ACTA MÉDICA PORTUGUESA 1994; 7: 9-14

COMPARATIVE STUDY OF VARIOUS BIOLOGICAL MARKERS IN INFECTION BY HIV1

J. E SERRA, V. POMBO, R. CÔRTE-REAL, SARAIVA DA CUNHA, A. MELIÇO-SILVESTRE
Infectious Diseases Clinic, Coimbra University Hospital, Coimbra.

SUMMARY

A study was performed on 51 human immunodeficiency virus (HIV 1)- infected patients with a previous history of drug abuse. By the CDC staging system for HIV infection they were mainly in advanced stages of that infection, 67% were in IV-A and 8% in IV-CI. Patients were divided in two groups, one composed of 33 individuals who needed AZT therapy and the other of those who did not need that medication (18 patients). Between 3 and 18 months several parameters were assessed on 3 different occasions, according to standard tecnhiques: red blood cells and platelet numeration, CD4 and CD8 cell counts, HIV antigen (p24 Ag), ß2microglobulin, high density serum lipoproteins (HDL), and anticardiolipin antibodies (ACA). In the patients treated with AZT the first bioserologic evaluation was performed before starting this therapy. Finally it was observed that p24 AG and ACA were present in 21% of the patients, all of them in advanced stages of HIV infection and under AZT therapy. A significant correlation was only found between CD4 counts and ß2-microglobulin (R=0,34; P=0,0001).

INTRODUCTION

Infection by the human immunodeficiency virus (H.I.V.) leads to a complex and progressive immunosuppression, which, in its final stages, is manifested by the Acquired Immunodeficiency Syndrome (AIDS)[1].

The immune deficit in this infection is primarily cellular, shown by a reduction in the number and function of CD4 positive lymphocytes, there also being monocyte and macrophage infection. These alterations, on the whole, propitiate the advent of the typical A.I.D.S. complications, the infections and tumors which define this syndrome[1,2]. There is also a compromise of the humoral immunity, which will lead to several anomalies, of which we emphasise: the polyclonal stimulation of B lymphocytes, as well as the occurrence of reduced immune response of antibodies (Ab.s) to antigenic stimulation, to name but a few[1,3].

For a long time attempts have been made to anticipate the occurrence of the typical complications of the final phases of this infection, by the quantity of T lymphocytes, and namely their subclasses[1,2,4,9]. Thus the CD4 cell count becomes increasingly important given the tropistic recognition of HIV for these cells. It is also known that complications related with HIV infection arise, as a rule, when the number of CD4 lymphocytes is greatly reduced[1,2,3,10]. However, it was verified that in the asymptomatic seropositive the monitoring of CD4 cells, isolated, did not *serve* as a prognostic marker, given the great individual variability in the speed of its decline[4,8,11].

On the other hand, there is a strong suggestion that immune activation is an important aspect in the pathogenesis of HIV infection, which has come to be confirmed by the monitoring of different serologic markers[3,4,6]. Thus we quote the staged dosages of Neopterin and ß2 microglobulin as an example of the most exhaustively studied. Their staged assessment in a subject who is a carrier of HIV infection has made it possible to foresee the occurrence of AIDS, either in conjunction with the CD4 determinations or still separately[2,4,6,7,12].

Thus, it is clearly established today that a sole biological marker, trustworthy marker of the progression of the disease, does not exist in this infection. This has conditioned the experimentation of multiple parameters to anticipate the progression of HIV infection[1,2,4,7,13-18].

However, the importance of monitoring these parameters does not exhaust their value, as prognostic markers, in what concerns this infection. It has also become important for the choice of the ideal moment to begin prophylactic and suppressive medication as well as the assessment of the response to this treatment[1,2,6,9,15,19,20,21].

Received for publication: 5 November, 1994

Even in what concerns CD4 cell count (the most widely used marker in the follow up of the HIV+ subject) there are doubts as to its relative value. Quoting, as an example, the preference given to the percentage of CD4 in studies on the HIV+ male homosexual[7], to the detriment of its absolute value, preferred by those who have studied drug users infected by these viruses[10]. There are still those who disregard the CD4 count in favour of the CD4/CD8 association, in the immunological follow up of the HIV seropositive subject[6].

The general consensus, however, indicates that in the correct monitoring of the asymptomatic HIV+ subject the T lymphocyte subclass count should be made use of along with one or more markers of immune activation, namely neopterine and/or ß2 microglobulin, to mention only two amongst the most important[1,3,6,7].

Other multiple tests and experiments have been made with this purpose, that is, to monitor the subject infected by the human immunodeficiency virus, there being some controversy regarding the validity of the results obtained[2,3,5,8,10,13,19,22]. On this issue we may mention the variable interpretation from the prognostic point of view due to the presence of anti-cardiolipine Ab.s (A.C.A.) in what concerns HIV infection[14,17,18,23-25]. However, there is at least a consensus on the suggestion forwarded by most Authors that the presence of these Ab.s, at least, must be linked with an altered immunological response related with HIV infection, independently of whether or not its prognostic value is legitimate regarding this infection[17,18,23-26]. In an attempt to stress how, even today, the monitoring of a patient infected with HIV is not peaceful, a decade after the first cases of this infection were described, it remains to be determined which *parameter* (if it does exist) allows a rigorous prevision of the course of this infection[1,2,14,15].

Taking these bases into account, we were also tempted to seriately assess some of these parameters, as well as their variation after the administration of anti-viral therapy with different doses of AZT.

METHODS

We conducted a prospective study involving a population of 51 ex-HIV+ drug addicts, the majority in an advanced stage of the disease. The population studied was divided into 2 large groups, according to whether they need (group B-n=33) or not (group A-n=18) anti-viral therapy with Azidotimidine (AZT). The group of 33 subjects under AZT therapy was subdivided into 5 sub-groups, according to the doses administered (group C-n=15, AZT 500 mg/day; group D-n=18, AZT 1000 mg/day) and the duration of its administration (group E-n=6, AZT <3; group F-n=13, AZT>3<9 months; group G-n=14, AZT>9 months).

The parameters assessed in 3 seriate samples, consisted of: haemogramme with platelet count and leukocyte formula, seriate HDL, study of the lymphocyte populations, ß2 dosage, and studies of the presence or absence of p24 Antigen (Ag p24) and of A.C.A.

In the group of 18 subjects who did not do AZT, the 3 samples were collected in series over a period of 18 months. In the remaining population, 33 subjects under AZT therapy, the 1st sample was collected before beginning anti-viral therapy. The duration of AZT administration corresponded to the date of the 3rd sample.

For the study of the lymphocytic populations monoclonal antibodies were used as markers and the reading made in *Epias Profile II* flux cytometry. The study of Ag p24, A.C.A. and dosage of ß2 was made according to an immunoenzymatic method.

The results were analysed statistically by the *Student* τ test for matched values, with values of $p \leq 0,05$ being considered significant.

RESULTS

Sex and Age - Males were the most represented being 83% of the total number of subjects studied, the remaining 17% females. Ages varied between 20 and 45 years, 89% in the age group between 20 and 30 years.

Race and Nationality - The subjects were all Caucasians. The predominant nationalities were Spanish and Italian, 51% and 41% of the cases respectively; only 6% were Portuguese and 2% Polish.

Epidemiology - The group studied consisted of ex-drug users of narcotics of parenteral administration (heroin and cocaine). The time of previous consumption of these drugs varied between 3 and 16 years, with an average of 9 years. The time of abstinence varied between 1 and 8 years in 65% of the cases, the remaining 35% varying between 14 days and 11 months.

All subjects affirmed heterosexual habits. In 95% of the cases the number of partners per year was greater than 4; none of the subjects affirmed the regular use of condoms.

C.D.C. classification for HIV infection - The whole population studied was infected with type 1 HIV, distributed in the following way, according to the CDC classification: 1,9% of cases belonged to group II, 11,7% to group III and IV-E, 66,7% to groups IV-A and the remaining 7,8% to group IV-C1.

Comparative Analysis of the Biological Markers studied - Of the analysis of table 1 we may observe the attempt to establish a comparison between the results obtained by the seriate study of the various markers analysed, namely the study of the lymphocytic populations, the dosage of B2 and HDL, with the subgroups formed in the population studied.

There was always the concern of knowing the distribution of patients, in each subgroup, according to the CDC classification for HIV infection. For a greater systematization we will attempt to compare the results obtained by concurrent analysis of the following groups, as regards:

- 1st The absence or presence of treatment, groups A and B respectively;
- 2nd The dose of AZT applied, groups C and D;
- 3rd The duration of AZT use, groups E,F,G.

1st – Group A consisted of 18 patients without AZT, assessed over 18 months. A discrete reduction (↓) of CD4 was observed, in its absolute number (from 817/mm^3 to 728/mm^3), in its percentage (from 30.6% to 28.6%),

Table 1 – Comparative Study of various biological markers in infection by HIV

Total	CDC	Sample	N.º CD4 [cell/mm³]	CD4 [%]	CD8 [cell/mm³]	CD4/CD8	ß2 [mg/l]	HDL [mg/l]
N=51	II-1							
	III-6	1 st	506±334	23.3±9.7	1036±473	.52±.35	2.39±.84*	40.4±13.56
	IVA-34	2 nd	490±298	23.0±9.7	1088±461	.50±38	2.16±.60	38.6±12.2
	IVC1-4	3 rd	490±307	22.5±10.6	1186±690	.48±.34	2.27±.60	40.2±13.19
	IVE-6							
N=18	II-1	1 st	817±356	30.6±9.12	1198±527	.67±.37	1.84±.46	38.94±8.03
[S/AZT]	III-6	2nd	731±322	29.6±10	1094±496	.72±.49	1.81±.35	38.44±6.41
group A	IVA-11	3 rd	728±328	28.6±11.5	1309±743	.67±.43	1.97±40◊	40.63±17.03
N=33	IVA-23	1 st	336±119	19.39±7.21	948±423*	.39±.25	2.69±85*	41.27±15.84
[C/AZT]	C1-4	2 nd	358±157	19.42±6.41	1084±449	.37±.20	2.35±.63	38.68±14.56
group B	IVE-6	3 rd	331±153	18.55±7.87	1107±655	.35±.19	2.47±.63	40.63±17±03
N=15	IV-14	1 st	342±119	18.00±4.62	1109±351	.33±.14	2.64±.84*	40.46±17.65
group C	IVC1-1	2 nd	377±146	18.90±5.6	1067±405	.36±.19	2.34±.63	36.33±10.25
AZT 500 mg		3 rd	351±184	18.00±7.11	11119±630	.32±.14	2.54±.63	40.90±20.51
N=18	IV-10	1 st	331±177	20.55±8.78	814±432*	.44±.31	2.70±89*	41.94±14.66
groupD	IVC1-3	2 nd	343±168	19.83±7.10	1098±494	.37±0.2	2.30±.65	40.83±17.40
AZT 1000mg	IVE-5	3 rd	317±132	18.93±8.56	1098±693	.39±.22	2.42±.64	40.43±14.90
N=6	IVA-5	1 st	374±111	18.33±2.65*	1172±446	.32±.07	2.56±.41	30.16±8.20
groupE	IVE-1	2 nd	384±97.4	20.50±2.07	1151±452	.41±.25	2.05±.24	34.66±7.28
AZT<3M								
N=13	IVA-12	1 st	316±83	19.76±5.23	936±423	.39±.15	2.57±.80	46.42±21.16
groupF	IVE-1	2 nd	360±141	20.76±7.45	1086±440	.38±.19	2.31±.54	43.15±18.26
AZT>3<9M		3 rd	361±137	20.38±7.76	1138±631#	.38±.20	2.47±.42	45.76±21.58
N=14	IVA-6	1 st	339±210	19.50±9.96	863±409	.41±.36	2.85±1.04	39.07±10.88
group G	IVC1-4	2 nd	346±195	17.71±6.54	1054±485	.34±.20	2.52±.78	36.50±12.70
AZT	IVE-4	e rd	294±162	16.85±7.87	1083±722	.31±.17	2.47±.79#	35.85±9.96
>9M								

*p between 1 st and 2 nd<0.05; #p between 1 st and 3 rd<0.05; ◊p between 2 nd and 3 rd<0.05

although it remains, always, within the normal figures. That is, in time, a ↓ of those figures was verified, but without significant differences between the samples collected. Despite the increase (↑) in CD8, only the value of B2 increased significantly between the 2nd and 3rd samples. From these initial results, we would be led to conclude that despite the *constant* values of CD4, the ↑ in CD8 and particularly B2 seemed to indicate the progression of the infection, in this group of 18 asymptomatic HIV+ subjects, if we consider the increase in ß2 as a good prognostic marker in HIV infection[1-4,6,7,12,16,20,27].

In group B, which consisted of 33 patients under antiviral treatment, a transitory ↑ in the N° of CD4, was observed, as would be expected (the % remaining constant), after the administration of AZT, develops without significant differences between the samples collected. This could be explained by the short assessment time of our patients, by the heterogeneity of the group studied, as regards the stage of HIV infection, and also the dosage and duration of AZT, as well as by the possible transitory anti-retroviral effect of AZT[2,19,20,21,28-31]. The CD8 and ß2 values have already shown significant differences. These last results will concur not only in what concerns the ↑ found for CD8, on one hand, with what is known of the natural history of HIV infection[1,3,6,15], as well as the effects of AZT on those cells; the ↓ of the ß2 values, on the other hand, may be interpreted as an indirect marker of AZT antiviral effect, with a different and independent significance to that obtained through CD4 monitoring, as was shown here[2,6,7,20,21,27].

2nd – We now move on to the analysis of the results of the parameters studied, according to the doses of antiviral given they were 500 or 1000 mg/day, groups C and D respectively. Only the ↓ of ß2 in both groups can be referred to as significant ↑ in the value of CD8 was verified in both groups. However they only proved to be significant , between the 1st and 2nd samples, and for the group of patients of group D. Also, in what concerns the side effects of AZT, significant macrocytosis was found in both groups after its introduction, that is, between the 1st and 2nd samples.

Nevertheless, in the group of patients under a greater dose of AZT, this type of toxicity endured, manifesting

itself also by a significant increase between the 1st and 3rd samples ($p<0.0001$). These findings are in agreement with what has been stated in literature, that is: lower doses of AZT will have anti-retroviral effects similar to the larger doses (if not even greater), will have more long-lasting benefits, also presenting its lower toxicity as an advantage[29,31]. The most effective doses of AZT remain, however, to be determined, as well as the best times to begin administering the drug and to substitute it with other anti-virals[20,29,31,32].

3rd – In the series of patients grouped by duration of AZT medication we now compare the results obtained in the above mentioned parameters. The patients of this series were subdivided into three groups according to the duration of therapy with AZT, being <3 months; >3<9 months; and >9 months. Despite the duration of AZT medication being fundamentally different, the three groups presented some similarity in what concerns the doses of the drug applied. As a matter of fact the majority of patients in groups E and F were on 500mg of AZT, 4 of the 6 patients in group E and 8 of the 13 patients in group F, respectively. From the results obtained it is worth pointing out as significant the increase in the percentage of CD4 found only in group E, among the 2 samples analysed. It would be bold to draw any conclusion from this finding. However, we do not wish to omit the fact that the 6 patients of this group were in earlier stages of HIV infection, in relation to the others of the remaining groups, as may be verified in Table 1, and therefore with base CD4 values relatively greater than that of the remaining groups. Group F only presented significant values in the increase of CD8 between the 1st and 3rd samples. A possible explanation for this isolated finding in relation to the other two groups could be the moment of the 2nd collection of samples: on average, this was done at 5 months in group F; in group E the 2nd and last collection of samples was made at 12 weeks, probably conditioning an insufficient duration of treatment!; in group G that collection was made, on average, at twelve months, at which point resistance to AZT would already be possible[2,19,28,32].

Lastly, in group G we found a reduction close to the values considered statistically significant, for HDL, with a value of $p<0.051$ between the 1st and 3rd samples, this result being in accordance with what has been stated by some Authors[2]. A significant reduction in the value of B2 between the 1st and 3rd samples was also observed only in group G in this series. Once more we are tempted to explain this finding with the duration of assessment time, relatively longer in this group as regards the remaining groups, groups E and F[2,4]. However, there are those who advise the seriate dosage of B2 to verify the efficacy of anti-viral treatment, as an earlier indicator of the benefits of that treatment[20,21].

In the population studied it was only possible to find a significant correlation between the n° of CD4 and B2 microglobulin ($R=0.34$; $p=0.0001$) *(Figure 1)*.

Let us now turn to the study of the results presented in Table 2, noting the relative prevalence of ACA and Agp24, according to the groups previously described, always bearing in mind the stages of the patients.

DISCUSSION

Therefore, in the total population studied consisting of 51 seropositive patients for anti-HIV1 an overlapping relative prevalence was observed, regarding the presence of ACA and Agp24, in other words, their presence was of 21.6% and 20% respectively.

Table 2 – Comparative study of the various biological markers in HIV1 infection

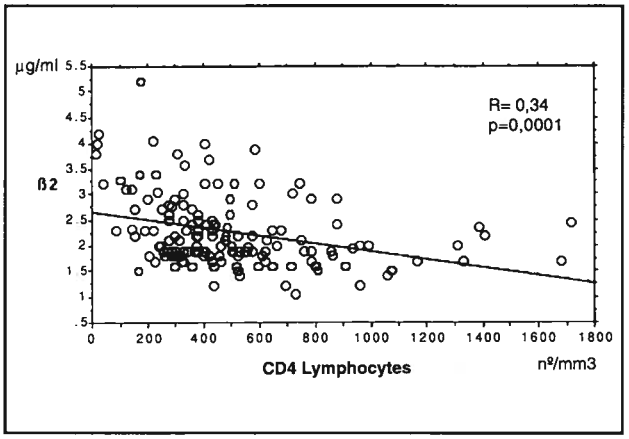
	CDC	ACA	Agp24	ACA+Agp24*
	II-1	0	0	
	III-6	0	0	
	IV-A-34	7	6	
	IV-C1-4	3	2	
N=51	IV-E-6	1	2	
	% Total	(21.6%)	(20%)	
[without AZT]				
	II-1	0	0	
(group A)	II-6	0	0	
N=18	IV-A-11	0	0	
	% Total	(0%)	(0%)	
[with AZT]	IV-A-23	7	6	3
(group B)	IV-C1-4	3	2	1
N=33	IV-E-6	1	2	
	% Total	(33%)	(30.3%)	
AZT 500mg	IV-A-14	3	5	2
(group C)N=15	IV-C1-1	1	1	1
	% Total	(33.3%)	(40%)	
AZT 1000mg	IV-A-10	3	1	1
(group D)	IV-C1-3	2	1	
N=18	IV-E-5	1	2	
	% Total	(33.3%)	(22.2%)	
AZT <3M	IV-A-5	1	0	
(group E)	IV-E-1	0	1	
N=6	% Total	(16.6%)	(16.6%)	
AZT >3>9M	IV-A-12	3	3	1
(group F)	IV-E-1	0	0	
N=13	% Total	(23%)	(23%)	
AZT>9M	IV-A-6	3	2	2
(group G)	IV-C1-4	3	2	1
N=14	IV-E-4	1	2	
	% Total	(50%)	(40.2%)	

CDC - CDC classification; ACA - Anticardiolipine antibodies; Agp24 - Antigen p24; *Only 4 common patients in total: 3 of group IV-a and 1 of group IV-C1

It is in a way important to point out that this occurred only in the subjects in need of anti-retroviral treatment. They were always absent in the group A subjects. On a first analysis of these results it seems possible to conclude from their presence, only in patients in advanced stages of HIV infection, an obvious prognostic connotation, which has presently become widely contested, regarding the ACA[17,18,23,26].

In addition, and contrary to what has been stated by some, the greater prevalence of ACA in HIV infection may be due to subjects who are carriers of Immune Thrombocytopenia[25], this would not be the case in our patients, in which of the 11 presenting ACA, only one was a carrier of thrombocytopenia. Moreover, if there are doubts as to the prognostic value of ACA in what concerns HIV infection, there is unanimity regarding their high prevalence in this infection[14,17,18,25,26].

In what concerns Agp24, and despite the possibility of its prevalence varying with race[12,13], it is still advisable to monitor its response to anti-retroviral treatment[13,19,29].



Graph 1 – Sample linear correlation between CD4 and ß2 microglobuline

The majority of Authors agree with the idea that its value could be a result if not of the HIV infection itself[1,6], at least from the complications induced by its inherent immunologic disturbances[22].

To end off, we could conclude that, in harmony with what has been repeatedly stated regarding the complex monitoring of infection by the human immunodeficiency virus, we are also led to believe, by our study, that the combined seriate dosage of B2 and CD4 lymphocytes continues to amply accomplish this requirement.

REFERENCES

1. CHAISSON R E, VOLBERDING P A: Clinical Manifestations of HIV infection. In: Principles and practice of infectious diseases, Mandell GL, Douglas RG, Bennett JE, Eds. Third edition, New York, Churchill Lvingstone 1990: 1059-1092
2. WAIGMANN H R, SCHRODER B, BESERT L, et al: Markets for HIV-Disease progression in untreated patients and patients receiving AZT: Evaluation of viral activity, AZT resistence. setum cholesterol, ß2-micmglobulin, CD4+ cell couts, and HIV antigen. Infection 1991;19 (2): 77-82
3. OSMOND H, SHIBOSKI S, BACCHETTI P, WINGER E E, MOSS A R: Immune activation markers and AIDS prognosis. AIDS 1991; 5: 505-511
4. KLEINSCHMIDT A, MATUSCHKE A, GOEBEL FD, et al: Serological markers as prognostic criteria for the course of HIV infection. Infection 1991; 19 (2): 89-92
5. BURCHAM J, MARMOR M, DUBIN N, et al: CD4% is the bestpredictor of development of AIDS in a cohon of HIV-infected homossexual men. AIDS 1991; 5: 365-372
6. FAIJEY J L, TAYLOR J M G, DETELS R, et al: The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus tupe I. N Eng J Med 1990; 332: 166-72
7. HOFMANN B, WANG Y CUMBERLAND G, el al: Serum ß2-microglobulin level increases in HIV infection: relation to seroconversion, CD4 T- cell fall and prognosis. AIDS 1990; 4: 207-214
8. MUÑOZ A, BLAHOV D, SOLOMON L, et al: Prognostic indicators for development of AIDS among intravenous drug users. J Acquir Immune Defic Syndr 1992; 5: 694-700
9. HOOVER D R, GRAHAM N M H, CHEN B, et al: Effect of CD4+ cell count measurement variability on staging HIV-i infection- J Acquir Immune Defic Syndr 1992; 5: 794-802
10. CRUZ E D, DESCO M, MONTES M G, et al: Immunological and serological markers presictive of progression to AIDS in a cohort of HIVinfected drug users. AIDS 1990; 4: 987-994
11. VENET A, LU W, BELDJORD K, ANDRIEU J M: Correlation between CD4 cell counts and celular and plasma viral load in HIV-1 seropositive individuals. AIDS 1991; 5: 283-288
12. JORGENSEN A F, JENSEN V G, SHAO J F et al: ß2-microglobulin as a prognostic marker for patients with AIDS in Dar es Salaam, Tanzania. AIDS 1990, 4: 1168-1169
13. BROWN C, KLINE R, ATIBU L, et al: Prevalence of HIV-1 p24 antigenemia in African and North American populations and correlaction with clinical status. AIDS 1991; 5: 89-92
14. KAYE B R: Rheumatologic manifestations of unfection with human immunodeficiency virus (HIV). (HIV) Ann Intern Med 1989; 111: 158167
15. BOGNER J R, GOEBEL F D: Lymphocyte subsets as rurrogate markers in antiretroviral therapy. Infection 1991;19 (2):103-108
16. WITT PL, SPEAR G T, LINDSTROM M J, et al: 2',5' - Oligoadenulate synthtase, neopterin and ß2 microglobulin in asymptomatic HIVinfected individuals. AIDS 1991; 5: 289-293
17. LAFEUILLADE A, DELBEKE P, CHAFFANJON P, et al: Intérête diu dosage des anticorpos anticardiolipine au cours de l'infection par le virus de límmunodefience humaine. Press Med 1990; 39: 1225-1227
18. RIVERA J, MONTEAGUDO I, LONGO JL, et al: Anticardiolipin anibodies in drug addicted patients with AIDS, Ann Rheum Dis 1991; 50: 338
19. WILLIAMS I G, GABREL G, KELLY G, et al: Respnse of serum p24 antigen and antibody to p24 antigen in patients with AIDS and AIDSrelated complex treated with zidovudine. AIDS 1990; 4: 909-912
20. BASS H Z, HARDY W D, MITSUYASU R T, et al: The effect of zidovudine treatment on serum neopterin and ß2-microglobulin levels in mildly symptomatic, HIV type 1 seropositive individuals. J Acquir Immunc Defic Syndr 1992; 5: 215-221
21. JACOBSON M A, ABRAMS D I, VOLDERDING P A, et al: Serum ß2-microglobulin decreases in patients with AIDS or ARC treated with azidothymidine. J Infect Dis. 1989;159: 1029-1036
22. CLAYDON EJ, BENNETT J, GOR D, FORSTER S M: Transient elevation of serum HIV antigen levels associated with intercurrent infection. AIDS 1991; 5:113
23. PANZER S, STAIN C, HARTL H, DUDCZAK R, LECHNER K: Anticardiolipin antibodies are elevated in HIV-1 infected haemophiliacs but do not predict for disease progression. Thromb Haemostas 1989; 61: 81-85
24. INTRATOR L, OKSENHENDLER E, DESFORGES L, BIERLING P: Anticardiolipin antibodies in HIV infected patients with or without immune thrombocytopenic purpura. BR J Haematol 1988; 68: 269-270
25. STIMMLER M M, QUISMORIO F P JR, McGEHEE W G: Anticardiolipin antibodies in acquired immunodeficiency syndrome. Arch Intern Med 1989; 149: 1833-1835
26. DAROCA J L, CEBOLLADA J G, YAZBECK H, BERGÉS A, PRAT J R: Anticardiolipin antibodies and acquired immunodeficiency symdrome: prognostic marker or association with HIV infection? Infection 1992; 20: 140-142
27. LIFSON A R, HESSOL N A, BUCHBINDER S P, et al: Serum microglobulin and prediction of progression to AIDS in HIV infection. Lancet 1992; 339: 1436-1440
28. GRUTERS R A, TERPSTRA F G, LANGE J M A, et al: Differences in clinical course in zidovudine-treated asymptomatic HIV-infected men associated with T-cell function at intake. AIDS 1991; 5: 43-47
29. COOLIER A C, BOZZETTE S, COOMBS R W, et al: A pilot study of low - dose zidovudine in immunodeficiency virus infection. N Engl J. Med 1990; 323:1015-1021
30. GAZZARD B G: When should asymptomatic patients with HIV infection be treated with zidovudine? Br M J 1992; 304: 456-457
31. VOLDERDING P A, LAGAKOS S W, KOCH M A, et al:

Zidovudine in asymptomatic human immunodeficiency virus infection-A controlled trial in persons with fewer than 500 CD4-positive cells per cubic millimeter. N Engl J Med 1990; 322: 941-949

32. SWANSON C E, COOPER D A: Factors influencing outcome of treatment with zidovudine of patients with AIDS in Austrália. AIDS 1990; 4: 749-757