

Imipramine Induced Elevation of Prolactin Levels in Patients with HIV/AIDS Improved their Immune Status

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ABSTRACT

Prolactin is known to have significant immunomodulatory properties. Imipramine, a monoamine oxidase inhibitor, stimulates prolactin production because it decreases dopamine which inhibits secretion of prolactin. The study objective was to determine if use of imipramine can result in immunological benefits for HIV-positive patients by restoration and preservation of immunological function. A cohort of 19 retroviral positive patients was identified for the prospective study which continued for a 28-week period. Three patients dropped out before the study began. Inclusion criteria accepted only patients on the same highly active antiretroviral therapy (HAART) regimen for a nine-month period and who had reached a plateau with respect to the CD4 cell count and also had no prior history of antidepressant use for a 12-month period. This study had a “before and after” design, patients serving as their own control. The study drug imipramine was prescribed for a 12-week period up to visit 4, and then discontinued for 4-weeks (washout period) at which time blood investigations were done at visit 5. Finally, patients were prescribed the study drug for a further 12-week period to the end of the trial (visit 7). At the 95 per cent probability level, significant differences in average prolactin and CD4 levels from visit 4 to the end of the trial period were recorded. Results showed a trend of prolactin levels decreasing after washout ($p = 0.015$) and increasing by the end of the trial period once imipramine dispensation had recommenced ($p = 0.006$). With respect to the CD4 cell count, there was a significant increase after wash-out ($p = 0.022$). These results indicate a trend to immune boosting in HIV-positive patients who had obtained the maximum response from HAART.

La Elevación de Niveles de Prolactina Mediante Inducción con Imipramina Mejoró el Estatus Inmune de Pacientes con VIH/SIDA

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RESUMEN

Se sabe que la prolactina posee importantes propiedades inmunomodulatorias. La imipramina, un inhibidor de la monoamino oxidasa, estimula la producción de la prolactina porque disminuye la dopamina, que a su vez inhibe la secreción de prolactina. El objetivo de este estudio fue determinar si el uso de la imipramina puede traer beneficios inmunológicos a los pacientes VIH positivos mediante la restauración y preservación de la función inmunológica. Se identificó una cohorte de 19 pacientes retrovirales positivos, a fin de realizar este estudio prospectivo que continuó por un período de 28 semanas. Tres pacientes se retiraron antes de que el estudio comenzara. Los criterios de inclusión aceptaban sólo pacientes que tuvieran el mismo régimen de terapia antiretroviral altamente activa (HAART) por un período de nueve meses, que hubieran alcanzado un nivel de estabilización con respecto al conteo de células CD4, y que no hubieran además tenido con anterioridad una historia de uso de anti-depresantes por espacio de 12 meses. Este estudio tuvo un diseño “antes y después”, sirviendo los pacientes como su propio control. La imipramina para el estudio fue prescrita por un período de 12 semanas hasta la visita 4, y luego discontinuada por 4 semanas para un reposo farmacológico (período de lavado), realizándose en-

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tonces pruebas de sangre en la visita 5. Finalmente se prescribió el medicamento de estudio a los pacientes por un nuevo período de 12 semanas hasta el final del ensayo (visita 7).

En el nivel de probabilidad del 95 por ciento, se registraron diferencias significativas en los niveles promedio de prolactina y CD4 desde la visita 4 hasta el final del período de ensayo. Los resultados mostraron una tendencia de los niveles de prolactina a descender tras el lavado ($p = 0.015$) y a aumentar hacia el final del período de ensayo, una vez que la dispensación de imipramina hubiese recommenzado ($p = 0.006$). Con respecto al conteo de células de CD4, hubo un aumento significativo luego del lavado ($p = 0.022$).

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INTRODUCTION

Prolactin, a peptide hormone produced mainly in the anterior pituitary, has well known physiological functions in reproduction, mammary gland preparation, osmoregulation and cell proliferation and survival (1, 2). However, over the past 30 years, there has been compelling evidence that prolactin is involved in immune regulation. In hypophysectomized rats that became immunodeficient, replacement doses of prolactin completely restored immune competence (3). Moreover, administration of the dopaminergic drug, bromocriptine, which inhibits pituitary prolactin secretion, to intact rats, rendered them immune deficient. Replacement of prolactin restored immunocompetence.

Many autoimmune diseases are prevalent in women during their reproductive years, most notably, systemic lupus erythematosus (SLE) which occurs more frequently in females than males by a 9:1 ratio. Bromocriptine has been shown to suppress SLE in some patients and to reduce the number of lupus flares (4, 5). There is now evidence that prolactin is produced and secreted by lymphocytes and that the prolactin receptor is almost ubiquitous. The prolactin receptor is a member of the type-I cytokine receptor superfamily, which includes those for growth hormone, interleukin-2 (IL-2) to interleukin-7 (IL-7), erythropoietin, granulocyte-colony stimulating factor and macrophage colony stimulating factor. Prolactin and its receptor have been detected in mature lymphocytes and in thymocytes. In these tissues, prolactin can induce proliferative responses and the secretion of IL-2 and interferon - γ [INF- γ] (6, 7). Prolactin most likely has autocrine and paracrine functions in the immune system.

The significance of prolactin in immunomodulation has been challenged by evidence from knockout mice experiments (8). Knockout mice deficient in prolactin or its receptor are immunocompetent. However, the type-1 cytokine receptor family of which prolactin and its receptor are members, show functional gene redundancy (9). Therefore knocking out one or more members of the family induces compensatory mechanisms to overcome the deficit. The major signal transduction pathway which involves the Janus Kinase (JAK) and signal transducers and activators of transcription (STAT) nuclear regulatory factors, is shared by the type-I cytokines, prolactin and growth hormone. Signal transducers and activators of transcription knockout mice show severe immune deficiencies (10). Prolactin is under

inhibitory control by dopamine. Imipramine is a dopamine antagonist and as a result increases prolactin levels.

On the basis of current evidence, we hypothesized that increasing prolactin levels with the use of the monoamine oxidase inhibitor, imipramine, in patients with human immunodeficiency virus (HIV)/ acquired immune deficiency syndrome (AIDS) should induce an increase in their CD4 levels.

SUBJECTS AND METHODS

The population of interest was HIV seropositive patients treated at the Infectious Disease Clinic of the Princess Margaret Hospital, the premier acute tertiary care facility of The Bahamas. Patients having met the following inclusion criteria were chosen consecutively to enter the study:

- * HIV-1 infected males or females aged 18 years and over.
- * Females of childbearing age on reliable contraception.
- * CD4+ T-Lymphocyte count \geq 100 cells/ μ l.
- * Treatment experienced on HAART for a period of nine months or longer.
- * Plateau in CD4 levels while on individual HAART.
- * Acceptable screening laboratory values indicating adequate baseline organ function.
- * No prior history of antidepressant use for the previous twelve months.

Treatment experienced on HAART is defined as the combination of three or more antiretroviral agents taken concurrently to suppress HIV replication.

Excluded were:

- * Patients who are active substance abusers.
- * Women who are pregnant.
- * Patients on antidepressant
- * Women who are breast feeding.
- * Persons not willing to adhere to the requirements of the treatment protocol.
- * Patients receiving treatment for any opportunistic infection.
- * Patients on any of the following medications: phenothiazines, methyl dopa, cimetidine and barbiturates.

Nineteen potential persons met the study criteria, sixteen of whom were enrolled to participate. Enrollees' age

ranged from a low of 30 years to a high of 66 years, average age 49 (SD ± 11) years. There were seven males (44%) and nine females (56%).

This study had a “before and after” design. With this type of design, patients served as their own control; response to treatment was measured for each subject *before* and *after* a treatment is administered.

Patients’ blood investigations included basal prolactin levels, white blood counts with differential platelets, haemoglobin, CD4 lymphocyte counts and general chemistry values read for each subject *before* and *after* the antidepressant imipramine 25 mg was administered or discontinued. In addition, as a part of the routine management of HIV patients, viral load levels were also monitored at each measurement interval.

The study took place between September 2005 and September 2006. Persons who met the study inclusion criteria were assigned an identification number and the treatment protocol was explained. Each patient consented, in writing, to participate about four weeks prior to entering the study and was given the option to withdraw at any time before or during the study period. All patients in the cohort were followed for a 28-week period.

All blood investigations were drawn in the morning. Prolactin levels were measured by microparticle enzyme immunoassay (MEIA) by Abbott® IMX, CD4 lymphocyte counts by the flow cytometry on the Coulter® Epic XL cytometer (Beckman coulter® Epics® XL) and viral load levels using the Bayer® system 340 analyzer.

The treatment regime for participants was as follows:

Beginning of Trial – visit 1/pre-entry (day 1):-

- * Initial blood investigations.
- * Study drug imipramine dispensed for a three-month period.

Visit 4 (end of week 12):-

- * Blood investigations repeated.
- * Study drug discontinued for one month.

Visit 5 (end of week 16):-

- * Blood investigations ordered following washout period.
- * Study drug dispensed for three months.

End of Trial – Visit 7 (end of week 28):-

- * Final blood investigations ordered.

The above study protocol was approved by the ethics committee of the Public Hospitals Authority.

In order to determine the effect of the antidepressant imipramine on prolactin levels, CD4 count and viral load, visits were paired with ‘a’ successive visit for each investigation. Final combinations are as below, pair 2 assessing the significance of differences in laboratory values after the 1-month washout period in all instances.

Blood investigation:

- 1) Prolactin levels: Visit 1 ↔ Visit 4 ↔ Visit 5 ↔ Visit 7
Pair 1 Pair 2 Pair 3
- 2) CD4 count: Pre-entry ↔ Visit 4 ↔ Visit 5 ↔ Visit 7
Pair 1 Pair 2 Pair 3
- 3) Viral Load: Visit 4 ↔ Visit 1 ↔ Visit 5
Pair 1 Pair 2

The statistical software package SPSS for Windows version 11.5 was used for data analyses.

Data Modification/Variable Types

Viral load data were classified into two discrete categories: ‘0’ for viral loads of ≤ 50 HIV copies per milliliter of blood (undetectable), and ‘1’ for all viral loads above 50 HIV copies per milliliter of blood (detectable). Prolactin levels and CD4 count were analyzed as scaled interval type variables. Means and standard deviations have been used to represent the average and typical spread of values.

Where an individual’s blood investigation values were missing at any measurement interval for prolactin levels and CD4 count, data were estimated. This was obtained by applying the mean of paired differences between visits for those participants completing the full requirements of the study to the known values for participants missing data. Many blood investigation results for viral load as were reported as categorical type variable and calculation of estimates were not possible.

Statistical tests of significance applied included the paired *t*-test for assessing *before* and *after* differences in prolactin levels and CD4 count, and the McNemar for monitoring changes in the viral load.

RESULTS

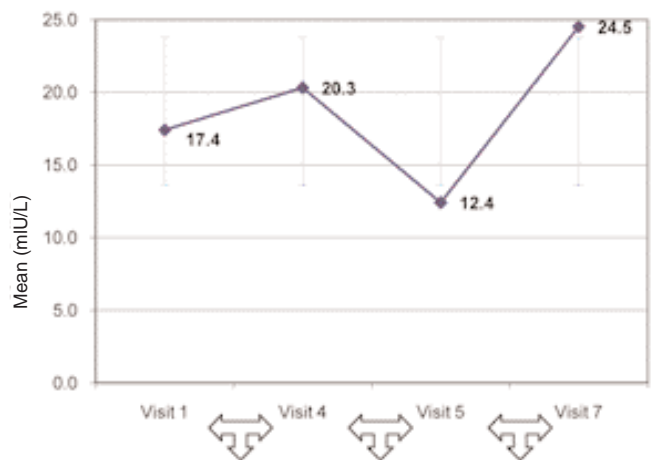
The final samples of participants included in the analysis were sixteen for basal prolactin level and CD4 lymphocytes, and eleven for viral load. Very few results were available for viral load at visit 7 due to several challenges experienced in obtaining results from the Canadian reference laboratory engaged to provide these results, and so these data were excluded from the analysis. Major results for the three blood investigations are as follows (Fig. 1–3 and Annexes 1–3):

Prolactin Levels

Normal levels of prolactin are, for women, < 580 mIU/L and for men < 450 mIU/L. All subjects were within the normal range throughout the 28-week study (Annex 1).

At the onset of the study, there was no significant difference in average prolactin levels between the initial visit (visit 1) and visit 4, after being on imipramine for three months ($p > 0.05$, Annex 1).

The differences in average prolactin levels between visits four to seven were significant. A decline in the average



TREATMENT PROTOCOL – IMPRIMINE: Administered Discontinued* Administered*

Fig. 1: Mean prolactin levels (± 1 SD) of patients after imipramine treatment protocol. Asterisk (*) indicates statistically significant results (paired t-test, $p \leq 0.05$); $n = 16$ patients completing all prolactin level testing.

Annex 1
Prolactin levels of patients during the study (mIU/L)

Patient #	Visit 1	Visit 4	Visit 5	Visit 7
P001		drop-out		
P002	5.6	5.0	3.9	6.8
P003	8.3	7.7	8.0	36.1
P004	8.9	12.2	8.7	49.3
P005	45.3	14.9	12.4	14.8
P006	10.2	5.9	3.8	48.5
P007	32.7	36.3	11.3	11.5
P008	15.8	11.4	5.1	20.0
P009	5.4	2.5	2.9	8.9
P010	12.5	7.9	12.1	4.0
P011	9.3	36.1	8.5	7.9
P012	10.6	27.2	5.2	5.3
P013		drop-out		
P014	8.6	6.6	13.7	14.9
P015	8.8	20.6	11.3	23.4*
P016	8.6	40.4	12.1	37.1
P017	7.7	7.4	5.4	17.5*
P018		drop-out		
P019	79.4	81.9*	73.7*	85.8*
mean	17.4	20.3	12.4	24.5
standard deviation	19.7	20.6	16.7	22.0
p-value**		0.429⁽¹⁾	0.015⁽²⁾	0.006⁽³⁾

* Data estimated based on available data for the 13 patients completing full requirements of the study from pre-entry through visit 7.

** Probability value for differences between: (1) Visits 1 and 4; (2) Visits 4 and 5; and (3) Visits 5 and 7.

prolactin level at visit 5 was registered following the washout period (Visit 4 = 20.3 mIU/L, Visit 5 = 12.4 mIU/L, $p = 0.015$), which subsequently rose to an average of 24.5 mIU/L at visit 7 after dispensation of imipramine had recommenced ($p = 0.006$) [Fig. 1].

CD4 Count

Only one subject at pre-entry and the beginning of the trial/visit 1 had AIDS (that is CD4 count < 200 cells/mm³) with no more than one individual at any given instance throughout the duration of the study falling below this value. At each measurement interval, more than half of the participants maintained CD4 counts within the 'healthy' threshold of 500 – 1450 cells/mm³ (Annex 2).

At the end of week 12 (visit 4), blood investigations showed no significant impact of imipramine on CD4 levels ($p > 0.05$). However, by visit 5 and continuing throughout the end of the trial period, both patterns exhibited were significant. That is, following the washout period, mean CD4 count increased ($p = 0.022$). This suggests a sustained immune boost despite being taken off imipramine, with CD4 count averaging its highest (596.1 cells/mm³) over the entire 28-week study period. Further, while there was a decline in CD4 levels after ingestion of imipramine for three more months ($p = 0.027$), on average, participants continued to maintain immune levels within the healthy threshold (510.4 cells/mm³) [Annex 2].



TREATMENT PROTOCOL – IMPRIMINE: Administered Discontinued* Administered*

Fig. 2: Mean CD4 count (± 1 SD) of patients after imipramine treatment protocol. Asterisk (*) indicates statistically significant results (paired t-test, $p \leq 0.05$); $n = 16$ patients completing all CD4 count testing.

VIRAL Load

No fewer than five persons maintained undetectable viral thresholds (50 HIV copies per milliliter of blood and under) at any given measurement interval. The change during the wash-out and treatment periods (Fig. 3) were not statistically significant ($p > 0.05$) which means that participant's viral loads were not impacted by the presence or absence of this antidepressant (Fig. 3, Annex 3).

Annex 2
CD4 Count for patients during the study (cells/mm³)

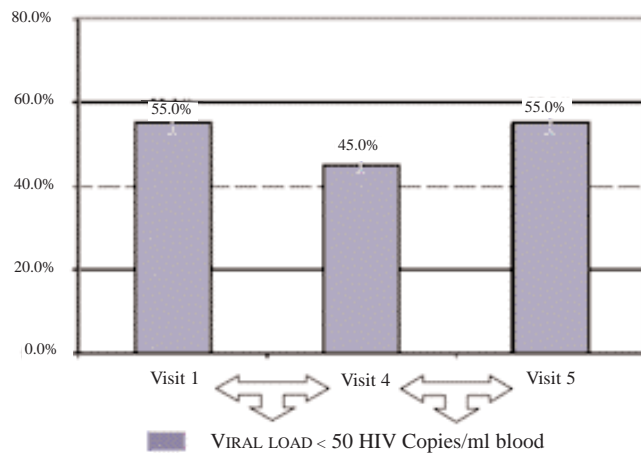
Patient #	Pre-entry	Visit 4	Visit 5	Visit 7
P001		<i>drop-out</i>		
P002*	496*	406	496	362
P003	791	649	949	865
P004	921	698	545	342
P005	105	234	305	199
P006	427	538	678	607
P007	791	579	643	412
P008	759	651	1063	754
P009	426	680	548	565
P010	615	525*	619	704
P011*	666	375	383	321*
P012*	635	943	1077*	708
P013		<i>drop-out</i>		
P014	344	320	321	425
P015	228	167	181	256
P016	568	355	613	532
P017	368	383	412	473
P018		<i>drop-out</i>		
P019*	660	570*	704*	642*
mean	550.0	472.6	596.1	510.4
standard deviation	22.9	234.2	261.0	192.9
p-value**		0.181⁽¹⁾	0.022⁽²⁾	0.027⁽³⁾

* Data estimated based on available data for the 12 patients completing full requirements of the study from pre-entry through visit 7.

** Probability value for differences between: (1) Pre-entry and Visit 4; (2) Visits 4 and 5; and (3) Visits 5 and 7.

DISCUSSION

Many patients indicated that they felt much better about themselves after entering the study and admitted to near 100 per cent of compliance with the study drug. In fact several participants insisted on remaining on the study drug for a fur-



TREATMENT PROTOCOL – IMPRIMINE: Administered Discontinued

Fig. 3: Proportion of clients with an undetectable viral load ($\pm 5\%$) after imipramine treatment protocol. Asterisk (*) indicates statistically significant results (McNemar test, $p \leq 0.05$); n = 11 patients completing all viral load testing.

Annex 3
Viral load for patients during the study (HIV copies/ml blood)

Viral Type	Number of Patients		
	Visit 1	Visit 4	Visit 5
Undetectable (? 50 copies/ml)	6	5	6
Detectable (50+ copies/ml)	5	6	5
No data*	1	2	3
p-value**		1.000⁽¹⁾	1.000⁽²⁾

* Patients did not complete full requirements of study and were not included in the analyses.

** Probability value for differences between: (1) Visits 1 and 4; and (2) Visits 1 and 5.

ther 12-weeks after the end of the study. One of the participants with a pre-entry CD4 cell count at 759 cells mm³, maintained an undetectable viral load throughout the study and also demonstrated a dramatic increase in CD4 count readings between visit 4 (651 cells/mm³) and visit 5 (1063 cells/mm³). This represents an increase over 60 per cent at the end of the washout period. This participant had no complaints and had an excellent adherence record. It was note worthy that two participants who remained on the study drug after the completion of the study, had CD4 count readings escalating to levels above 1000 cells/mm³ with undetectable viral loads. During the study period after commencing imipramine, results showed that no more than one participant at any given measurement interval recorded CD4 counts below 200 cells/mm³, the CDCs classification for AIDS. The CD4 count is a measure of strength of the immune system. The CD4 level gives an indication of the robust state of the immune system. It is important to note that the CD4 cell counts can exhibit diurnal changes, with the lowest levels at 12:30 pm and peak values at 8:30pm (11). The CD4 cell provides vital information to make therapeutic decisions regarding antiviral treatment and prophylaxis for opportunistic infections.

The CD4 count at initiation acts as the dominant prognostic factor in patients starting HAART (12, 13). The CD4 cells are the primary target of HIV and numbers decline as HIV advances. Highly active antiretroviral therapy often leads to increases in the CD4 T cell count of $\geq 100 - 200$ cells/mm³/year (14).

Results of this study showed a trend of CD4 cells increasing significantly ($p = 0.022$) by visit 5, which points to a sustained increase in CD4 count following the washout period. Further, despite the decline of CD4 count once imipramine was re-introduced, significantly high levels were maintained (510 cells/mm³) which indicates the need to maintain administration of the drug for at least a four-month period to sustain increasing levels.

This information was encouraging as it indicated further strengthening of the immune system after the effects of HAART had reached a plateau. Continued viral load sup-

pression is more likely for those patients who achieved higher CD4 T cell counts during therapy (15). Although viral load is the strongest single predictor of long-term clinical outcome, it is important to consider also sustained rises in CD4 T cell counts as a significant rise in the CD4 T cell count reflects partial reconstitution of the immune system that results in reduced HIV-related morbidity and mortality (16).

There was no evidence of treatment failure in this study. Treatment failure is defined as clinical, immunological or virological. Clinical failure is clinical disease progression with the development of an opportunistic infection or malignancy after more than 3 months on therapy, excluding immune reconstitution syndrome. None of the participants developed an opportunistic infection while on the study drug. Immunological failure can be defined as a fall of over 30 per cent in CD4 counts from the peak value or a return to or below the pre-therapy baseline (17). Virological failure has no uniform accepted definition but repeated detectable viraemia is indicative of incomplete viral suppression, HIV RNA > 400 copies/ml after 24 weeks, > 50 copies after 48 weeks or > 400 copies after viral suppression (18). High viral loads also indicate a deterioration of HIV disease. Non-adherence in patients on HAART was the strongest predictor for failure to achieve viral suppression below detection (19). Certain factors that increase viral load include: 1) progressive disease 2) failing antiretroviral therapy due to inadequate potency 3) non-adherence 4) active infections *eg* active TB which increases viral load 5- to 160-fold (20). None of the participants in this study had to change HAART due to high viral loads over 55 000. Throughout the trial period, many participants while receiving the study drug imipramine, maintained an undetectable viral load (generally <50 copies /ml).

Data from clinical trials demonstrate that lowering plasma HIV RNA to < 50 copies/ml is associated with increased duration of viral suppression, compared with reducing HIV RNA to levels of 50–500 copies/ml (21). All participants who qualified for entry into the study were HAART experienced. Highly active antiretroviral therapy has been associated with immunological restoration, a slowing of disease progression and improvement of the quality of life. In order to qualify for the study, it was a requirement for participants to have been on the HAART for a period not less than nine months, thus allowing the designed HAART regimen to stabilize HIV disease progression and allow the patient to obtain optimum benefits from the HAART regimen. Hence only patients who had good compliance with HAART regimens were accepted in this study. The response to HAART correlates strongly with adherence. Suboptimal adherence has been reported to decrease virologic control and has been associated with increased morbidity and mortality (22). Suboptimal adherence also leads to drug resistance, limiting the effectiveness of therapy (23). Many of the participants had been on the same HAART well over the required period. None of the participants needed a change in HAART after entering the study. Partial reconstitution of

immune function induced by HAART might allow elimination of unnecessary therapies [*eg* therapies used for the prevention and maintenance against opportunistic infection] (24, 25). Hence further strengthening of the immune system with imipramine might also allow elimination of unnecessary preventive therapies.

A number of side effects have been associated with the study drug imipramine. These include constipation, dry mouth, urinary retention, cardiac arrhythmia, drowsiness, hypotension, confusion and dizziness. Imipramine can also be seen as a rare cause of grey blue facial pigmentation (26). None of the participants reported any side effects.

The average cost of HAART comprising brand drugs can vary from \$1000 (US) to \$1500 (US) monthly. This obviously can be a tremendous burden on poor countries in Africa and the Caribbean region. Subsidized HAART with brand drugs and also cheaper generic drugs can be obtained in several countries throughout the globe. Patients who had reached a maximum response to HAART demonstrated a further immune boost after commencing the study drug. This trend of immune strengthening continued throughout the study period. The patients who requested to remain on the medication after completion of the study continued to show an immune boost. A large scale multicentre trial is necessary to provide further information on the positive benefits of the drug imipramine to HIV-infected individuals. In addition, a study for patients who are HAART naïve is also necessary to determine the benefits of the drug in this group. We propose that in all poverty stricken areas of the world persons living with HIV/AIDS could be given the drug imipramine intermittently for the purpose of boosting the immune system.

The study drug imipramine is inexpensive and can be obtained with ease throughout the Caribbean and the rest of the world, the cost being US\$9 per patient per annum.

Limitations of the study

The study investigators experienced a number of challenges throughout the study period. The investigators engaged the services of a reference laboratory in Canada for CD4 count and viral load testing. Blood samples were sent *via* special courier but there were occasions when specimens were delayed in transit. This impacted on results being received and recorded on a timely basis. The investigators were also concerned that the delay in specimens arriving at the reference laboratory could impact CD4 count readings. Further research in this area is warranted.

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