987; 46: 91-4.

16. Stephenson CB; Alvarez JO; Kohatsu J. et al. Vitamin A is excreted in the urine during acute infection. Am J Clin Nutr 1994; 60:388-92.

17. Ward BJ; Humphrey JH; Clement L et al. Vitamin A status in HIV infection. Nutr Res 1993; 13: 157-166.

18. Karter DL; Karter AJ; Yarrish R et al. Vitamin a deficiency in Non-Vitamin-Supplemented patients with AIDS: A Cross-Sectional Study. J Acquir Immune Def Syn 1995; 8: 199-203.

2 Aim and Objectives

2.1 Aim

To determine the serum vitamin A status amongst HIV-seropositive patients and its relationship with clinical stage and controlling for the effects of immune function and the acute phase response.

2.2 Objectives

The objectives of this study are:

(i) to compare the proportion of patients without major active opportunistic infections with low plasma retinol levels within WHO clinical stages 1 to 4 HIV-infection,

(ii) to determine the relationship between plasma retinol and CD4 lymphocyte levels and the CD_4:CD_8 lymphocyte ratio

(iii) to determine the relationship between plasma retinol and the acute phase response as reflected by plasma levels of C-reactive protein, zinc and retinol-binding protein (RBP) ;

(iv) to determine whether plasma retinol is independently associated with clinical stage 4 (AIDS) adjusting for potential confounding, intermediate and effect modifying variables. Variables that could be included are body mass index

(BMI), Total lymphocyte count (TLC), CD_4 lymphocyte levels, Haemoglobin, Haematocrit, CRP, RBP and zinc and

(v) to examine potential predictor variables of low plasma retinol levels which are clinically relevant such as WHO clinical stage, BMI, TLC, CD_4 lymphocyte levels, Haemoglobin, Haematocrit , CRP and zinc.

3 Literature Review

3.1 Vitamin A

Vitamin A has been described as the "anti-infective vitamin" since the 1920s (1). Today it is recognised as an essential micronutrient for immunity, cellular differentiation, maintenance of epithelial surfaces, haematopoiesis, growth, reproduction and vision (2,3).

Clinical vitamin A deficiency is characterised by clinical indicators such as night blindness and xeropthalmia. Subclinical deficiency is associated with reduced biochemical indicators such as serum retinol and liver retinol levels in the absence of clinical manifestations. The level of depletion at which physiological functions begin to be impaired is not entirely clear. What is known is that the integrity of epithelial barriers and the immune system are compromised before the visual system is affected (4).

3.2 Vitamin A, immunity and infection

Experimental studies indicate that vitamin A deficiency-induced immune abnormalities include atrophy of the thymus and spleen, reduced lymphocyte counts and lymphocyte mitogenic responses and impairment of macrophage function. Antibody affinity, production and response are also impaired. Vitamin A

deficiency also results in decreased cellular turnover, stratification of epithelial cells and ultimately squamous metaplasia, keratinisation and desquamation of cells (2).

The evidence that vitamin A is essential for immunity in humans has been summarised by Semba et al. based upon epidemiological observations and clinical trials as follows:

(i) Infectious diseases are associated with vitamin A deficiency;

(ii) Vitamin A deficiency is associated with increased morbidity and mortality from infectious diseases;

(iii) Specific immune alterations take place during vitamin A deficiency in humans;

(iv) Vitamin A and its metabolites are essential to T-and B-cell growth and function;

(v) Vitamin A supplementation enhances immunity in humans and

(vi) Vitamin A supplementation reduces morbidity and mortality from infectious diseases in children (3).

It should be noted that the effect of vitamin A deficiency on the immune system can be obscured by changes in immune responsiveness induced by accompanying protein-energy malnutrition (PEM). Scrimshaw et al. were the first to suggest an overall hypothesis of synergistic interaction between malnutrition and infection, largely based upon investigations among malnourished children in developing

countries (5). Human and experimental studies have confirmed that protein-energy malnutrition (PEM) and deficiencies of iron, vitamin B6 and zinc are all associated with a depressed cell mediated immune response (6,7).

In a study on vitamin-A-deficient children in which the confounding effect of PEM was reduced by the exclusion of acutely malnourished children according to anthropometric criteria, the vitamin-A-deficient children had lower CD_4 lymphocyte levels and a decreased CD_4: CD_8 lymphocyte ratio, compared to non-deficient children. High dose vitamin A supplementation reversed these abnormalities (8).

In a meta-analysis of 20 controlled trials of vitamin A supplementation for the prevention of death or morbidity from infectious disease, it was concluded that high dose vit. A supplementation reduced morbidity and mortality from infectious diseases such as measles, pneumonia and diarrhoea in children in developing countries. Results suggested a reduction in all cause mortality of 30% (95% CI: 21%; 38%) (9).

The biological mechanisms by which vitamin A affect morbidity and mortality are unclear. Two main types of mechanisms have been considered namely, the effects of vitamin A on epithelial integrity and on the immune system.

3.3 Vitamin A, infection and the acute-phase response

During infections serum retinol levels may drop due to decreased food intake, decreased absorption, decreased mobilisation of hepatic reserves of retinol during

the acute-phase response, an increased utilisation of retinol by target tissues and increased urinary losses of vitamin A (3,10,11). The inflammatory response to infection or the acute-phase response has been summarised by Koj & Gordon (1985) as "an early and unspecific highly complex reaction of the animal organism to a variety of injuries, such as bacterial or parasitic infection, mechanical or thermal trauma, malignant growth or ischaemic necrosis" (12). The systemic acute phase responses include fever, alterations in vascular permeability, leucocytosis, increased plasma copper, decreased plasma iron and zinc, increased glucocorticoid concentrations and changes in hepatic plasma proteins. Bacterial infection frequently gives a stronger stimulus to the acute-phase response than viral or parasitic infection. The acute-phase response still occurs in severe malnutrition, but is reduced (13).

"Positive acute-phase proteins" are those proteins whose serum concentrations increase during the acute phase response which include C-reactive protein (CRP), serum amyloid A protein (SAA), a_1-antitrypsin, a_1-acid glycoprotein, fibrinogen and caeroluplasmin. In contrast, "negative acute-phase proteins" are those proteins whose serum concentrations decrease which include albumin, pre-albumin, retinol-binding protein (RBP) and transferrin. The functions of the acute-phase proteins are not fully understood but include immunoregulation, removal of foreign material from the body, inhibition of proteases, binding of minerals and promotion of blood clotting (13,14).

During the acute phase response plasma zinc and iron levels decrease due to hepatic sequestration, whereas copper levels increase due to an increase in caeruloplasmin. Serum retinol levels have been negatively associated with markers of the acute-phase response such as elevated CRP levels. in adults undergoing orthopaedic surgery. and in Zimbabwean schoolchildren with schistosomiasis (15,16). Decrease retinol levels may be caused by a reduced mobilisation of RBP from the liver due to hypozincaemia, since RBP is a zinc-dependent protein. RBP may also be lowered because of increased vascular permeability during the systemic acute-phase response (2,13,14).

Although lowered retinol levels during the acute-phase response have been attributed to a reduced mobilisation of retinol from the liver, Campos et al. found that a single episode of chicken pox in children was associated with the depletion of liver vitamin A stores, suggesting that vitamin A requirements are increased during infection (10).

The effects of vitamin A supplementation on the acute phase response in Ghanaian children was investigated in a randomised, controlled trial. Acute-phase responses to fever and cough were not affected by supplementation. Children with vomiting or diarrhoea who received vitamin A supplementation demonstrated higher levels of acute-phase proteins (17).

The precise effect of the acute-phase response on the metabolism of vitamin A is not clear, but it is well documented that infections result in lowered serum retinol

levels and hepatic vitamin A stores. In view of the close relationship between the availability of vitamin A and immune effector cell function, low serum levels of vitamin A during infection may therefore have detrimental effects on the immune response (3).

3.4 Vitamin A status in HIV infection/AIDS

Factors that may result in a deficient vitamin A status in HIV infection include a poor nutritional intake, diarrhoeal disease or malabsorption, and repeated episodes of infection.

Studies in the USA have reported deficient serum vitamin A levels in HIV-1 seropositive individuals and in AIDS patients.

Beach et al. studied 100 asymptomatic HIV-positive homosexual men and 42 age-matched seronegative controls and found that the prevalence of vitamin A deficiency at baseline (defined as serum retinol levels less than 30 μg/dl) was 11% (18). Baum et al. using a similar cohort, found that the mean baseline serum retinol level of the HIV-positive men (43 ± 11 μg/dl) was significantly lower than the seronegative controls (51 ± 13 μg/dl), despite a very high intake of vitamin A in the HIV-positive men (19).

In a randomly selected sub-sample of 179 seropositive and seronegative individuals from a cohort of 2000 intravenous drug users, the prevalence of vitamin A deficiency in the seropositive group, was 15% (20).

In a study consisting of only 25 HIV-positive outpatients (8 symptomatic patients and 15 asymptomatic patients), patients with AIDS not taking a daily multivitamin supplement containing modest amounts of vitamin A, demonstrated the lowest serum retinol levels in the sample (21).

In a cross-sectional study amongst hospitalised AIDS patients not receiving vitamin A supplementation, 22% of patients had severe vitamin A deficiency (defined as serum retinol levels less than 20 μg/dl). Two subgroups were described with lower mean serum retinol levels compared to the mean serum retinol level of the sample, namely patients with diarrhoea and those with non-Pneumocystis carinii pneumonia. Karter et al. commented that 27% of patients with an adequate dietary vitamin A intake during the preceding 4 weeks before hospitalisation, still demonstrated deficient serum retinol levels (22).

Maternal vitamin A deficiency has been reported in 58% and 32% of seropositive pregnant women in Africa and in the USA, respectively. These high prevalences in pregnant seropositive women could be partly explained by the increased requirements for vitamin A by the developing foetus. Both studies suggested a four-to-five fold increased risk of mother-to-child transmission of HIV in those who were vitamin A deficient compared to those who were not deficient. The

increased risk of transmission may result from altered mucosal immunity, altered pathology of the reproductive tract, abnormalities of the placenta or increased foetal susceptibility to infection (23,24).

Thus, a wide range of reported vitamin A deficiency prevalence in HIV disease could reflect the inclusion of individuals at various stages of disease, those with or without active opportunistic infections, as well as the use of multivitamin or vitamin A supplements.

3.5 Vitamin A and the progression of HIV disease

The relatively high prevalence of micro-nutrient abnormalities in early HIV-infection has inspired interest in determining whether abnormalities such as vitamin A deficiency in particular, contribute to the progression of HIV disease to AIDS or to the survival of AIDS patients.

In a prospective cohort study of 281 HIV-positive men, Tang et al. investigated the association between the daily intake of micronutrients from food and supplements in early HIV-infection with the rate of progression to the development of AIDS. A Cox proportional hazards model was adjusted for energy intake, age, presence of HIV-symptoms, CD_4 lymphocyte count at baseline and the use of antiretrovirals and Pneumocystis carinii pneumonia prophylaxis. Smoking and alcohol intake were not included in the final model since neither were related to disease progression and therefore not considered as confounders. They showed that the relationship

between the dietary intake of vitamin A and the progression to AIDS was U-shaped, with the middle two quartiles of vitamin A intake being associated with significantly slower progression to AIDS (25).

In another prospective study in 296 HIV-seropositive men, Abrams et al. could not demonstrate any association between the dietary intake of 11 micronutrients at baseline and progression to AIDS after adjusting for energy intake, age, baseline CD_4 lymphocyte count, HIV-symptoms and smoking. A higher vitamin A intake was associated with higher CD_4 lymphocyte levels at baseline after controlling for age, smoking, symptoms and total energy intake (26). It should be noted that both these studies related the risk of progression to AIDS to dietary intake at baseline and the influence of subsequent dietary change was not evaluated.

Vitamin A deficiency (defined as serum retinol levels less than 1.05 μmol) predicted mortality in a sample of 179 seropositive and seronegative individuals from a cohort of intravenous drug users, after adjusting for CD_4 counts of less than 200 cells/ml and Hepatitis B surface antigen (RR=6.3; 95%CI:2.1;18.6). Current use and duration of IV drug use, antiretroviral treatment, alcohol consumption and history of diarrhoea were not significantly related to mortality using a proportional hazards model (20). A nested case control study within the same cohort on 50 AIDS patients who had died compared to 235 controls who had survived, showed that a 4-fold increase in the risk of death if vitamin A deficiency was recorded during their last study visit before death, after adjusting for CD_4 counts and body weight at their last visit (27).

Baum et al. examined the relationship between micronutrient status at baseline and over time with immunological markers of disease progression in a prospective study. They showed that the development of vitamin A deficiency was associated with a decline in CD_4 levels, while normalisation of serum vitamin A levels was associated with higher CD_4 cell counts (28).

Finally, another prospective study with 311 seroprevalent men was unable to show any association between serum vitamin A levels and disease progression, CD_4 cell decline or mortality. The absence of any significant finding was attributed to the vitamin A status of the cohort being in the normal to high range (median retinol level = 69.9 μg/dl) (Tang 1997).

References

1. Green HN; Mellanby E. Vitamin A as an anti-infective agent. BMJ 1928; 2:691-6.

2. Hussey GD; Labadarios D. Vitamin A - beyond vision. CME 1992; 10(8): 1383-1391.

3. Semba RD. Vitamin A, Immunity and Infection. CID 1994:19:489-99.

4. World Health Organisation. Global Prevalence of Vitamin A deficiency. Micronutrient Deficiency Information System Working Paper 2; 1995.

5. Scrimshaw NS; Taylor GJ; Gordon JS. Interactions of nutrition and infection. Am J Med Sci 1959; 237 : 367-381.

6. Beisel WR. Single nutrients and immunity. Am J Clin Nutr 1982; 35S:417-68.

7. Chandra RK. 1990 McCollum Award Lecture. Nutrition and immunity: lessons from the past and new insights into the future. Am J Clin Nutr 1991; 53: 1087-1101.

8. Semba R; Muhilal; Ward BJ et al. Abnormal T-cell subset proportions in vitamin A-deficient children. Lancet 1993; 341: 5-8.

9. Glasziou PP; Mackerras DE et al. Vitamin A supplementation in infectious disease: a meta-analysis. BMJ 1993; 306(6874): 366-70.

10. Campos FACS; Flores H and Underwood B. Effect of an infection on vitamin A status of children as measured by the relative dose response (RDR). Am J Clin Nutr 1987; 46: 91-4.

11. Stephenson CB; Alvarez JO; Kohatsu J. et al. Vitamin A is excreted in the urine during acute infection. Am J Clin Nutr 1994; 60:388-92.

12. Koj A and Gordon AH (ed). The Acute Phase Response to Injury and Infection. Amsterdam: Elsevier; 1985.

13. Raynes JG. Carbohydrate Binding Proteins and Immune responses. Biochem Soc Trans 1994; 22:69-74.

14. Fleck A. Clinical and nutritional aspects of changes in acute-phase proteins during inflammation. Proc Nutr Soc 1989; 48: 347 -354.

15. Louw JA; Werbeck A; Louw ME; Kotze T; Cooper R; Labadarios D. Blood vitamin concentrations during the acute-phase response. Crit Care Med 1992; 20(7): 934-41.

16. Friis H; Ndhlovu P; Kaondera K; Sandstrom B; Michaelsen KF; Vennervald BJ; et al. Serum concentration of micronutrients in relation to schistosomiasis and indicators of infection: a cross-sectional study amongst rural Zimbabwean schoolchildren. Eur J Clin Nutr 1996; 50(6):386-91.

17. Filteau SM; Morris SS; Raynes JG; Arthur P; Ross DA; Kirkwood BR et al. Vitamin A supplementation, morbidity, and serum acute-phase proteins in young Ghanaian children. Am J Clin Nutr 1995; 62: 434-8.

18. Beach RS; Mantero-Atienza E; Shor-Posner G et al. Specific nutrient & abnormalities in asymptomatic HIV-1 infection. AIDS 1992; 6(7): 701-708.

19. Baum M; Cassetti L; BonvehiP; Shor-Posner G; Lu Y and Sauberlich H. Inadequate Dietary intake and Altered Nutrition Status in Early HIV-1 Infection.Nutrition 1994; 10(1):16-20.

20. Semba RD; Graham NMH; Caiaffa WT et al. Increased Mortality Associated With Vitamin A Deficiency During Human Immunodeficiency Virus Type 1 Infection. Arch Intern Med 1993; 153 : 2149- 2154.

21. Ward BJ; Humphrey JH; Clement L et al. Vitamin A status in HIV infection. Nutr Res 1993; 13: 157-166.

22. Karter DL; Karter AJ; Yarrish R et al. Vitamin a deficiency in Non-Vitamin-Supplemented patients with AIDS: A Cross-Sectional Study. J Acquir Immune Def Syn 1995; 8: 199-203.

23. Semba RD; Miotti PG; Chiphangwi JD et al. Maternal vitamin A deficiency and mother-to-child transmission of HIV-1. Lancet 1994; 343: 1593-1597.

24. Greenberg BL; Semba RD; Vink PE; Farley JJ; Sivapalasingam M; Steketee RW et al. Vitamin A deficiency and maternal-infant transmission of HIV in two metropolitan areas in the United States. AIDS 1997; 11:325-32.

25. Tang AM; Graham NMH; Kirby AJ et al. Dietary Micronutrient Intake and Risk of Progression to Acquired Immunodeficiency syndrome (AIDS) in Human Immunodeficiency Virus Type 1 (HIV-1) - infected Homosexual Men. Am J Epi 1993; 138(11): 937-951.

26. Abrams B; Duncan D; Hertz-Picciotto I. A Prospective Study of dietary intake and Acquired Immunodeficiency Syndrome in HIV-seropositive Homosexual Men. J Acquir Immune Def Syn 1993; 6: 949-958.

27. Semba RD; Caiffa WT; Graham NMH et al. Vitamin A deficiency and Wasting as Predictors of Mortality in Human Immunodeficiency Virus-Infected Injection Drug Users. J Infect Dis 1995; 171:1196-202.

28. Baum MK; Shor-Posner G; Lu Y et al. Micronutrients and HIV-1 disease progression. AIDS 1995; 9(9): 1051-6.

29. Tang AM; Graham NMH; Semba RD and Saah AJ. Association between vitamin A and E levels and HIV-1 disease progression. AIDS 1997; 11:613-20.

4 Methods

4.1 Study design

A cross-sectional study.

4.2 Time frame

October 1995 to October 1996.

4.3 Study population

Male and female HIV-antibody-positive patients with heterosexually acquired HIV-infection, attending the Groote Schuur Hospital HIV-outpatient clinic in whom routine blood tests were requested by the attending physician. HIV-seropositivity was determined according to WHO guidelines by means of three positive enzyme-linked immunosorbent assays (ELISAs) that uses different antigens and have differing test principles (1).

Exclusion criteria:

- less than 18 years of age
- active major opportunistic infection (requiring hospitalisation)
- pyrexia > 38.0 °C at enrolment

- patients who had received TB treatment for less than 12 weeks
- clinical diagnosis of liver cirrhosis
- anti-retroviral therapy e.g. AZT
- any multivitamin or vitamin A supplements during the preceding 6 months
- pregnancy (patients were asked re. the date of their last menstrual period)
- prisoners

4.4 Sample size

One study in the USA reported the proportion of AIDS patients with low serum retinol levels who were not receiving vitamin supplements as 22% (2). In the current study, the proportion of patients with WHO Clinical Stage IV (AIDS) with low serum vitamin A levels was estimated at 40%.

Clinic attendance records showed that attendance was evenly distributed amongst patients within clinical stages 1 and 2, clinical stage 3 and 4 HIV-infection. The Chi-square test for trend was used to derive different scenarios, assuming that the proportion of patients with a low serum vitamin A status differed between the clinical strata by greater or equal to 10%.

Binomial distribution : $n = (100/D)^2 \times 1.96^2 \times p(1-p)$

P Clinical Stage 1+2	p Clinical Stage 3	p Clinical Stage 4	D	N	χ^2_{trend}
0.2	0.3	0.4	0.075	143	4.3 (p=0.0381)
0.2	0.3	0.4	0.05	322	10.43 (p=0.0012)

A sample size of 322 subjects was not attainable within the specified time period, since this is much larger than the annual patient attendance at the Groote Schuur Hospital Outpatient Clinic. Due to methodological reasons it was decided not to include other outpatient clinics in the present study. The required sample size at a precision level of 7.5% is 143 subjects.

4.5 Measurements

4.5.1 Clinical Stage

Patients were classified according to the WHO Staging system for the classification of HIV disease. The WHO Staging system consists of both a clinical and a laboratory axis. The clinical axis is represented by HIV-associated clinical conditions that are considered to have prognostic significance and are subdivided into one of four clinical stages: **stage I**: asymptomatic infection/ persistent generalized lymphadenopathy; **stage II**: early disease; **stage III**: intermediate disease and **stage IV**: late disease (Appendix 1) WHO Stage IV is

equivalent to the 1987 Centres for Disease Control (CDC) definition of AIDS (3,4). The clinical stage of each patient was assessed by a physician according to the WHO Clinical Staging system and recorded on a standard data sheet. The initial AIDS-defining illness was recorded for Stage IV patients.

It has been demonstrated that patients with WHO stage I and II HIV-disease display similar survival curves (5). Therefore, during data analysis patients with Stage I and II HIV- infection were grouped together, representing early HIV-disease.

4.5.2 Retinol

Serum levels of vitamin A is the most commonly used biochemical measure of vitamin A status, but serum levels become predictive of an individual's status only when body reserves have been critically depleted or overfilled. It should be noted that the Relative Dose Response Test (RDR) show greater validity as a marker of marginal liver vitamin A stores than a single serum sample. The RDR requires a fasting blood sample followed by a small dose of vitamin A and another blood sample to be taken after 5 hours (6,7). This method was not feasible in this study since patients only attend the clinic during the afternoon.

The WHO has recommended that the following criteria should be used for the interpretation of serum vitamin A levels: Deficient: < 10 μg/dl (< 0.35 μmol/l); Low: 10 - 19 μgl/dl (0.35 - 0.69 μmol/l); Normal: 20 - 50 μg/dl (0.7 - 1.75 μmol/l)

and High: > 50 μg/dl (> 1.75 μmol/l) (8). However, US population-based data suggest that values in the range of 20-30 μg/dl (0.7 - 1.05 μmol/l) may be inadequate in particular for post-adolescent age groups (6,9). Therefore, in this study blood retinol status was categorised as follows:

(i) **borderline** retinol levels defined as levels less than 30 μg/dl (1.05 μmol/l);

(ii) **low** retinol levels defined as levels less than 20 μg/dl (0.7μmol/l) and

(iii) **deficient** retinol levels defined as levels less than 10 μg/dl (0.35 μmol/l).

Pregnant patients were excluded from this study because serum retinol levels decrease during pregnancy even among well-nourished women and are therefore not a good reflection of vitamin A status (10).

Venous blood samples were taken in heparinised tubes covered with tin foil to protect from light. Samples were refrigerated immediately until centrifugation was possible, after which samples were frozen at -20 °C. It has been shown that under these storage conditions, vitamin A is stable for several years (11).

Plasma retinol was determined using the fluorometric method (12). Each batch of samples was calibrated against two retinol standards and one plasma control using the spectrophotofluorometer. The interassay coefficient of variation (CV) was 7%. The intra-assay CV was previously determined at 7.55%.

4.5.3 Possible confounding, intermediate or effect modifying variables

4.5.3.1 Body weight, height and Body Mass Index (BMI)

The Body Mass Index (BMI) or Quetelet index is defined as body weight divided by the square of the height (BMI= kg/m2). A BMI of less than 18.5 had been suggested as the cut off point for identifying adults who are suffering from chronic energy malnutrition in developing countries (13).

The degree of body wasting, and specifically lean body mass wasting has been associated with mortality in AIDS patients (14; 15). Wasting (> 10% loss of usual weight) is included in the WHO staging system for HIV disease and has been shown to be a risk factor for mortality in AIDS patients (15). However, it has been shown that a low BMI is a better predictor of mortality in HIV-disease than wasting. It has been suggested that the weight loss criterion which is included in the WHO Staging system should be replaced with the BMI criterion because this allows clinicians and researchers to determine the body weight status at a single point in time, rather on relying on weight change over an unspecified interval (16).

Subjects were asked to remove heavy clothing and shoes. A balance scale which was calibrated at regular intervals was used to measure body weight to the first decimal point. A standard measuring stick was used to measure height to the nearest 0.5 cm. The BMI was calculated for each subject.

4.5.3.2 Total Lymphocyte Count (TLC), CD_4 Count and CD_4/CD_8 ratio

T-lymphocyte levels may be measured by the absolute number or percentage of CD_4 T-cells, the number of CD_8 T-cells or the ratio of CD_4 to CD_8 T-cells.

A significant increase in CD_4 lymphocyte counts has been demonstrated after 3 months of TB therapy in seropositive patients with pulmonary Tuberculosis (PTB) (17). This potential confounding effect of TB on the CD_4 count was controlled in this study by restricting the inclusion criteria to patients who had received TB treatment for at least 12 weeks.

The total white cell count was used as an input value for the determination of TLC, CD_4 and CD_8 T-lymphocyte levels by flow cytometry. Variability in CD_4 and CD_8 T-lymphocyte levels between and within laboratories occur due to techniques used in cell labeling, type of antibody used, type of machine, expertise of the operators and standardization of machine alignment, number of cells counted and normal variations in cell populations (18). To avoid inter-laboratory variation one laboratory was used (Department of Clinical Immunology, Department of Medicine, UCT). The intra-assay CV has previously been determined at 4%.

CD_4 lymphocyte counts do show diurnal variation and therefore blood samples should ideally be taken in the morning. This was not feasible in this study. Blood samples were taken in heparinised tubes. Samples were stored at room temperature and analysed within 24 hours after collection.

The reference ranges for TLC, CD_4 levels and the CD_4:CD_8 lymphocyte ratio are depicted in Table 4.1.

Table 4.1 Reference ranges for TLC, CD_4 levels and the CD_4:CD_8 lymphocyte ratio

	Reference range
Total lymphocyte count	1500 - 4000 x 10^6/l
CD_4 T-cells	500 - 2010 x 10^6/l
CD_4:CD_8 ratio	> 1.5

4.5.3.3 Haemoglobin and Haematocrit

Haematopoiesis is reflected by haemoglobin and haematocrit levels. Full blood counts was performed on all blood specimens with the use of a blood counter (Technicon H2 systems). The reference ranges for haemoglobin and haematocrit are given in Table 4.2 below.

Table 4.2 Reference ranges for haemoglobin and haematocrit for males and females

	Haemoglobin (g/dl)	Haematocrit (%)
Males	13.3 - 17.3	0.37 - 0.53
Females	11.6 - 15.6	0.32 - 0.48

4.5.3.4 C-reactive protein (CRP), plasma zinc and retinol-binding protein (RBP)

C-reactive protein (CRP) is described as a marker of the acute phase response, in that following an acute stimulus the plasma CRP concentration rises within 6 hours, reaching a peak at 50 hours. Similarly, on cessation of a stimulus, the plasma CRP concentration declines in an exponential manner in view of its short life of 5 to 7 hours (19). During the acute phase response serum zinc levels decrease due to hepatic sequestration of zinc and may influence serum retinol via its effect on retinol binding protein (RBP) which is a zinc-dependent protein (20,21).

The puncture site was cleaned with trace-element free alcohol to avoid contamination of the blood sample with zinc. Venous blood was placed in plastic tubes with plastic tops, since vacutainers with rubber stoppers contain zinc. Samples were refrigerated immediately until centrifugation was possible.

Plasma CRP and RBP levels were determined using a Behring nephelometer. This method was standardised against an international reference preparation for plasma proteins (22). The normal CRP concentration in adults may be regarded as less than 10mg/l, although a few healthy individuals may have a slightly higher value (19). The adult reference range for RBP is 3.5-9 mg/dl (23).

Plasma zinc levels show diurnal and postprandial variation and therefore fasting blood samples should ideally be taken in the morning (23). This was not feasible in this study. Plasma zinc concentration was determined using atomic absorption spectrophotometry. Each batch of samples was calibrated against five standards which were 20 μg/dl, 50 μg/dl, 100 μg/dl, 150 μg/dl and 200 μg/dl. The intra-assay coefficient of variation (CV) was 5-9%. The interassay CV was previously determined at 6%. The reference range for zinc in plasma is 70-150 μg/dl (23).

4.6 Pilot study

A pilot study was initiated during October 1995 amongst 10 male and female patients attending the GSH HIV Outpatient Clinic. Patient folders were screened to identify patients in whom routine blood tests were requested by the attending physician and according to the inclusion criteria. A 10ml venous blood sample was taken in a heparinised tube with a plastic screw top, in addition to the routine blood tests requested for each patient. This blood sample was protected from light with tin foil and immediately refrigerated. All samples were centrifuged within 12 hours after collection and put into aliquots before it was stored at -20 °.

Consent was taken from all subjects and a short questionnaire for the collection of demographic variables such as age and proxies for socio-economic status such as educational level, current employment, type of housing, and the availability of electricity or a refrigerator (24,25).

References

1. Martin DJ; Blackburn NK; O'Connell KF; Brant ET and Goetsch EA. Evaluation of the World Health Organisation antibody-testing strategy for the individual patient diagnosis of HIV infection (Strategy III). S Afr Med J 1995; 85(9):877-80.

2. Karter DL; Karter AJ; Yarrish R et al. Vitamin a deficiency in Non-Vitamin-Supplemented patients with AIDS: A Cross-Sectional Study. J Acquir Immune Def Syn 1995; 8: 199-203.

3. WHO International Collaborating Group for the Study of the WHO Staging System. Proposed "World Health Organization Staging System for HIV Infection and Disease": preliminary testing by an international collaborative cross-sectional study. AIDS 1993; 7:711-8.

4. Centres for Disease Control. Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. MMWR Morb Mortal Wkly Rep 1987; 36(suppl 1S):1S-15S.

5. Post FA; Wood R and Maartens G. CD4 and total lymphocyte counts as predictors of HIV disease progression. Q J Med 1996; 89:505-8.

6. Underwood B. Methods for Assessment of Vitamin A Status. J Nutr 1990; 120: 1459-63.

7. Underwood B. Biochemical and histological methodologies for assessing Vitamin A status in human populations. Methods in Enzymology 1990; 190: 242-51.

8. World Health Organisation. Vitamin A deficiency and xerophthalmia. WHO Tech Rep Ser 1976; 590:1-88.

9. Pilch SM. Analysis of Vitamin A Data from the Health and Nutrition Examination Surveys.J Nutr 1987; 117:636-40.

10. Underwood BA. Maternal vitamin A status and its importance in infancy and early childhood. Am J Clin Nutr 1994;59(suppl):517S-24S.

11. Comstock GW; Alberg AJ and Helzlsouer KJ. Reported effects of Long-Term Freezer storage on Concentrations of Retinol, β-carotene and α-Tocopherol in serum or Plasma Summarized. Clin Chem 1992;39(6): 1075-8.

12. Thompson JN; Erdody P; Brien R and Murray TK. Fluorometric Determination of Vitamin A in Human Blood and Liver. Biochemical Medicine 1971; 5: 67 - 89.

13. James WPT; Ferro-Luzzi A; Waterlow JC et al. Definition of chronic energy deficiency in adults. Eur J Clin Nutr 42:969-981; 1988.

14. Kotler DP; Wang J; Pierson RN. Body Composition studies in patients with the Acquired Immunodeficiency Syndrome. Am J Clin Nutr 1985; 42: 1255-1265.

15. Semba RD; Caiffa WT; Graham NMH et al. Vitamin A deficiency and Wasting as Predictors of Mortality in Human Immunodeficiency Virus-Infected Injection Drug Users. J Infect Dis 1995; 171:1196-202.

16. Lifson AR; Allen S; Wolf W; Serufilira A; Kantarama G; Lindan CP et al. Classification of HIV Infection and Disease in women from Rwanda. Ann Intern med 1995;122:262-70.

17. Martin DJ, Sim GM, Sole GJ et al. CD4 Lymphocyte Count in African Patients Co-infected with HIV and Tuberculosis. J Acquir Immune Def Syn Human Retrovir 1995; 8:386 -391.

18. Stein DS; Korvick JA and Vermund SH. CD4 Lymphocyte Enumeration for Prediction of Clinical Course of human Immunodeficiency Virus Disease: A Review. J Infect Dis 1992; 165: 352 -363.

19. Young B; Gleeson M and Cripps AW. C-Reactive Protein: A Critical Review. Pathology 1991; 23:118-24.

20. Hussey GD; Labadarios D. Vitamin A - beyond vision. CME 1992; 10(8): 1383-1391.

21. Fleck A. Clinical and nutritional aspects of changes in acute-phase proteins during inflammation. Proc Nutr Soc 1989; 48: 347 -354.

22. Baudner S. Manufacture and Characterisation of a new Reference Preparation for 14 Plasma Proteins/ CRM 470 = RPPHS Lot 5. J Clin Lab Anal 1994; 8:177-90.

23. Tietzman NW.(Ed).Fundamentals of Clinical Chemistry. 3rd ed. WB Saunders, Philadelphia, 1987.

24. Liberatos P; Link BG and Kelsey JL. The measurement of social class in epidemiology. Epi Rev 1988; 10 : 87 -121.

25. Bourne LT. Dietary intake in an urban African population- with special reference to the nutrition transition. [Dissertation] Cape Town, South Africa: University of Cape Town, 1996. 300pp.

5 Statistical Analysis

Data was computerised and analysed on Epi-info, version 6 and SAS Statistical package, version 6.03. The normality of all the continuous variables was assessed by examining the stem-and leaf and the box plots, the normal probability plots and by the Shapiro-Wilk test for normality.

The relationship between clinical stage and plasma retinol levels was analysed with plasma retinol as a categorical and as a continuous variable. The proportion of patients with borderline plasma retinol levels (<30 μg/dl) and low plasma retinol levels (<20 μg/dl) within clinical stages 1 and 2, clinical stage 3 and clinical stage 4 was compared, using the Chi-square test for trend. The median plasma retinol levels within clinical stages 1 and 2, clinical stage 3 and clinical stage 4 was compared using the Kruskal-Wallis test.

The relationship between plasma retinol levels and absolute CD_4 lymphocyte cell count and the CD4:CD8 ratio was assessed by examining the linear association, using the Spearman correlation coefficient. The median plasma retinol levels was compared in patients with an absolute CD_4 lymphocyte cell count of less than and above 200 cells/μl and in patients with a CD4:CD8 ratio of less than and above 0.15 with the Wilcoxon rank-sum test.

C-reactive protein (CRP) was categorised as follows: < 10 mg/l, 10-40 mg/l and 40 mg/l. The relationships of plasma CRP levels with retinol, retinol-binding protein and zinc were assessed by the comparison of the median plasma levels of these variables within the three categories of CRP, using the Kruskal-Wallis test.

Correlations between all the continuous variables such as plasma retinol, body weight, BMI, CD_4 lymphocytes, TLC, haemoglobin, haematocrit, RBP,CRP and zinc was performed using the Spearman correlation coefficient. The presence of collinearity ($r \geq 0.8$) was examined in order to prevent the inclusion of strongly correlated variables into the multivariate model.

Multivariate logistic regression was used to determine whether plasma retinol is independently associated with clinical stage 4 as outcome variable. Potential confounding variables such as body weight, BMI, absolute CD_4 lymphocyte cell count and CD4:CD8 ratio, haemoglobin, haematocrit, CRP and serum zinc levels were entered into a multivariate model. Variables showing collinearity were entered independently into the model. Interaction terms were evaluated. The same procedure was followed to examine a logistic regression model with a borderline plasma retinol as outcome variable.. Models were assessed in terms of model fit, statistical and clinical significance. Residual analysis was performed on the final multivariate models to determine the effect of individual observations on the parameter estimates of each model and to identify observations that were not well fitted by each of the models.

6 Ethics

Informed consent was obtained from all participants before entering the study. This study was approved by the Research Ethics Committee, UCT.

7 Results

A total of 247 patients with heterosexually acquired HIV-disease attended the HIV-positive outpatient clinic at Groote Schuur Hospital during the period October 1995 to December 1996 (excluding January 1996). Blood tests were not requested in 38 patients. The following patients were excluded according to the exclusion criteria: less than 18 years (n=1), the presence of active opportunistic infection at the time of the clinic visit (n=9), patients who received TB therapy for less than 3 months (n=30), clinical diagnosis of liver disease (n=3), multivitamin supplements at the time or during the preceding 6 months (n=17), pregnancy (n=9), prisoners (n=1) and problems with venous access (n=2). One patient refused to participate in the study.

Data was collected on 136 subjects. All subjects had a temperature less than 38°C recorded at the time of the study. One blood sample was haemolysed making it unsuitable for analyses. Clinical review of the data further excluded 1 patient with a Cryptococcal infection who had received treatment for less than 3 weeks and 2 patients in whom M. tuberculosis was diagnosed shortly after they were included in the study.

The total number of 132 patients suitable for data analysis consisted of 51, 48 and 33 patients with Stage 1/2, 3 and 4 HIV-infection, respectively. The initial AIDS-

defining illness of Stage 4 patients and the time period between the initial AIDS-defining illness and enrollment into this study, is illustrated in Table 7.1.

Table 7.1 Initial AIDS-defining illnesses of Clinical Stage IV patients (n=33)

AIDS-defining Illness	No. of patients	Time period between AIDS-defining illness and study enrollment (months)
Extrapulmonary Tuberculosis	20	11.2 ± 10.7
Pneumocystis carinii pneumonia	2	3 ± 1.4
Cryptococcosis	1	3
Toxoplasmosis	1	0
Herpes simplex virus	1	0
Kaposi's sarcoma	3	7.3 ± 10.2
Oesophageal candidiasis	3	1.7 ± 2.9
HIV-Wasting syndrome	2	0

7.1 Demographic characteristics

The mode of HIV-transmission for all subjects was heterosexual transmission. Table 7.2 shows that women are overrepresented in the sample, which reflects the current trend in the HIV-epidemic in South Africa. There were no significant differences between the three clinical stage categories for gender or age.

Due to logistic problems, variables such as educational level, current employment, the availability of electricity or a refrigerator are only available on 118 patients. However, sub-analysis revealed no significant differences in terms of plasma retinol levels between those patients on whom information was available and the total number of patients in each clinical stage category.

No differences between the clinical stage categories were noted in terms of type of housing and the availability of electricity or a refrigerator. Seventy seven percent (77%) of subjects with stage 4 disease did not have high school education compared to 41% of subjects with early disease ($p < 0.01$). Only thirteen percent (13%) of stage 4 subjects and 14% of stage 3 subjects were employed at the time of the study, compared to 43% of subjects with early disease ($p < 0.05$).

Table 7.2 Characteristics of HIV-positive individuals with WHO Clinical Stage I to IV HIV-Infection

	WHO STAGE I & II	WHO Stage III	WHO Stage IV
Age (n=132)	(n=51) 32.9 ± 9.5	(n =48) 33.9 ± 10.6	(n = 33) 32.5 ± 10.9
Gender			
Male	15/51 (29%)	17/48 (35%)	15/33 (45%)
Female	36/51 (71%)	31/48 (65%)	18/33 (55%)
Education (n=118)			
< Std 5	6/44 (14%)	6/44 (14%)	9/30 (30%)
Std 5	12/44 (27%)	22/44 (50%)	14/30 (47%)
Std 8	13/44 (30%)*	11/44 (25%)*	4/30 (13%)*
Std 10	10/44 (23%)*	4/44 (9%)*	2/30 (7%)*
> Std 10	3/44 (6%)	1/44 (2%)	1/30 (3%)
Current Employment (n=118)			
Yes	19/44 (43%)*	6/44 (14%)*	4/30 (13%)*
No	25/44 (57%)	38/44 (86%)	26/30 (87%)
Electricity (n=118)			
Yes	31/44 (70%)	33/44 (75%)	20/30 (67%)
No	13/44 (30%)	11/44 (25%)	10/30 (33%)
Refrigerator (n=118)			
Yes	20/44 (45%)	16/44 (36%)	8/30 (27%)
No	24/44 (55%)	28/44 (64%)	22/30 (73%)

* $p < 0.01$

7.2 Retinol

Table.7.3 shows that the median plasma retinol levels are significantly different between clinical stages amongst all the patients (p=0.002). The median plasma

retinol level for patients with early disease was 34μg/dl, compared to 30.7 and 23.7 μg/dl for patients with Stage 3 and 4 disease, respectively. The median plasma retinol levels between clinical stage categories is also significantly different in the case of male (p=0.001) and female (p=0.02) patients.

The plasma retinol levels of male patients with Stage 1 and 2 HIV-infection were significantly higher than female patients being 46.4 ± 15.8 μg/dl (median = 42.9μg/dl) compared to 30.8 ± 10.4 μg/dl (median = 30.1 μg/dl), respectively. However, the plasma retinol levels of male and female subjects within the other clinical strata in the sample, were similar (Table 7.3).

The median plasma retinol level of Stage 4 patients with HIV-wasting syndrome (n=3) **at study entry** was 21.1μg/dl, compared to the median level of 23.7 μg/dl for all Stage 4 patients (NS). The median plasma retinol level of Stage 4 patients with extrapulmonary tuberculosis (n=7) **at study entry** was 20.6 μg/dl, however, this difference was also not statistically significant.

In Stage 3 patients the median plasma retinol level of subjects with oral candidiasis (n=13) **at study entry** was 27.4 μg/dl, compared to the median level of 30.7 μg/dl for all stage 3 patients (NS).

Table 7.3 Plasma retinol levels of patients with WHO Stage I to IV HIV-infection

Plasma retinol µg/dl	WHO Stage I and II (n =51)	WHO Stage III (n = 48)	WHO Stage IV (n =33)	p
BOTH SEXES (N=132)				
Mean ± SD	35.4 ± 14.1	30.4 ± 11.2	25.2 ± 11.1	
Median	34.0	30.7	23.7	0.002
MALES (n=47)				
Mean ± SD	46.4 ± 15.8	28.3 ± 10.2	26.9 ± 11.7	
Median	42.9*	29.2	24.3	0.001
FEMALES (n=85)				
Mean ± SD	30.8 ± 10.4	31.6 ± 11.8	23.8 ± 10.6	
Median	30.1*	31.6	21.9	0.02

* **p < 0.001**

The proportion of patients with borderline plasma retinol levels (< 30 µg/dl) versus low plasma retinol levels (<20 µg/dl) in each clinical stage category is shown in Table 7.4.

Table 7.4 The proportion of patients with WHO Stage I to IV HIV-infection with a borderline (< 30 μg/dl) versus a low (< 20 μg/dl) plasma vitamin A status

Plasma retinol < 30 μg/dl (< 1.05 μmol/l)	WHO Stage I & II	WHO Stage III	WHO Stage IV	χ^2 trend
Both sexes (n=132)	20/51 (39%)	23/48 (48%)	26/33 (79%)	0.0007
Males (n= 47)	2/15 (13%)	9/17 (53%)	12/15 (80%)	0.0003
Females (n=85)	18/36 (50%)	14/31 (45%)	14/18 (78%)	0.1

Plasma retinol < 20 μg/dl (< 0.7 μmol/l)	WHO Stage I & II	WHO Stage III	WHO Stage IV	χ^2 trend
Both sexes (n=132)	7/51 (14%)	8/48 (17%)	11/33 (33%)	0.04
Males (n=47)	0/15	3/17 (18%)	3/15 (20%)	
Females (n=85)	7/36 (19%)	5/31 (16%)	8/18 (44%)	0.08

Table 7.4 shows that there is a significant linear increase in the proportion of patients with a borderline retinol status (< 30 μg/dl) from those with early disease to those with advanced disease., especially in the case of male patients.

Thirteen percent (13%) of male patients with early disease had a borderline retinol status, compared to 53% and 80% of Stage 3 and 4 patients, respectively (p=0.003).

A higher proportion of female patients with early disease displayed a borderline retinol status compared to the male patients, namely 50%. Therefore, a significant linear trend across clinical stage categories was not apparent in female subjects.

7.3 Body weight and Body Mass Index (BMI)

No gender differences in terms of body weight and BMI within the three clinical staging categories, were demonstrated. The relationship between clinical stage and body weight or BMI was significant (p=0.02) (Table 7.5).

Although the data is incomplete in terms of BMI, only 6/29 (21%) Stage 4 patients had a BMI < 18.5 and could therefore be categorised with chronic energy malnutrition.

Table 7.5 Body weight and Body Mass Index (BMI of patients with WHO Stage I to IV HIV-infection

	WHO Stage I & II	WHO Stage III	WHO Stage IV	p
Weight (Kg)	(n=51)	(n=48)	(n=33)	
Mean ± SD	69.0 ± 19.8	61.2 ± 11.0	57.3 ± 13.5	
Median	66	60.5	58.0	0.01
BMI (kg/m^2)	(n =45)	(n = 42)	(n = 29)	
Mean ± SD	26.4 ± 6.5	23.7 ± 4.8	21.9 ± 4.5	
Median	24.5	22.7	21.7	0.01

7.4 CD4 lymphocyte count, CD4:CD8 lymphocyte ratio and Total lymphocyte count (TLC)

Six blood samples were insufficient for full blood count analysis, therefore data is presented on 126 subjects. For 129 patients the CD_4:CD_8 lymphocyte ratio could be calculated.

No gender differences in terms of CD_4 lymphocyte count, CD_4:CD_8 lymphocyte ratio or TLC within the three clinical staging categories, were demonstrated. Table 7.6 shows a significant difference between the median CD_4 lymphocyte levels and CD_4:CD_8 lymphocyte ratios ($p < 0.001$) between the three clinical stage categories. The difference in the median Total lymphocyte counts approached significance between the three categories ($p=0.1$).

Table 7.6 CD4 lymphocyte levels , CD4: CD8 lymphocyte ratio and Total lymphocyte count (TLC) of patients with WHO Stage I to IV HIV-infection (n=126)

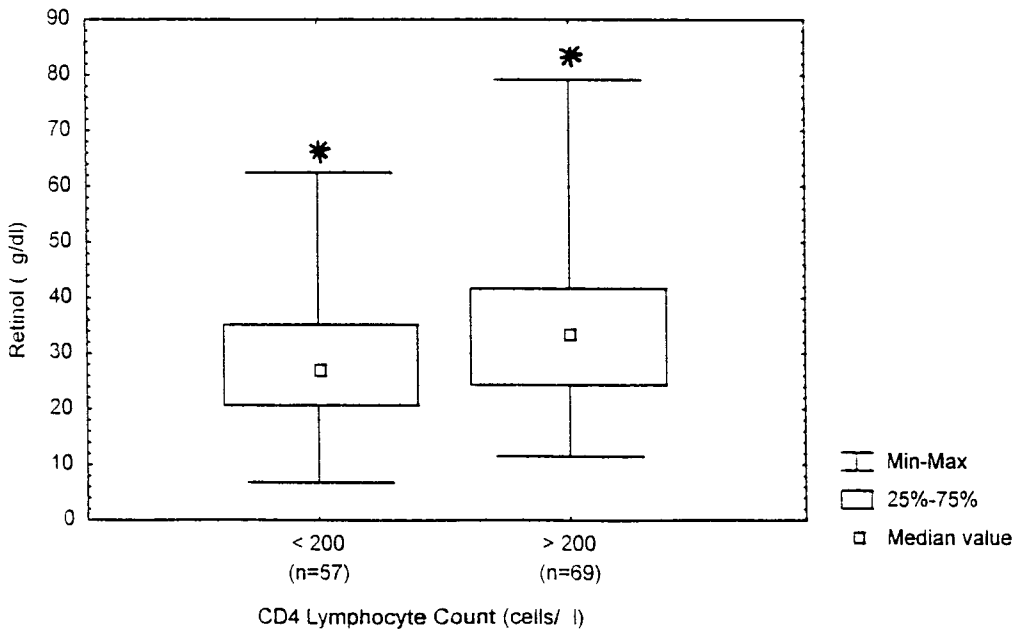
	WHO Stage I & II (n = 47)	WHO Stage III (n = 47)	WHO Stage IV (n = 32)	P
CD4 lymhocytes cells/μl				
Mean ± SD	403 ± 198	205 ± 177	155 ± 136	
Median	369	165	141	< 0.001
CD4:CD8 lymphocyte ratio				
Mean ± SD	0.5 ± 0.3	0.2 ± 0.2	0.1 ± 0.1	
Median	0.4	0.2	0.1	< 0.001
Total lymphocyte count (TLC) cells/μl				
Mean ± SD	1883 ± 757	1583 ± 701	1573 ± 948	
Median	1820	1464	1392	0.1

CD_4 lymphocyte levels and the CD_4:CD_8 ratio were dichotomised to < 200, ≥ 200 cells/μl and ratios of < 0.15, ≥ 0.15, respectively. In patients with CD_4 lymphocyte levels of less than 200 cells/μl the median retinol level was 27.1 μg/dl (28 ± 14.7 μg/dl), compared to 34 μg/dl (34 ± 13.3 μg/dl) in patients with CD_4 lymphocyte levels above or equal to 200 cells/μl (Fig. 7.1) (p=0.01). The median retinol level of patients with a CD_4:CD_8 ratio of less than 0.15 was 25.4 μg/dl (26.3 ± 11.5 μg/dl)

versus 32.4 μg/dl (33.3 ± 13.3 μg/dl) in patients with a ratio equal or above 0.15 (Fig 7.2) (p=0.005).

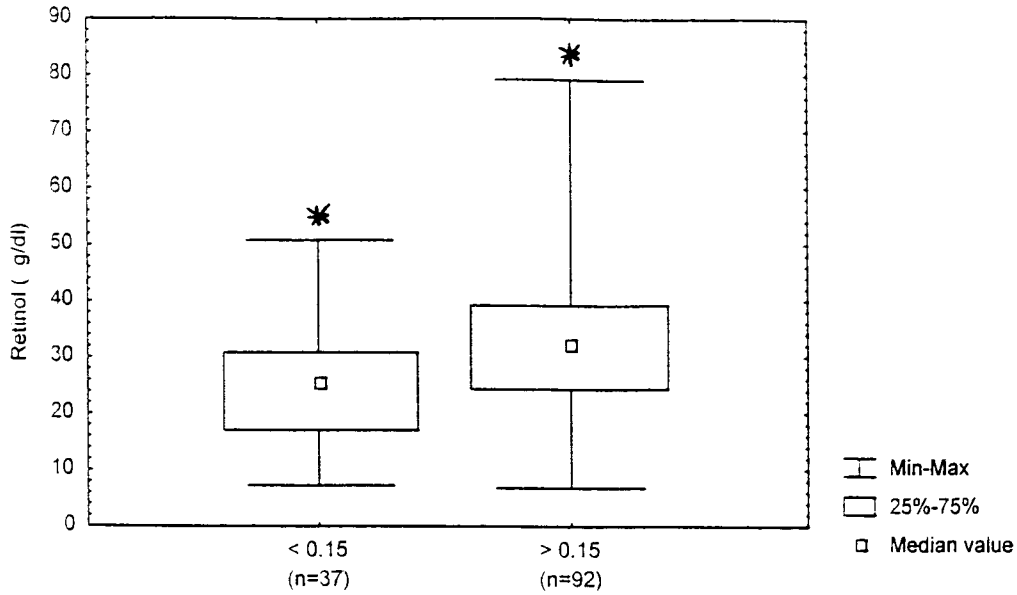
When TLC was dichotomised to < 1250 and ≥ 1250 cells/μl the median retinol levels in the respective categories were 27.8 μg/dl (29 ± 14.7 μg/dl) and 31.6 μg/dl (32.4 ± 12.2 μg/dl), respectively (Fig. 7.3). This difference in retinol levels approached significance (p=0.1).

FIG. 7.1 Plasma retinol levels grouped by CD4 Lymphocyte count (n=126)



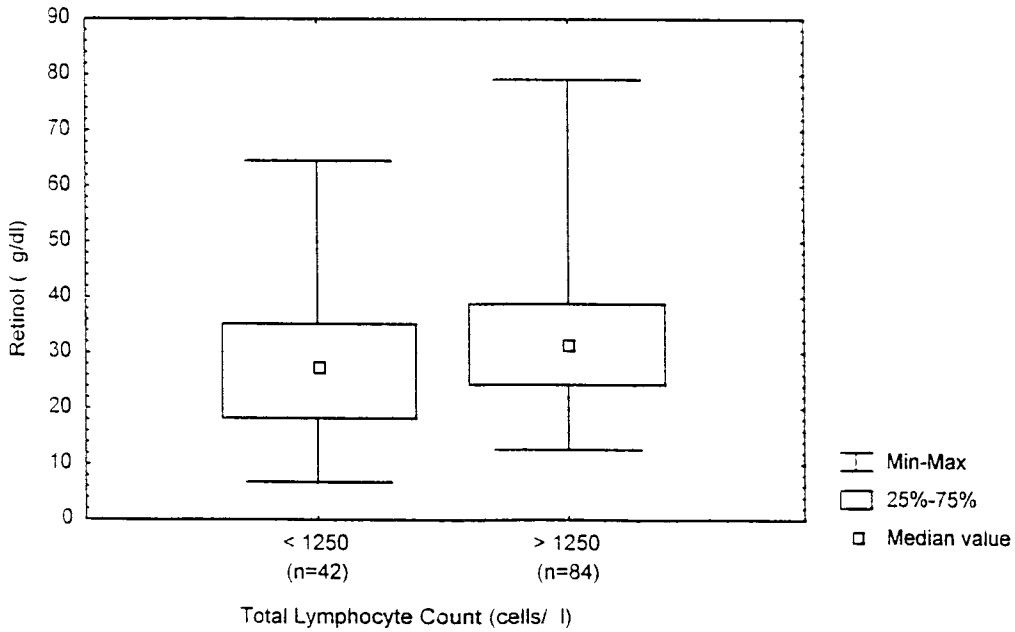
* p = 0.01

FIG. 7.2 Plasma retinol levels grouped by the CD4:CD8 Lymphocyte ratio (n=129)



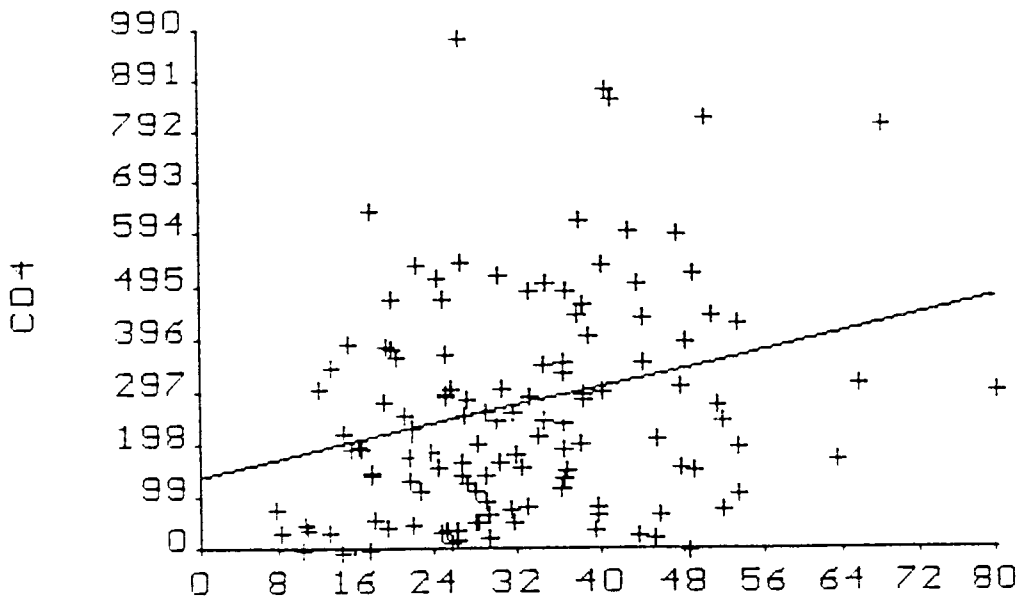
* P = 0.005

FIG. 7.3 Plasma retinol levels grouped by Total Lymphocyte Count (n=126)



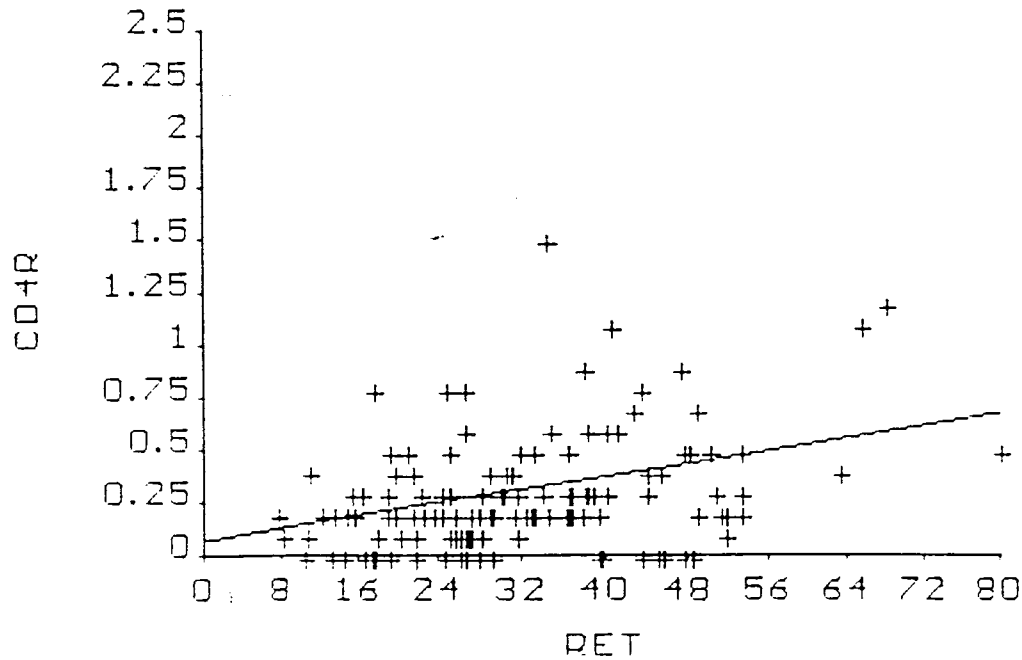
A significant association between plasma retinol and CD_4 lymphocyte levels (r = 0.27; 95% CI: 0.1; 0.43) and the CD_4:CD_8 ratio (r=0.33 95 CI%: 0.1; 0.42) for 126 subjects can be seen from Fig.7.4 and 7.5, using the Spearman correlation coefficient.

FIG.7.4 Scatter diagram of plasma retinol levels and CD_4 lymphocyte levels (n=126)



r=0.27
95% CI:(0.1;0.43)
p=0.003

FIG 7.5 Scatter diagram of plasma retinol and the CD_4:CD_8 ratio (n=129)



r=0.33
95% CI:(0.1;0.42)
p=0.0002

7.5 C-reactive protein (CRP)

All the subjects with the exception of one subject, did not show highly elevated CRP levels above 100mg/l. One patient with clinical stage 3 disease had a CRP level of 174 mg/l. This observation was not excluded. A higher proportion of patients with Stage 3 or 4 disease (48%; 42%) had CRP levels ≥ 10mg/l, compared to patients with early disease (23%). (Table 7.7).

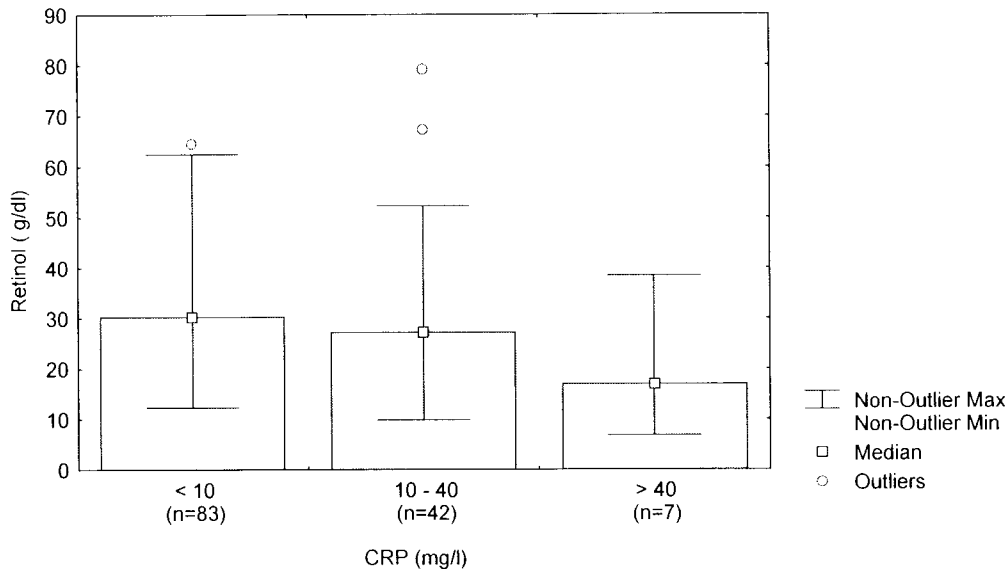
Table 7.7 CRP levels of patients with WHO Stage I to IV HIV-infection (n=132)

CRP	WHO Stage I & II (n = 51)	WHO Stage III (n = 48)	WHO Stage IV (n = 33)	χ^2 trend
< 10 mg/l	39 (77%)	25 (52%)	19 (58%)	3.93 (p=0.05)
≥ 10 mg/l	12 (23%)	23 (48%)	14 (42%)	

7.5.1 CRP and retinol

CRP levels were then categorized according to the following categories: < 10 mg/l; 10-40 mg/l and > 40 mg/l. Plasma levels of retinol were examined according to these categories (Fig. 7.6).

FIG. 7.6 Plasma retinol levels according to different categories of C-reactive protein (CRP) (n=132)

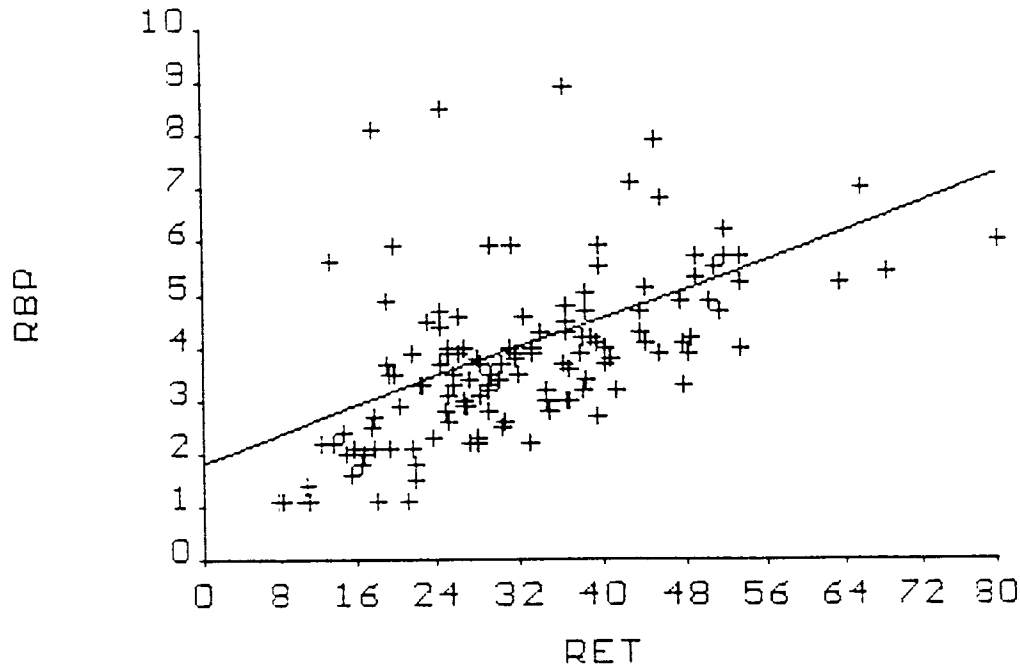


The mean retinol level of patients with CRP levels above 40 mg/l was 19.7 ± 13.3 mg/l (median=16.8 mg/l), compared 30.3 ± 14.7 mg/l (median = 27.3mg/l) in patients with CRP levels between 10 and 40 mg/l and 32.4 ± 11.5 mg/l (median = 30.2 mg/l) in patients with CRP levels below 10 mg/l, respectively. The median retinol levels across the different categories of CRP was statistically significant (p=0.04).

7.6 Retinol-binding protein (RBP)

A strong correlation between plasma retinol and RBP levels for all the subjects was shown using the Spearman's correlation coefficient (Fig.7.7)

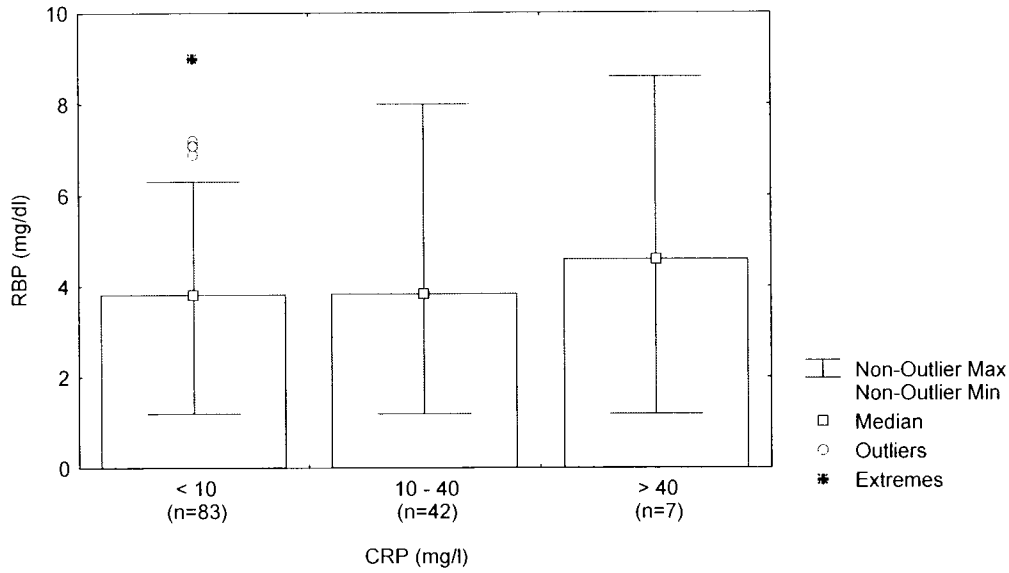
Fig 7.7 Scatter diagram of plasma RBP and retinol levels (n=132)



r=-0.62
95%CI: (0.53: 0.73)
p=0.0001

Plasma RBP levels were also categorised according to the following categories of CRP , namely < 10 mg/l; 10-40mg/l and > 40 mg/l (Fig. 7.8). No statistical differences in terms of plasma RBP levels across the three categories of CRP were found (p=0.94).

FIG.7.8 Plasma retinol binding protein (RBP) levels according to different categories of C-Reactive Protein (CRP) (n=132)



(vii) Zinc

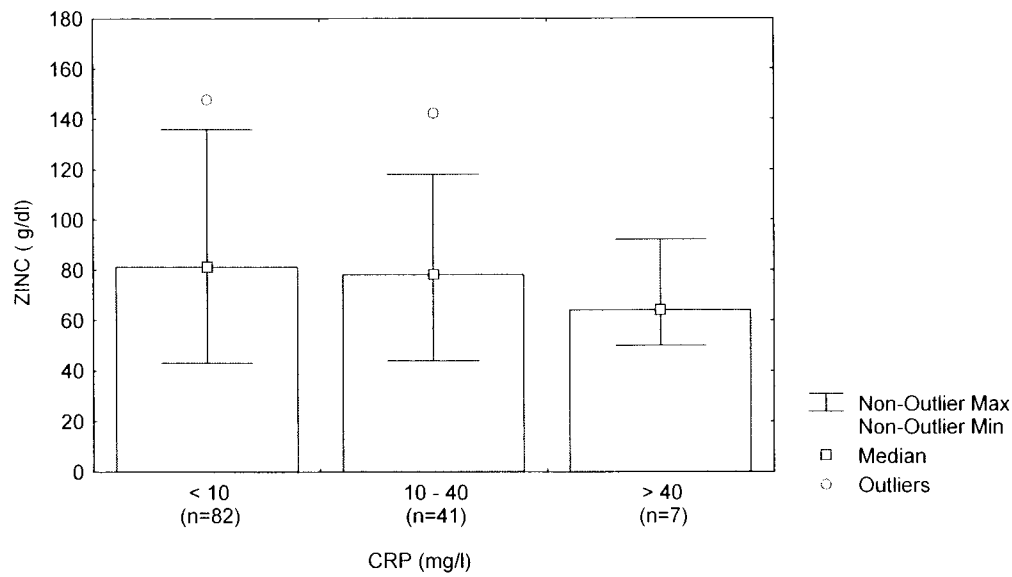
Two blood samples were contaminated with zinc during the blood sampling procedure and were identified as extreme values and therefore data is presented on 130 subjects. The difference in the median zinc levels between clinical stage categories approached significance (p=0.06) (Table 7.8). Plasma zinc levels were similar between genders within the three clinical staging categories.

Table 7.8 Plasma zinc levels of patients with WHO Stage I to IV HIV-infection (n=130)

	WHO Stage I and II (n =50)	WHO Stage III (n = 47)	WHO Stage IV (n =33)	p
Plasma zinc µg/dl				
Mean ± SD	85.8 ± 22.6	79.1 ± 21.6	74.7 ± 18.1	
Median	84	78	72	0.06

Plasma zinc levels were also categorised according to the following categories of CRP , namely < 10 mg/l; 10-40mg/l and > 40 mg/l (Fig. 7.6).

FIG. 7.9 Plasma zinc levels according to different categories of C-Reactive Protein (CRP) (n=130)



The relationship between plasma zinc and the three categories of CRP was similar to that observed between plasma retinol and CRP, although not statistically significant.

The mean zinc level of patients with CRP levels above 40 mg/l was 67.4 ± 14.9 μg/dl (median=64 μg/dl), compared to 77.9 ± 22.7 μg/dl (median = 78 μg/dl) in patients with CRP levels between 10 and 40 mg/l and 83 ± 21 μg/dl (median = 81 μg/dl) in patients with CRP levels below 10 mg/l, respectively (p=0.1).

7.7 Multivariate analysis with clinical stage IV (AIDS) as dependent variable

Univariate analysis showed a significant association between plasma retinol with Clinical Stage 4 HIV-infection or AIDS as dependent variable. Plasma retinol levels were dichotomised as < 30μg/dl; ≥ 30 μg/dl and as < 20μg/dl; ≥ 20 μg/dl. Risk factors for the development of AIDS reported in the literature such as CD_4 lymphocyte count, CD_4:CD_8 lymphocyte ratio, haemoglobin, haematocrit and serum zinc levels were also found to be significant on univariate analysis. .Other factors which were significantly associated with Clinical Stage 4 were body weight and BMI. Total lymphocyte count, CRP levels, platelet count, gender and socio-economic variables such as employment and the availability of a refrigerator were not significant during univariate analysis.

A strong correlation between retinol and RBP levels ($r= 0.62$; $p<0.001$) resulted in the inclusion of only retinol in the final multivariate model. Due to collinearity between CD_4 lymphocyte count and CD_4:CD_8 lymphocyte ratio ($r=0.78$; $p=0.0001$) two multivariate models are presented including each of these variables.

Haemoglobin and haematocrit were also highly correlated ($r=0.97$; $p=0.0001$) and it was decided to use haemoglobin in the final model. Haemoglobin was dichotomised to < 12g/dl and ≥ 12 g/dl, respectively. In a previous study examining the role of serum retinol with survival in AIDS patients, haemoglobin was excluded from the final multivariate model due to collinearity with serum retinol (Semba 1995). In our study haemoglobin was included in the final model, since the Spearman's correlation coefficient for haemoglobin with retinol was 0.43 ($p<0.001$).

Because of the incompleteness of the data in terms of BMI, it was decided to include body weight in the final model since these variables were also highly correlated ($r=0.83$;$p<0.001$).

No interaction terms between any of the variables were found to be significant.

Table 7.9 **Unadjusted and adjusted odds ratios (OR) and 95% confidence intervals (CI) of logistic regression for potential risk factors as predictors of Clinical Stage IV HIV-infection or AIDS.**

MODEL 1 (n=125)

Variable	$OR_{Unadjusted}$ (95%CI)	$OR_{Adjusted}$ (95%CI)
Plasma retinol **< 30 μg/dl**	4.7 (2.9-7.6)	3.3 (2.0-5.6)
CD_4 count **< 200 cells/μl**	3.7 (2.4-5.7)	2.5 (1.6-4.0)
Haemoglobin **< 12 g/dl**	2.6 (1.7-4.1)	1.5 (0.9-2.4)
Plasma zinc **(< 70 μg/dl)**	2.5 (1.7-3.9)	1.7 (1.07-2.8)
Body weight **(per 5kg increase)**	0.83 (0.77-0.91)	0.95 (0.86-1.01)

Table 7.10 **Unadjusted and adjusted odds ratios (OR) and 95% confidence intervals (CI) of logistic regression for potential risk factors as predictors of Clinical Stage IV HIV-infection or AIDS.**

MODEL 2 (n=125)

Variable	$OR_{Unadjusted}$ (95%CI)	$OR_{Adjusted}$ (95%CI)
Plasma retinol **< 30μg/dl**	4.7 (2.9-7.6)	2.9 (1.7-4.9)
CD_4:CD_8 ratio **< 0.15**	6.1 (3.9-9.5)	3.9 (2.4-6.5)
Haemoglobin **< 12 g/dl**	2.6 (1.7-4.1)	1.5 (0.94-2.5)
Plasma zinc **(< 70 μg/dl)**	2.5 (1.7-3.9)	1.3 (0.8-2.2)
Body weight **(per 5kg increase)**	0.83 (0.77-0.91)	0.96 (0.88-1.05)

The association between **borderline** plasma retinol levels (< 30μg/dl) and Stage 4 disease remained significant when adjusted for potential confounding variables such as CD_4 lymphocyte count, CD_4:CD_8 lymphocyte ratio, haemoglobin, zinc and body weight.

The association between **low** plasma retinol levels (<20μg/dl) and Stage 4 disease was significant on univariate analysis, but did not remain significant when adjusted for potential confounding variables. This could be explained by the small number of patients with retinol levels of less than 20 μg/dl, resulting in a lack of statistical power.

Both models are highly significant ($p < 0.001$). Model 7.10 shows a better model fit than model 7.9, using the -2 Log L and the AIC criterion (See Appendix). Residual analysis of both models identified four observations that were not well explained by the model. These observations were Stage 4 subjects who had retinol levels above 30μg/dl, CD_4 lymphocyte counts above 200/μl, or CD_4:CD_8 lymphocyte ratio above 0.15, plasma zinc levels above 70 μg/dl and haemoglobin levels above 12g/dl.

7.8 Multivariate analysis with retinol as dependent variable

In this logistic regression model, plasma retinol levels of < 30 μg/dl was used as the outcome variable. Predictor variables of a **borderline** retinol status that were

examined during univariate analysis included variables which are available in routine clinical practice such as TLC, CD_4 lymphocyte count or CD_4:CD_8 lymphocyte ratio, body weight and BMI, haemoglobin, haematocrit, CRP and zinc levels.

Due to collinearity between CD_4 lymphocyte count and CD_4:CD_8 lymphocyte ratio($r=0.78$; $p=0.0001$) two multivariate models are presented including each of these variables, as in section 7.6. Haemoglobin levels and body weight were included in the final multivariate model, as in section 7.6.

No interaction terms between any of the variables were found to be significant.

Table 7.11 Unadjusted and adjusted odds ratios (OR) and 95% confidence intervals (CI) using logistic regression analysis for predictors of borderline plasma retinol levels (< 30 μg/dl)

MODEL 1 (n=126)

Variable	$OR_{Unadjusted}$ (95%CI)	$OR_{Adjusted}$ (95%CI)
WHO Clinical Stage IV	4.8 (3-7.6)	3.4 (2.1-5.7)
CD_4 count < 200 cells/μl	3.6 (2.4-5.6)	1.4 (0.9-2.1)
Haemoglobin < 12 g/dl	3.3 (2.3-4.8)	2.4 (1.6-3.5)
Body weight (per 5kg increase)	0.82 (0.77-0.98)	0.87 (0.80-0.93)

8 Discussion

8.1 Retinol and clinical stage

Our study demonstrated that the proportion of patients with borderline plasma retinol levels (< 30 μg/dl) is greater in symptomatic disease, specifically in Clinical Stage IV disease or AIDS. The confounding effect of opportunistic infections was limited in this study by means of the study exclusion criteria and by clinical review of the data.

79% of all AIDS patients had borderline retinol levels (< 30 μg/dl) compared to 39% of patients with early disease (p <0.001). 33% (11/33) of AIDS patients had low retinol levels (< 20 μg/dl) compared to 14% (7/51) of patients with early disease (p=0.04). In a study by Semba et al. borderline retinol levels .(< 30 μg/dl) were present in 50% of AIDS patients at their last visit before death (1). The mean plasma retinol level of the AIDS patients in our study (25.2 ± 11.1μg/dl) was significantly lower than previously reported in hospitalised AIDS patients not receiving vitamin A supplementation in an industrialised country (36.5 ± 22.53 μg/dl) (p <0.01) (2).

Only four patients in this study were vitamin A deficient . Three patients with Stage 3 disease and one patient with Stage 4 disease demonstrated plasma retinol levels of less than 10 μg/dl.

Table 7.12 Unadjusted and adjusted odds ratios (OR) and 95% confidence intervals (CI) using logistic regression analysis for predictors of borderline plasma retinol levels (< 30 μg/dl)

MODEL 2 (n=126)

Variable	$OR_{Unadjusted}$ (95%CI)	$OR_{Adjusted}$ (95%CI)
WHO Clinical Stage IV	4.8 (3-7.6)	3.0 (1.8-5.1)
CD_4:CD_8 ratio < 0.15	3.6 (2.4-5.6)	2.0 (1.2-3.2)
Haemoglobin < 12 g/dl	3.3 (2.3-4.8)	2.4 (1.6-3.6)
Body weight (per 5kg increase)	0.82 (0.77-0.98)	0.88 (0.81-0.94)

WHO Stage 4 disease or AIDS was independently associated with a threefold increased risk of **borderline** plasma retinol levels (< 30 μg/dl) after controlling for CD_4 lymphocyte count or the CD_4:CD_8 lymphocyte ratio, haemoglobin levels and body weight. Haemoglobin levels of less than 12 g/dl and body weight were both

also independently associated with low retinol levels. CD_4 lymphocyte counts of less than 200 cells/μl and a CD_4:CD_8 lymphocyte ratio of less than 0.15 were significant on univariate analysis, but were not significant in the multivariate model. TLC was not associated with retinol levels during univariate analysis. Plasma zinc and CRP levels were evaluated in the multivariate model, but did not contribute significantly to the model, nor did it change the adjusted odds ratios of any of the covariates in the model.

Both models are highly significant ($p < 0.001$). Model 7.12 shows a better model fit than model 7.11, using the -2Log L and the AIC criterion (See Appendix). Residual analysis showed that only one observation could not be explained by the model. This observation was a subject with stage 4 disease with a retinol level of 43 μg/d.

A recent study in a local urban African population has shown that selected proxies for higher socio-economic status such as formal housing, the availability of electricity and the presence of a refrigerator were associated with a higher micronutrient intake in women aged 19-44 years, especially with respect to vitamin A (3). Our study showed that 43% of patients with early disease were employed at the time of the study, compared to 13% and 14% of patients with stage 3 and stage 4 disease, respectively. This could be explained by the nature of HIV disease. As the disease progresses, so unemployment due to disability increases. Also, 27% of patients with advanced disease had a refrigerator compared to 45% of patients with early disease, although this was not statistically significant. Therefore, although dietary intake of micronutrients and specifically vitamin A intake was not assessed, it is likely that socio-economic factors contributed to a poorer micronutrient status in those patients with advanced disease who were included in our study.

The difference in plasma retinol levels between male and female patients with early disease in our study cannot be readily explained. No gender differences are apparent in the other two clinical stage categories in terms of plasma retinol. Socio-economic variables between male and female patients with early disease, were similar. We were unable to assess any differences in parity between female patients in the three clinical categories in this study against the background of increased physiological needs during pregnancy and lactation (4).

It has been suggested that diarrhoea may contribute to low retinol levels in HIV disease. In a study by Semba et al. patients with histories of oral thrush and

diarrhoea were more likely to be vitamin A deficient (1). In our study we were unable to show any significant finding because of the inclusion of a very small number of patients with diarrhoea. No significant differences in plasma retinol levels between patients with oral or oesophageal thrush compared to the rest of the Stage 3 or Stage 4 patients, were found.

8.2 Retinol and immune function

Retinoids are known to enhance cellular differentiation and have an important role in the differentiation of haemopoietic cells and other cell lineages (5). Thus, retinol status may be important for the differentiation of CD_4 cells.

Lower CD_4 counts have been associated with lower retinol levels amongst HIV-seropositive and HIV-seronegative individuals (6,7). In this study lower retinol levels were associated with a low CD_4 count and CD_4:CD_8 lymphocyte ratio. Patients with CD_4 counts of < 200 cells/μL had a mean plasma retinol of 28 μg/dl (median= 27.1 μg/dl) compared with a mean level of 34 μg/dl (median 33.6 μg/dl) in patients with CD_4 counts ≥ 200 cells/μL (p=0.01). Plasma retinol levels were slightly lower in patients with a CD_4:CD_8 lymphocyte ratio of < 0.15 when compared with those with a low CD_4 count a (median= 25.4 μg/dl). The association between plasma retinol levels and CD_4 counts and CD_4:CD_8 lymphocyte ratios was confirmed by means of significant Spearman's correlation coefficients (r=0.27 95% CI: 0.1;0.43 ; r=0.33 95% CI: 0.1;0.42).

Many infectious diseases are associated with a lowered CD_4 lymphocyte levels, CD_4: CD_8 lymphocyte ratio such as HIV, Epstein-Barr virus, measles, cytomegalovirus, candidosis and pneumonia. High dose vitamin A supplementation in children with measles and in post-operative adult patients have been associated with an increase in absolute T lymphocyte cell count (8,9). In HIV disease, high dose vitamin A supplementation has been shown to reduce the morbidity associated with diarrhoeal disease in HIV-infected children (OR = 0.51; 95% CI = 0.27,0.99) (10). Another clinical trial investigated the effect of high dose vitamin A supplementation on the immune function of 75 children with AIDS without active infections. Supplementation was associated with a significant increase in serum retinol, absolute lymphocyte count, CD_4 and CD_{29} counts at 4 weeks after supplementation (11).

Short term intervention studies in HIV-infected individuals have suggested that ß-carotene supplementation may increase white blood cell count and CD_4 counts (12). A recent clinical trial conducted amongst 72 HIV-positive patients failed to demonstrate any significant effect of ß-carotene supplementation after 3 months on T cell subsets and other immune markers. Coodley et al. suggests that the addition of a multivitamin supplement to both arms of the study may have masked any differences between the two groups, since sufficient retinol concentrations were observed in both groups (13).

8.3 Retinol and the acute phase response

Interleukin-6 (IL-6) is thought to be the main mediator of CRP production, one of the major acute-phase proteins (14). HIV-infection per se is known to stimulate macrophages resulting in increased IL-6 levels, even in asymptomatic HIV-positive patients (15). It has been suggested that the IL-6 level increases with the progression of HIV-infection (16). However, normal CRP levels (< 10 mg/l) have been reported in asymptomatic HIV-positive subjects and in HIV-positive subjects after recovering from pneumonia, suggesting that increased IL-6 levels alone seems insufficient to increase CRP production (17,18).

In contrast to the studies cited above, Tang et al. showed in a cohort of 312 men of whom 81% were asymptomatic, that 55% had elevated serum CRP levels (≥ 8 mg/l) (19). In our study there was an association between raised CRP levels (≥ 10 mg/l) and the clinical stage of disease. The proportion of subjects with raised CRP levels who were asymptomatic was 23%, compared to 48% and 42% of subjects with Stage 3 and 4 disease, respectively (p=0.05). It should be noted that in the majority of our subjects the CRP levels were only mildly elevated. One patient with clinical stage 3 disease showed highly elevated CRP levels (> 100 mg/l). This could indicate the presence of an underlying infection in this subject despite the exclusion of patients with pyrexia in our study. In the majority of our subjects, a mildly raised CRP level may be attributable to a direct effect of HIV-1 activity.

Our study showed that the median retinol level of patients who had CRP levels greater than 40 mg/l was significantly lower (16.8 μg/dl) than in patients with CRP levels between 10 and 40 mg/l (27.3 μg/dl) and compared to those with CRP levels within the normal range (30.2 μg/dl). This suggests that the acute phase response contributed to low plasma retinol levels only in a small number of our subjects (n=7). Tang et al. showed despite their observation that CRP levels were elevated in 55% of their subjects, that the retinol levels were in the high-normal range (median= 70 μg/dl). In view of this, they suggested that a better vitamin A status can protect against drops in serum retinol into the deficient range during the acute phase response (19).

Serum zinc levels decrease during acute illness or infection due to the presence of the acute phase response and is negatively correlated with CRP levels (20,21,22). Decreased serum zinc levels have been reported in AIDS patients with opportunistic infections (23). Graham et al. showed in a nested case control study embedded in a cohort, that serum zinc levels in seropositive individuals who progressed to AIDS were significantly lower when compared to those in whom the disease did not progress. He also showed in a logistic regression model that low serum zinc and high serum copper levels predicted progression to AIDS independently of baseline CD4 lymphocyte count, age and the calorie-adjusted dietary intakes of both nutrients. Serum zinc and copper are both acute phase reactants and Graham et al. postulated that the observed effect of zinc and copper on disease progression was attributable to a direct effect of HIV-1 viral activity

resulting in the acute phase response, since patients with opportunistic infections were excluded in their study. Markers of the acute-phase response such as CRP levels, were not measured in their study (24).

Although not statistically significant, our study demonstrated a similar relationship between plasma levels of CRP and zinc to that observed between plasma retinol and CRP (p=0.1). In addition, the median plasma zinc level was lower in subjects with advanced disease compared to subjects with early disease, this difference approached significance (p=0.06). Therefore, our results suggest that the decrease in plasma zinc status with advancing disease could only be partly explained against the background of the acute phase response as a result of HIV- viral activity.

This study demonstrates that markers of the acute phase response such as raised CRP levels were associated with low plasma retinol levels in a small number of subjects. Other contributory factors such as a poor dietary intake, malabsorption and regular intercurrent infections could result in lowered serum retinol levels due to depleted hepatic retinol stores in patients with advanced HIV-disease.

8.4 Multivariate analysis with clinical stage IV (AIDS) as dependent variable

Low levels of retinol (< 30μg/dl) was independently associated with a 3-fold increase in the risk of having AIDS in this study, after adjusting for potential confounding and intermediate variables such as CD_4 lymphocyte count, CD_4:CD_8

lymphocyte ratio, haemoglobin, zinc and body weight (Table). CD_4 counts of < 200 cells/μl and a CD_4:CD_8 lymphocyte ratio of < 0.15 were associated with a 2.5 and 4-fold increase in the risk of having AIDS. Haemoglobin, zinc and body weight were not independently associated with AIDS.

Decreased CD_4 T-cells and a decreased CD_4 to CD_8 lymphocyte ratio have both been shown to be strong predictors of the progression of HIV-disease to AIDS (25,26,27,28,29). Furthermore, it has been suggested that a low TLC is also an independent predictor of HIV-disease progression and a high correlation between CD_4 T-cells and TLC has been demonstrated in patients with symptomatic HIV-disease (29,30). In this study, TLC was not significantly associated with Stage 4 disease on univariate analysis.

Semba et al. showed that wasting occurred in 38% of AIDS patients at their last visit before death. His study showed that low retinol levels (OR=4.6; 95%CI:1.8;11.3) and wasting (8.8; 95%CI:2.7-28.2) were both independent predictors of mortality (1). In our study only 6/29 (21%) of subjects with AIDS had a low BMI (< 18.5). Wasting was therefore not likely to be a strong confounder in the relationship between low plasma retinol levels and AIDS in our study.

The inclusion of haemoglobin levels < 12g/dl in the multivariate analysis resulted in a 8-10% decrease in the regression coefficient of retinol in both models. Reduced haemoglobin and haematocrit levels have been associated with low serum retinol levels in HIV-infected patients (1,6). Haemoglobin may be an intermediate

variable in the relationship between vitamin A and AIDS, since haematopoiesis is vitamin A dependent. It appears that vitamin A-deficient children are prone to the development of a mild iron-deficiency anaemia and that vitamin A supplementation may improve haematopoiesis in children who are vitamin A deficient . Vitamin A may therefore be involved in the regulation of iron release from hepatic stores (31,32,33).

However, the development of anaemia in advanced disease may signify the degree of involvement of the bone marrow via direct infection of progenitor cells by HIV (34). Several studies have shown that a low haematocrit or haemoglobin are associated with progression of HIV-disease or a decrease in CD4 counts and shorter survival in AIDS patients (25,35,36,37).

Although plasma zinc levels < 70 μg/dl was significantly associated with AIDS during univariate analysis, we were unable to demonstrate any significant relationship after adjusting for the other covariates in the multivariate model.

The inclusion of CRP levels into the multivariate model did not contribute significantly to the model, nor did it change the adjusted odds ratio of any of the covariates in the model. The lack of a significant association between raised CRP levels and AIDS is similar to the findings of Tang et al. who showed in a prospective study that raised CRP levels were not significantly associated with an increased risk of progression to AIDS (19).

Although our study showed that low serum retinol levels were associated with clinical stage IV HIV-disease or AIDS after controlling for potential confounding factors such CD_4 lymphocyte count, the cross-sectional design precludes causal inferences about this association.

8.5 Multivariate analysis with retinol as dependent variable

In this study subjects with WHO clinical stage 4 disease or AIDS had a threefold risk of having retinol levels less than 30 μg/dl, after adjusting for CD_4 lymphocyte count, body weight and haemoglobin levels.

As mentioned before, because haematopoiesis is vitamin A dependent, it is therefore not surprising that low haemoglobin levels were independently associated with low retinol levels in the multivariate model. The confounding effect of PEM was controlled by the inclusion of body weight in the final multivariate model.

CD_4 lymphocyte count and the CD_4:CD_8 lymphocyte ratio were not independent predictors of a low retinol status in this study, after adjusting for clinical stage. Clinical stage was therefore a stronger predictor of a low vitamin A status in this study. There was also no relationship between TLC and retinol.

Finally, raised CRP levels and lowered zinc levels were not significantly predictors of retinol levels less than 30 μg/dl.

References

1. Semba RD; Caiffa WT; Graham NMH et al. Vitamin A deficiency and Wasting as Predictors of Mortality in Human Immunodeficiency Virus-Infected Injection Drug Users. J Infect Dis 1995; 171:1196-202.

2. Karter DL; Karter AJ; Yarrish R et al. Vitamin a deficiency in Non-Vitamin-Supplemented patients with AIDS: A Cross-Sectional Study. J Acquir Immune Def Syn 1995; 8: 199-203.

3. Bourne LT. Dietary intake in an urban African population- with special reference to the nutrition transition. [Dissertation] Cape Town, South Africa: University of Cape Town, 1996. 300pp.

4. Underwood BA. Maternal vitamin A status and its importance in infancy and early childhood. Am J Clin Nutr 1994;59(suppl):517S-24S.

5. Nuget J and Clark S. Retinoids, differentiation and disease. Ciba Foundation Symposium Sept 1984, London, Pitman Publishing 1985.

6. Semba RD; Graham NMH; Caiaffa WT et al. Increased Mortality Associated With Vitamin A Deficiency During Human Immunodeficiency Virus Type 1 Infection. Arch Intern Med 1993; 153 : 2149- 2154.

7. Baum MK; Shor-Posner G; Lu Y et al. Micronutrients and HIV-1 disease progression. AIDS 1995; 9(9): 1051-6.

8. Cohen BE. Reversal of postoperative immunosuppression in man by vitamin A. Surg Gynecol Obstet 1979;149:658-62.

9. Coutsodis A; Kiepiela P; Coovadia HM; Broughton M. Vitamin A supplementation enhances specific IgG antibody levels and total lymphocyte numbers while improving morbidity in measles. Pediatr Infect Dis J 1992; 11:203-9.

10. Coutsoudis A; Bobat RA; Coovadia HM; Kuhn L; Tsai W and Stain ZA. The Effects of Vitamin A Supplementation on the Morbidity of Children Born to HIV-Infected Women. Am J Pub Health 1995; 85 (8): 1076- 1081.

11. Hussey GD; Hughes J; Potgieter S; Kessow G; Burgess J; Beatty D et al. Vitamin A status and supplementation and its effects on immunity in children with AIDS. Abstracts of the XVII IVACG meeting, Guatemala City p.6.

12. Coodley GO; Nelson HD; Loveless MO et al. Beta-carotene in HIV Infection. J AIDS 1993; 6: 272-276.

13. Coodley GO; Coodley MK; Lusk R; Green TR; Bakke AC et al. β-Carotene in HIV infection: an extended evaluation. AIDS 1996; 10:967-73.

14. Castell JV; Goméz-Lechón MJ; David M; Fabra R; Trullenque R; Heinrich PC. Acute-phase response of human hepatocytes: regulation of acute-phase protein synthesis by Interleukin-6. Hepatology 1990; 12:1179-86.

15. Breen EC; Rezai AR; Nakajima K; Beall GN; Mitsuyasu RT; Hirano T et al. Infection with HIV is associated with elevated IL-6 levels and production. J Immunol 1990; 144:480-4.

16. Honda M; Kitamura K; Mizutani Y; Oishi M; Arai N; Okura T et al. Quantitative analysis of serum IL-6 and its correlation with increased levels of serum IL-2R in HIV-induced diseases. J Immunol 1990; 145:4059-64.

17. Storgaard M; Laursen AL and Andersen PL. The C-Reactive Protein Responses in HIV-infected Patients with Pneumonia. Scand J Infect Dis 1993; 25:305-9.

18. Syrjälä H; Lähdevirta J; Ruutu P; Jokiph L; Jokiph AMM and Ruutu T. Acute phase response in Pneumocystis carinii Pneumonia. Scand J Infect Dis 1990; 22:713-6.

19. Tang AM; Graham NMH; Semba RD and Saah AJ. Association between vitamin A and E levels and HIV-1 disease progression. AIDS 1997; 11:613-20.

20. Sobocinski PZ; Canterbury WJ Jr; Mapes CA et al. Involvement of hepatic metallothioneins in hypozincemia with bacterial infection. Am J Physiol 1978; 234: E399-406.

21. Falchuk KH. Effect of acute disease and ACTH on serum zinc proteins. N Engl J Med 1977; 296:1129-34.

22. Craig GM; Evans SJ and Brayshaw BJ. An inverse relationship between serum zinc and C-reactive protein levels in acutely ill elderly hospital patients. Postgrad Med J 1990; 66(782):1025-8.

23. Falutz J; Tsoukas C; Gold P. Zinc as a cofactor in human immunodeficiency virus-induced immunosuppression. JAMA 1988; 259: 2850-2851.

24. Graham N; Sorenson D; Odaka N et al. Relationship of Serum Copper and Zinc Levels to HIV-1 Seropositivity and Progression to AIDS. J Acquir Immune Def Syn 1991; 4: 976-980.

25. Moss AR; Bacchetti P; Osmond D et al. Seropositivity for HIV and the development of AIDS or AIDS-related condition: three year follow up of the San Fransisco General Hospital cohort. BMJ 1988; 296:745-50.

26. Eyster ME; Ballard JO; Gail MH; Drummond JE and Goedert JJ. Predictive Markers for the Acquired Immunodeficiency Syndrome (AIDS) in Hemophiliacs: Persistence of p24 antigen and Low T4 Cell Count. Ann Intern Med 1989; 110:963-9.

27. Fahey JL; Taylor JMG; Detels R; Hofmann B; Melmed R; Nishanian P et al. The Prognostic value of Cellular and Serologic Markers in Infection with Human Immunodeficiency Virus Type 1. N Eng J Med 1990; 322(3):166-72.

28. MacDonell KB; Chmiel JS; Poggensee; Wu S and Phair JP. Predicting Progression to AIDS: Combined Usefulness of CD4 Lymphocyte Counts and p24 Antigenemia. Am J Med 1990; 89:706-11.

29. Post FA; Wood R and Maartens G. CD4 and total lymphocyte counts as predictors of HIV disease progression. Q J Med 1996; 89:505-8.

30. Beck EJ; Kupek EJ; Gompels MM and Pinching AJ. Correlation between total and CD4 lymphocyte counts in HIV infection: not making the good an enemy of the not so perfect. Int J STD AIDS 1996;7:422-8.

31. Mejiia LA; Chew F. Hematologic effect of supplementing anemic children with vitamin A alone or in combination with iron. Am J Clin Nutr 1988; 48:595-600.

32. Semba RD; Muhilal; West KP Jr et al. Impact of vitamin A supplementation on hematological indicators of iron metabolism and protein status in children. Nutr Res 1992; 12:469-78.

33. Hussey GD; Labadarios D. Vitamin A - beyond vision. CME 1992; 10(8): 1383-1391.

34. Zon LI and Groopman JE. Hematologic Manifestations of the Human Immune Deficiency Virus (HIV). Sem Hematol 1988; 25(3): 208-17.

35. Apolonio EG; Hoover DR; He Y; Saah AJ; Lyter DW; Detels R et al. Prognostic Factors in Human Immunodeficiency Virus-Positive Patients with a CD4+ Lymphocyte Count <50/μL. JID 1995; 171:829-36.

36. Blatt SP; McCarthy WF; Bucko-Krasnicka B; Melcher GP; Boswell RN; Dolan MJ et al. Multivariate Models for Predicting Progression to AIDS and Survival in Human Immunodeficiency Virus-Infected Persons. JID 1995;171:837-44.

37. Lifson AR; Allen S; Wolf W; Serufilira A; Kantarama G; Lindan CP et al. Classification of HIV Infection and Disease in women from Rwanda. Ann Intern med 1995;122:262-70.

9 Limitations

9.1 Study design

The ideal study design to investigate risk factors such as low plasma vitamin A status with the development of AIDS as endpoint or outcome measure would be a prospective cohort study. Although logistic regression indicated a significant relationship between plasma retinol and advanced disease, the cross-sectional study design used precludes any causal inferences about the association between plasma vitamin A status and the progression of HIV-disease. A modification of the basic cohort design involves inserting a case-control study into a prospective cohort study, which is often referred to as a nested case-control design. For example, blood samples could be collected from a subgroup of the cohort at baseline and at certain intervals during the follow-up period. When a sufficient number of AIDS cases had accrued, the blood results for these cases could then be compared with those subjects who had not yet developed AIDS (controls). Such an approach would be particularly efficient, in terms of both time and cost, compared to a cohort design.

Furthermore, the validity of this study may also be criticised due to the lack of HIV-seronegative controls.

9.2 Sample size

A precision level of 5% for the estimation of sample estimates is viewed as an acceptable level of precision. However, a sample size at a precision level of 5% was not attainable within the specified study period and therefore the precision of this sample was reduced to 7.5%.

Statistical significance is dependent on sample size and is derived from the standard error, which is directly related to the variability of the observation. The exclusive use of the 95% confidence level ($p < 0.05$) to assess significance has obvious drawbacks which may result in potentially clinically important observations being discarded (type II error). It has been suggested that the construction of confidence intervals provides a better interpretation of an association as it provides an indication of the magnitude of the association. Therefore, odds ratios derived from logistic modelling are presented as 95% confidence intervals.

Non-parametric statistics was used in this study due to the non-normality of the data. An obvious drawback that resulted from this is that there are no non-parametric post-hoc tests available for further investigation of a significant relationship between more than two variables.

Finally, the external validity or generalisability of this study to the HIV-positive population attending health facilities in South Africa is limited by the small sample size.

9.3 Bias and validity of instruments

Selection bias in this sample may explain the observed differences in selected proxies for socio-economic status such as educational status, employment and fridge ownership between subjects with early and advanced disease. On the other hand, it seems feasible that subjects with advanced disease would be more likely to become unemployed than subjects with early disease.

Plasma retinol is a commonly used biochemical measure of vitamin A status. It should be remembered that plasma levels only become predictive of an individual's status when body reserves has been critically depleted. Therefore, the Relative Dose Response test which was not employed in this study, show greater validity as a marker of marginal vitamin A stores than a single plasma sample. Plasma zinc levels show diurnal and postprandial variation and therefore the validity of plasma zinc levels in this study may also be critized in view of the fact that fasting blood samples were not taken.

Another limitation of this study is that the status of other micronutrients were not assessed. Deficiencies of other micronutrients such as vitamin E, B_6 , B_{12} and selenium may also influence cell-mediated immunity in HIV disease.

10 Conclusions and Recommendations

This study demonstrated that a significant proportion of subjects, especially those with advanced disease, presented with low plasma retinol levels in the absence of opportunistic infections, which highlights the potential magnitude of this public health problem.

Controlled trials are required to evaluate the effect of vitamin A supplementation on the progression of HIV disease and survival of AIDS patients in developing countries such as South Africa where the majority of the HIV- infected population are likely to have a marginal vitamin A status. High dose vitamin A supplementation has been associated with improved immune function in children with measles and AIDS and has also been associated with reduced morbidity in HIV-infected children (1,2,3). However, a recent clinical trial showed that multivitamins, not vitamin A, resulted in a significant increase in immune function of pregnant HIV-infected women. Multivitamin supplementation also decreased the risk of foetal death, low birthweight, severe preterm birth and small for gestational age. With regard to the lack of a beneficial evidence for the role of vitamin A, Fawzi et al. suggests that serum retinol concentrations are markers of the stage of HIV-

disease or intermittent infections due to the acute phase response, rather than causally related to adverse birth outcomes (4). The results of similar trials which are being conducted in South Africa, Malawi and Nepal, are awaited.

High circulating HIV viral load has been shown to be a risk factor for HIV progression (5). It has been suggested that the relationship between vitamin A levels and viral load is of potential importance, since in vitro studies suggest that vitamin A supplementation may up-regulate the expression of HIV (6). However, a recent randomized controlled trial of high dose supplementation in HIV-infected adults showed that vitamin A supplementation had no effect on viral load (7). This would suggest that vitamin A deficiency and viral load are independent risk factors for the progression of HIV disease.

In Sub-Saharan Africa where antiretroviral therapies are prohibitively expensive, the optimization of micronutrient status may be among the affordable strategies available for potentially prolonging survival in HIV disease and reducing vertical transmission of HIV. Vitamin A or β-carotene supplementation may prove to be an effective low cost intervention strategy to improve the morbidity and mortality of HIV-infected adults and children.

11 References

1. Coutsodis A; Kiepiela P; Coovadia HM; Broughton M. Vitamin A supplementation enhances specific IgG antibody levels and total lymphocyte numbers while improving morbidity in measles. Pediatr Infect Dis J 1992; 11:203-9.

2.Coutsoudis A; Bobat RA; Coovadia HM; Kuhn L; Tsai W and Stain ZA. The Effects of Vitamin A Supplementation on the Morbidity of Children Born to HIV-Infected Women. Am J Pub Health 1995; 85 (8): 1076- 1081.

3. Hussey GD; Hughes J; Potgieter S; Kessow G; Burgess J; Beatty D et al. Vitamin A status and supplementation and its effects on immunity in children with AIDS. Abstracts of the XVII IVACG meeting, Guatemala City p.6.

4. Fawzi WW; Msamanga GI; Spiegelman D; Urassa EJN; McGrath N; Mwakagile D et al. Randomised trial of effects of vitamin supplements on pregnancy outcomes and T cell counts in HIV-infected women in Tanzania. Lancet 1998; 351:1477-1482.

5.Ferre F; Moss RB; Daigle A; Richieri SP;Jensen F and Carlo DJ.Viral load in peripheral blood mononuclear cells as surrogate for clinical progression. J Acquire Immune Def Syndr Hum Retrovirol 1995; 10 Suppl 2:51-56.

6. Turpin JA; Vargo M and Meltzer MS. Enhanced HIV-1 replication in retinoid-treated monocytes:retinoid effects mediated through mechanisms related to cell differentiation and to direct transcriptional action on viral gene expression. J Immunol 1992; 148:2539-2546.

7. Semba RD. Vitamin A and human immunodeficiency virus infection. Proc Nutr Soc 1997; 56:459-469.

12 Appendices

APPENDIX I

Proposed World Health Organization Staging System for HIV Infection and Disease

Table 1. List of clinical conditions by clinical stage*.

Clinical stage 1
1. Asymptomatic infection (ASY)
2. Persistent generalized lymphadenopathy (PGL)
3. Acute retroviral infection (ARI)

Performance scale 1: asymptomatic, normal activity

Clinical stage 2
4. Unintentional weight loss (WL), < 10% of body weight
5. Minor mucocutaneous (mcs) manifestations (e.g. seborrheic dermatitis, prurigo, fungal nail infections, oropharyngeal ulcerations, angular cheilitis)
6. Herpes zoster (HZV), within the previous 5 years
7. Recurrent upper respiratory tract infections (URTI) (e.g., bacterial sinusitis)

And/or performance scale 2: symptoms, but nearly fully ambulatory

Clinical stage 3
8. Unintentional WL, > 10% of body weight
9. Chronic diarrhoea (DIA), > 1 month
10. Prolonged fever (PYR) (intermittent or constant) > 1 month
11. Oral candidiasis (ORC) (erythematous or pseudomembranous)
12. Oral hairy leukoplakia (HLP)
13. Pulmonary tuberculosis (PTB) (typical or atypical), within the previous year
14. Severe bacterial infections (BAC) (e.g., pneumonia, pyomyositis)
15. Vulvovaginal candidiasis (VVC, chronic (> 1 month) or poorly responsive to therapy

And/or performance scale 3: in bed < 50% of normal daytime, but > normal, during previous month

Clinical stage 4†
16. HIV wasting syndrome (CAC)
17. *Pneumocystis carinii* pneumonia (PCP)
18. Toxoplasmosis of the brain (TOXO)
19. Cryptosporidiosis with diarrhoea (CRS), > 1 month
20. Isosporiasis with diarrhoea (ISO), > 1 month
21. Cryptococcosis (CRC), extrapulmonary
22. Cytomegalovirus (CMV) disease of an organ other than liver, spleen or lymph node
23. Herpes simplex virus (HSV) infection, mucocutaneous (> 1 month) or visceral (any duration)
24. Progressive multifocal leukoencephalopathy (PML)
25. Any disseminated endemic mycosis (MYC) (e.g., histoplasmosis, coccidioidomycosis)
26. Candidiasis of the oesophagus, trachea, bronchi or lungs (OEC)
27. Atypical mycobacteriosis, disseminated (MAI)
28. Non-typhoid *Salmonella* septicaemia (SAL)
29. Extrapulmonary tuberculosis (ETB)
30. Lymphoma (LYM)
31. Kaposi's sarcoma (KS)
32. HIV encephalopathy (ADC)

And/or performance scale 4: in bed > 50% of normal daytime during previous month

See Appendix I for definitions of clinical conditions. *This is a modification of the original list proposed by the International Collaborating Group, and includes three additional clinical conditions: ARI (number 3), VVC (number 15), and ISO (number 20), which were not evaluated in the present study. †For conditions listed in Stage 4, either a definitive or a presumptive diagnosis is acceptable, according to Appendix I, which reflects, with slight modifications, criteria previously published by the Centers for Disease Control [12].

Appendices

I. Definitions and diagnostic criteria for the clinical conditions listed in Table 1

These consensus criteria were established during a Consultation convened by WHO/GPA during the VII International Conference on AIDS (Florence, June 1991), with the following participants: P. Cahn (Argentina); B. Tindall (Australia); R. Colebunders (Belgium); M. Schechter (Brazil); J.S.G. Montaner (Canada); A. Lazzarin (Italy); R. Coutinho, R. Keet (The Netherlands); J. González-Lahoz (Spain); R. Mugerwa (Uganda); J. Curran, A. Saah (USA); B. Kapita (Zaire); D. Heymann, M. Karam, P. Crocchiolo (WHO).

Definitions of HIV-related clinical conditions

Asymptomatic infection: the absence up to the time of examination, as determined by the subject's history, of any HIV-related symptom since the time of infection with HIV.

Persistent generalized lymphadenopathy: palpable lymphadenopathy (lymph node enlargement of at least 1 cm) at two or more extrainguinal sites persisting for more than 3 months in the absence of a concurrent illness or condition other than HIV infection to explain the findings.

Acute retroviral infection: unexplained acute febrile illness, with or without aseptic meningitis, associated with seroconversion for HIV antibody.

Unintentional weight loss: percentage of total body weight loss compared with baseline value or with the highest value recorded during preceding assessments.

Chronic diarrhoea: loose stools three or more times a day, continuously or episodically, for more than 1 month.

Prolonged fever: fever > 38°C, intermittent or constant for more than 1 month (if self-reported, documented at least once).

Oral candidiasis: erythematous: red area without removable plaques, often on palate, dorsum of the tongue and buccal mucosa; pseudomembranous: white or yellow removable plaques anywhere in the oral cavity.

Oral hairy leukoplakia: white, non-removable lesions, usually bilaterally on the margins of the tongue. The surface is corrugated, but could be non-corrugated if on the inferior surface of the tongue.

Pulmonary tuberculosis: typical: disease caused by acid-fast mycobacteria, involving the lungs (including pleuritis and parahilar lymphadenitis), presenting with apical cavitation and/or pleural effusion; atypical: as above, but presenting without apical cavitation and pleural effusion.

From: AIDS 1993; 7(5): 711 - 8.

APPENDIX II

QUESTIONNAIRE: VITAMIN A STUDY

RECORD NUMBER: GSH.....

DATE: ..../..../1996

1. PATIENT NAME:.....

   FOLDER NUMBER:.....

2. Gender:

Male 1	Female 2

3. Age at present:

4. Address: .....

   .....

SOCIO-ECONOMIC DATA: TO BE COMPLETED FOR ALL PATIENTS

5. What type of house do you live in?

Formal house 1	Informal housing 2

6. How many rooms are there (excluding toilets and bathrooms) in the house/ place that you live?

	rooms

7. How many people (including children) sleep in the house most of the week?

	people

8. Do you have electricity in the house?

Yes 1	No 2	Don't know 3

9. Is there a working fridge in your house ?

Yes 1	No 2	Don't know 3

10. Did you attend school ?

Yes 1	No 2

IF YES, GO TO 11.
IF NO, GO TO 12.

11. What is the highest standard that you passed ?

Less than Std 5 1	Std 5 2	Std 8 3	Std 10 4	Tertiary education 5

12. Do you work for money or goods at present ?

Yes 1	No 2

IF YES, GO TO 13.
IF NO, GO TO 14.

13. What type of work do you do for money or goods ?

Please specify .....

14.1 If you not working are you ?

Unemployed 1	Disability grant 2	Student 3	Homemaker 4	Other 5

If other, please specify .....

14.2 How long have you not been working ?

		months

15. Do you drink alcohol at present ?

Yes 1	No 2

IF NO, GO TO 16.
IF YES, GO TO 17.

16.1 Have you ever drunk alcohol in the past ?

Yes 1	No 2

16.2 How long ago did you stop drinking alcohol ?

			months

ANTHROPOMETRIC DATA: TO BE COMPLETED FOR ALL PATIENTS

17. Weight:

			,	kg

18. Height:

				cm

19. Body Mass Index (BMI):

		,	kg/m^2

CLINICAL DATA: TO BE COMPLETED FOR ALL PATIENTS

20. Time since HIV diagnosis:

			months

21. Temperature:

		,	C

APPENDIX III

The LOGISTIC Procedure

Data Set: WORK.DATA1
Response Variable: STAGE4
Response Levels: 2
Number of Observations: 125
Link Function: Logit

Response Profile

Ordered Value	STAGE4	Count
1	1	32
2	2	93

Model Fitting Information and Testing Global Null Hypothesis BETA=0

Criterion	Intercept Only	Intercept and Covariates	Chi-Square for Covariates
AIC	144.208	132.223	.
SC	147.036	149.193	.
-2 LOG L	142.208	120.223	21.985 with 5 DF (p=0.0005)
Score	.	.	20.543 with 5 DF (p=0.0010)

Analysis of Maximum Likelihood Estimates

Variable	DF	Parameter Estimate	Standard Error	Wald Chi-Square	Pr > Chi-Square	Standardized Estimate	Odds Ratio
INTERCPT	1	-5.0370	2.0456	6.0628	0.0138	.	.
RET30	1	1.2056	0.5109	5.5684	0.0183	0.333404	3.339
NEWCD4	1	0.9242	0.4705	3.8592	0.0495	0.254801	2.520
NEWHB	1	0.3733	0.4796	0.6058	0.4364	0.103238	1.453
WT	1	-0.0119	0.0183	0.4184	0.5178	-0.105558	0.988
ZINC70	1	0.5548	0.4778	1.3486	0.2455	0.142293	1.742

Association of Predicted Probabilities and Observed Responses

Concordant = 77.0%	Somers' D = 0.542
Discordant = 22.7%	Gamma = 0.544
Tied = 0.3%	Tau-a = 0.208
(2976 pairs)	c = 0.771

The LOGISTIC Procedure

Data Set: WORK.DATA1
Response Variable: STAGE4
Response Levels: 2
Number of Observations: 125
Link Function: Logit

Response Profile

Ordered Value	STAGE4	Count
1	1	32
2	2	93

Model Fitting Information and Testing Global Null Hypothesis BETA=0

Criterion	Intercept Only	Intercept and Covariates	Chi-Square for Covariates
AIC	144.208	128.460	.
SC	147.036	145.430	.
-2 LOG L	142.208	116.460	25.748 with 5 DF (p=0.0001)
Score	.	.	25.464 with 5 DF (p=0.0001)

Analysis of Maximum Likelihood Estimates

Variable	DF	Parameter Estimate	Standard Error	Wald Chi-Square	Pr > Chi-Square	Standardized Estimate	Odds Ratio
INTERCPT	1	-5.1798	2.0340	6.4853	0.0109	.	.
RET30	1	1.0684	0.5246	4.1473	0.0417	0.295471	2.911
NEWCD4R	1	1.3702	0.4947	7.6721	0.0056	0.346223	3.936
NEWHB	1	0.4250	0.4894	0.7539	0.3852	0.117521	1.530
WT	1	-0.00886	0.0186	0.2257	0.6347	-0.078821	0.991
ZINC70	1	0.2893	0.5071	0.3255	0.5683	0.074196	1.335

Association of Predicted Probabilities and Observed Responses

Concordant = 77.4%	Somers' D = 0.553
Discordant = 22.1%	Gamma = 0.556
Tied = 0.5%	Tau-a = 0.212
(2976 pairs)	c = 0.776

The LOGISTIC Procedure

Data Set: WORK.DATA1
Response Variable: RET30
Response Levels: 2
Number of Observations: 126
Link Function: Logit

Response Profile

Ordered Value	RET30	Count
1	1	65
2	2	61

Model Fitting Information and Testing Global Null Hypothesis BETA=0

Criterion	Intercept Only	Intercept and Covariates	Chi-Square for Covariates
AIC	176.546	159.358	.
SC	179.382	173.539	.
-2 LOG L	174.546	149.358	25.188 with 4 DF (p=0.0001)
Score	.	.	22.780 with 4 DF (p=0.0001)

Analysis of Maximum Likelihood Estimates

Variable	DF	Parameter Estimate	Standard Error	Wald Chi-Square	Pr > Chi-Square	Standardized Estimate	Odds Ratio
INTERCPT	1	-0.1116	1.3385	0.0069	0.9336	.	.
NEWCD4	1	0.3053	0.4122	0.5487	0.4589	0.084121	1.357
NSTAGE3	1	1.2248	0.5062	5.8550	0.0155	0.295108	3.404
NEWHB	1	0.8585	0.4013	4.5756	0.0324	0.237328	2.360
WT	1	-0.0287	0.0151	3.6101	0.0574	-0.257419	0.972

Association of Predicted Probabilities and Observed Responses

Concordant	= 72.8%	Somers' D	= 0.460
Discordant	= 26.9%	Gamma	= 0.461
Tied	= 0.3%	Tau-a	= 0.231
(3965 pairs)		c	= 0.730

The LOGISTIC Procedure

Data Set: WORK.DATA1
Response Variable: RET30
Response Levels: 2
Number of Observations: 126
Link Function: Logit

Response Profile

Ordered Value	RET30	Count
1	1	65
2	2	61

Model Fitting Information and Testing Global Null Hypothesis BETA=0

Criterion	Intercept Only	Intercept and Covariates	Chi-Square for Covariates
AIC	176.546	157.857	.
SC	179.382	172.038	.
-2 LOG L	174.546	147.857	26.689 with 4 DF (p=0.0001)
Score	.	.	23.893 with 4 DF (p=0.0001)

Analysis of Maximum Likelihood Estimates

Variable	DF	Parameter Estimate	Standard Error	Wald Chi-Square	Pr > Chi-Square	Standardized Estimate	Odds Ratio
INTERCPT	1	-0.6476	1.3752	0.2218	0.6377	.	.
NEWCD4R	1	0.6813	0.4791	2.0221	0.1550	0.171747	1.976
NSTAGE3	1	1.1102	0.5175	4.6033	0.0319	0.267493	3.035
NEWHB	1	0.8733	0.4012	4.7375	0.0295	0.241436	2.395
WT	1	-0.0268	0.0151	3.1509	0.0759	-0.240296	0.974

Association of Predicted Probabilities and Observed Responses

Concordant = 73.1%		Somers' D = 0.467	
Discordant = 26.5%		Gamma = 0.469	
Tied = 0.4%		Tau-a = 0.235	
(3965 pairs)		c = 0.733	