AFRICAN JOURNAL OF MEDICINE and medical sciences

VOLUME 35 NUMBER 1 MARCH 2006

ISSN 1116—4077

Afr. J. Med. med. Sci. (2006) 35, 53-57

Analysis of diurnal variation of lymphocyte subsets in healthy subjects in the Caribbean, and its implication in HIV monitoring and treatment

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Summary

Absolute values of the lymphocyte subsets are known to be influenced by various biological factors. We set out to determine if diurnal variations in lymphocyte subsets occur in our population. A prospective study was done on 25 randomly chosen healthy subjects. Persons were enrolled for the early morning to mid afternoon study i.e. 8:30, 12:30 and 15:30. In a second study, samples were collected at hourly intervals from 08:30 to 12:30. The EDTA samples were analyzed for lymphocyte subsets by flowcytomery. In the first study, the results have shown that there was a progressive increase in CD4 cell count throughout the day, while CD8 and CD19 cell counts increased between 08:30am and mid-day and then there was no further change between midday and mid afternoon. CD56 was uniform throughout the whole day. As most clinics and venesections take place in the morning, the aim of the second part of the study was to focus on the nature of the changes observed in the morning to midday phase. The results have shown that there were no significant changes in the lymphocyte subset counts before 11.30, thereafter there was a progressive increase in all of the lymphocyte subsets between 11:30 and 12:30 except for the CD56 cell count. This study has shown that diurnal rhythms influence the lymphocyte subsets in a normal population. This may have major implications in the use of CD4 subset analysis in the management of HIV infected persons as an indicator for initiation of treatment. In our setting, pending the results of diurnal variation studies on PLWHA, we have set the latest blood collection time at 11:30 am.

Keywords: *Lymphocyte, caribbean, HIV, monitoring, treament diurnal variation.*

Résumé

Dans la premiére étude, les résultats montraient que une augmentation progressive du taux des cellules CD4 toute la journée alorsque les cellules CD8 et CD19 augmentaient entre 8.30 am a midi et ensuite restaient uniforme.Les taux des cellules CD56 étaient uniforme tout au long de la journée. Comme la plupart des cliniques opérent en matinée, la deuxiéme experience montraient des changements non significative de taux des sous types cellulaires de lymphocyes avant 11.30am,une augmentation progressive de tous les sous tye de lymphocytes entre 11.30- 12.30 am à 1 exception du taux des cellules CD56. Cette étude demontre l influence du rythme duirne sur les sous type de lymphocytes dans une population saine.Ceci peut avoir des implications dans l usage du taux des sous type des cellules CD4 comme indicateur de l initiation du traitement pour le ménagement des patients du VIH. Dans notre environement en attendant les résultats des variations duirne aux PLWHA, nous avons mis la limite du temps de la collection des échanillons de sang à 11.30 am.

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Introduction

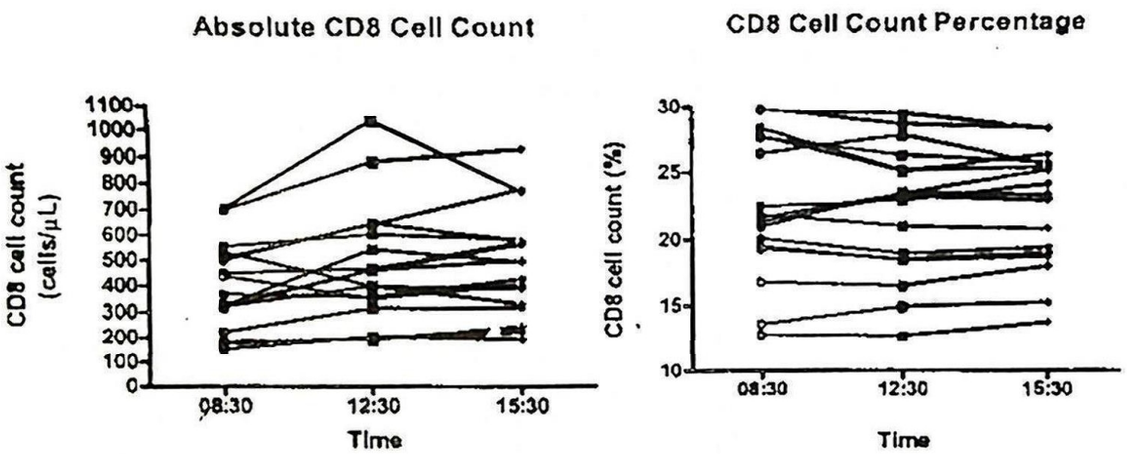
HIV affects the immune system and has an affinity for CD4 cells. Over time HIV depletes the number of $CD4^+$ cells causing damage to the immune system. $CD4^+$ cell count is therefore an important parameter used by clinicians to monitor the status of the immune system in Persons Living With HIV/AIDS (PLWHA), which is one of the landmarks used in the decision to commence Highly Active Anti Retroviral Therapy (HAART). Therefore the results that are produced must be accurate and reproducible.

Reports have shown that there can be variability in the measurements of T-lymphocyte subsets [1,2,3,4,5,6]. The two main types of variability seen are analytical and biological [1]. Analytical variability is dependent on the temperature of the specimen, type of anticoagulant used, time period it takes to analyze the sample, and machine and human error [1,3]. Whereas biological variability may include circannual, lunar, circadian and diurnal rhythms. The other factors that may influence these cell populations include age, sex, ethnic group, physical and psychological stresses, drugs, antilymphocyte auto-antibodies and splenectomy. Biological variability has a greater effect on lymphocyte subsets than analytical [1]. Diurnal rhythms are of greater importance since they impact on the lymphocyte subpopulations. These rhythms will create errors in clinical judgement if not standardized. In this study we examined the diurnal variation of all 4 lymphocyte subsets, namely the CD4 T helper cells, the CD8 Cytotoxic T lymphocytes, the CD19 B lymphocyte cells and the CD56 Natural Killer cells, as designated by their specific Cluster Designated markers.

Method and materials

Twenty-five healthy subjects were chosen randomly. In both studies, a 2ml EDTA sample was taken, the first at three intervals, 08:30, 12:30 and 15:30, and the second at five intervals, from 08:30 to 12:30. In the immunophenotyping analysis, 10µL of tetraCHROME CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 and CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5 was pipetted into appropriately labeled tubes. These monoclonal antibodies contain fluorescent dyes that bound to the Fc portion of the antibody and are commonly used for the identification of the cell surface antigens. 100µL of the EDTA samples was added, vortexed and allowed to stand for 10 minutes. The antibody links the dye to the cells making the cells detectable.

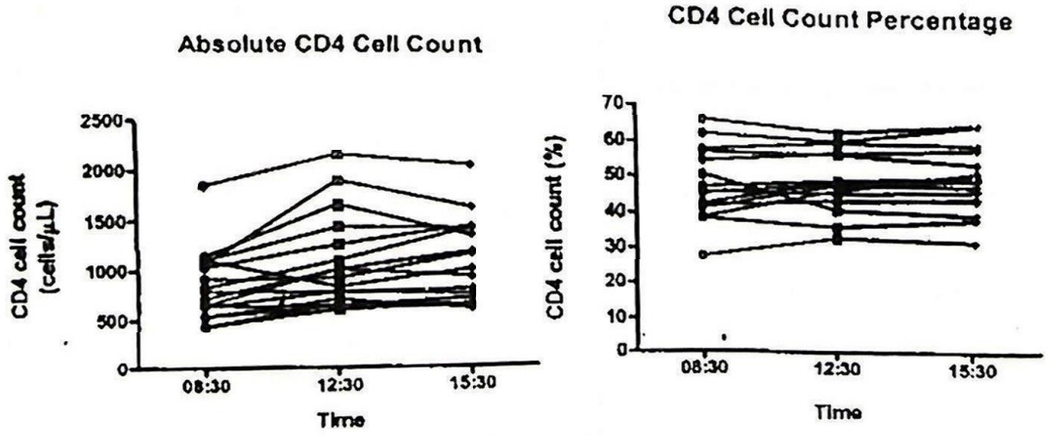
500µL of Optilyse C was added to the samples, vortexed and stood for 10 minutes. This reagent is used to lyse the red blood cells to prevent interference of these cells. 500µL of Phosphate Buffered Saline (PBS) was then added, vortexed and wait 5 minutes before analysis. At this stage, the PBS stops the shedding or internalization of the antigen/antibody complexes. 100mL of Flow-Count was also added to each sample and vortexed. The beads in the Flow-Count provide a precise count of the cells present. The samples were then placed on the Beckman Coulter Epics-XL for analysis by the Beckman Coulter tetraCHROME System with the XL System II software.



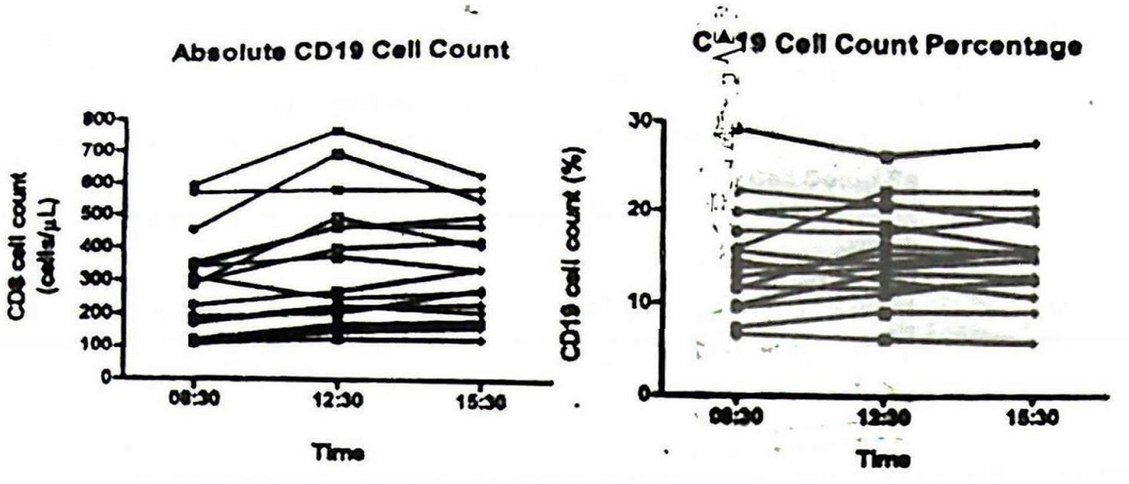
For the absolute CD8 count, $P<0.01$ 08:30 vs 12:30 and 15:30
For the CD8 %, $P>0.05$ 08:30 vs 12:30 and 15:30

Early-morning to late afternoon study

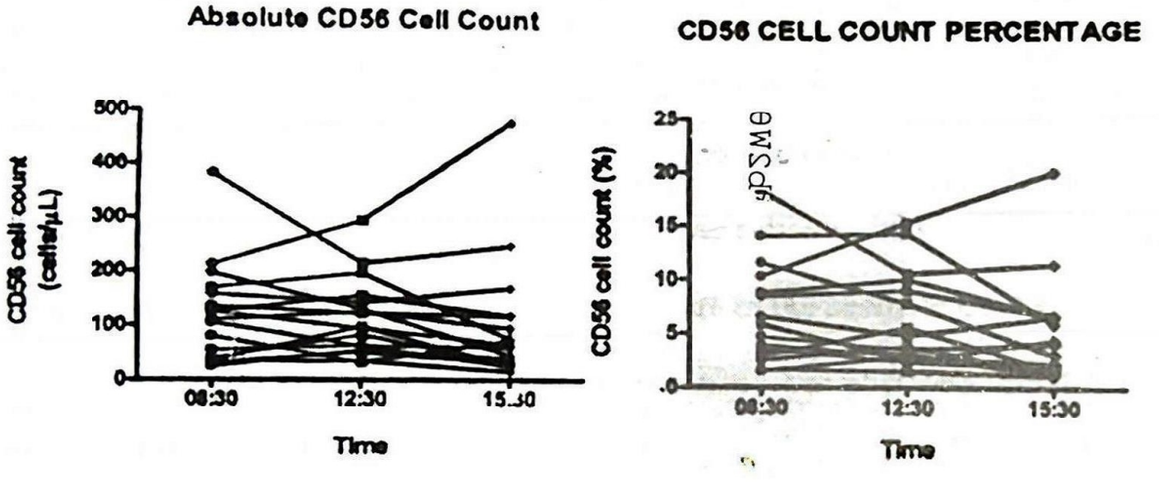
Early-morning to late afternoon study



For the absolute CD4 count, $P<0.05$ 08:30 vs 12:30 and 15:30
For the CD4 %, $P>0.05$ 08:30 vs 12:30 and $P<0.05$ 08:30 vs 15:30

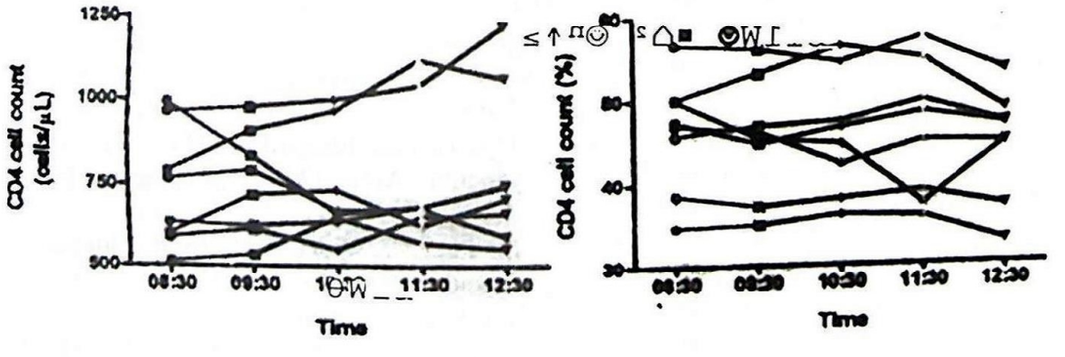


For the absolute CD19 count, $P<0.01$ 08:30 vs 12:30 and 15:30
For the CD19 %, $P>0.05$ 08:30 vs 12:30 and 15:30



For the absolute CD56 count, $P<0.05$ 08:30 vs 12:30 and 15:30
For the CD56 %, $P>0.05$ 08:30 vs 12:30 and $P<0.05$ 08 :30 vs 15:30

Early morning to mid-day study



For both absolute CD4 and CD4%, $P<0.05$ 08:30 vs all other timepoints.

This software does a four colour analysis where it provides necessary quality control reagents as well as automate quality control and sample analysis.

The statistical method used was the ANOVA (repeated measures test) with Dunnett's post-test.

Results

lute count for clinical decision making and the monitoring of PLWHA.

Both studies have shown that timing of sample collection for CD4 analysis can affect the result obtained and can influence the management of the patients where CD4 levels are critical decision making parameters. It is

Table 1: The lymphocyte subsets range at 8.30, 12.30 and 15.30

	T I M E					
Lympho Subsets	8:30		12:30		15:30	
	%	Abs	%	Abs	%	Abs
CD4	28.1-66.2	431-1845	33.3-62.9	608-2154	32.6-65.5	608-2048
CD8	12.8-29.8	155-703	12.7-29.3	192-1039	13.7-28.1	191-932
CD56	1.5-18.2	25-382	1.5-15.2	32-294	1.1-19.9	14-480
CD 19	6.7-29.2	106-588	6.3-26.4	124-756	5.8-27.9	123-620
CD8	12.8-29.8	155-703	12.7-29.3	192 - 1039	13.7-28.1	191 -932
CD56	1.5- 18.2	25-382	1.5- 15.2	32 -294	1.1- 19.9	14-480
CD 19	6.7-29.2	106-588	6.3-26.4	124-756	5.8-27.9	123 -620

Table 2: The mean lymphocyte subsets at 8:30, 12:30 and 15:30

Lymphocyte Subsets								
Time	CD4		CD8		CD56		CD19	
	a/o	ABS	%	ABS	%	ABS	%	ABS
8:30	47.9	846	22.4	401	6.6	118	14.9	276
12:30	49.4	906	22.1	482	5.9	114	15.7	348
15:30	50.2	1082	22.2	488	4.7	100	15.5	341

Conclusion

In the previous study by Jeffrey Laurence which focused was on CD4 and CD8 cells, an increase was seen in the CD4 cell count at 12:30. In our study, which was broken down into two studies, early morning to late afternoon and early morning to mid-day, we examined the full range of lymphocyte subsets. In the early morning to late afternoon study, there was a significant change in the absolute CD4 cell count between the morning and the afternoon samples. The same was observed for CD8 and CD19 absolute cell counts. In the second study, there was no significant change in any of the lymphocyte subsets before 11:30. Therefore it seems that 11:30 is a critical time before which blood be drawn for analysis.

CD56 cell count remained uniformed in both studies. Of significance is the finding that the percentages value of lymphocyte subsets did not show any variation throughout the day. This observation lends weight to the proponents of using CD4 percentages rather than absosuggested that all samples be drawn before noon for standardization of biological variation. It is recommended that further studies are conducted to determine the diurnal variation in PLWHA, especially those who are treatment naïve. Based on these results and to ensure reproducibility of quality results, we have set a ceiling on venesection for CD4 analysis at 11:30am.

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Received: 05/12/05
Accepted: 22/02/06