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AVALIAÇÃO DA CAPACIDADE FUNCIONAL, DOS SISTEMAS RESPIRATÓRIO, CARDIOVASCULAR E AUTONÔMICO EM PACIENTES INFECTADOS COM HIV

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Dissertação submetida ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal de Ciências da Saúde de Porto Alegre como requisito para a obtenção do grau de Mestre

Orientador: Dr. Pedro Dal Lago

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RESUMO

O advento da *highly active antiretroviral therapy* (HAART) produziu uma redução da mortalidade em pacientes portadores do vírus da imunodeficiência humana (HIV) e, com isso, esses pacientes têm sido acometidos por outras alterações crônicas. Objetivou-se estabelecer uma equação de predição e avaliar a capacidade funcional pelo teste de caminhada de seis minutos (TC6min), identificar a presença de alterações dos sistemas pulmonar, cardiovascular e autonômico de pacientes portadores do HIV com ou sem uso de HAART. Foram avaliados parâmetros de espirometria, manovacuometria, função autonômica, TC6min, variabilidade da frequência cardíaca (VFC), exames laboratoriais e dados relacionados às caracetrísticas da doença. Inicialmente, 155 pacientes foram avaliados no TC6min, sendo que 124 participaram da elaboração de uma equação de referência e 31 compuseram a validação da referida equação. Para a análise das funções cardiopulmonares e autonômica, 70 pacientes foram divididos em 2 grupos: 57 sob medicação (divididos em HAART-1 e HAART-2, conforme recomendações medicamentosas) e 13 pacientes formaram o grupo sem medicação. A partir dos resultados das distâncias percorridas (DP) dos TC6min, elaborou-se a seguinte equação: (DP) = 610,07 + (sexo (0 mulheres; 1 homens)*75,68) – (idade*3,15) + (ΔFC*1,43); (r^2 0,44). Nas demais avaliações verificaram-se diferenças entre o tempo de diagnóstico entre o grupo HAART e HIV^{+ve} (p = 0,006), concentração de hemoglobina entre o grupo HAART-2 comparado ao grupo HIV^{+ve} (p = 0,033). Não houve diferença na DP do TC6min, verificou-se diferença entre a frequência cardíaca ao final do teste entre os grupos HAART-2 e HIV^{+ve} (p = 0,030) e pressão diastólica final entre HAART e HIV^{+ve} (p <0,001). Nas análises de função pulmonar, ocorreu diferença apenas na pressão inspiratória máxima entre os grupos HAART e HIV^{+ve} (p = 0,025) e HAART-2 e HIV^{+ve} (p = 0,003). Não foi observada diferença nas variáveis analisadas na VFC. Na avaliação da função autonômica, verificou-se que a maioria dos pacientes apresentou classificação normal e 3 pacientes do grupo HAART apresentaram classificação anormal. Conclui-se que uma fórmula de predição específica é capaz de predizer a DP na avaliação da capacidade funcional de portadores do HIV. Além disso, pacientes de ambos os grupos não apresentam alterações respiratórias significativas, e não existe diferença na VFC entre os grupos avaliados. A maioria dos pacientes apresentam classificação normal de neuropatia

autonômica. Além disso, pacientes com controle adequado da doença apresentam, na sua maioria, condições clínicas compatíveis com sujeitos saudáveis.

Palavras-chave: Vírus da imunodeficiência humana, *Highly active antiretroviral therapy*, teste de caminhada de 6 minutos, alterações cardíacas, alterações respiratórias, função autonômica.

ABSTRACT

The advent of highly active antiretroviral therapy (HAART) produced a reduction of mortality in patients with the human immunodeficiency virus (HIV) and, therefore, these patients have been affected by other chronic changes. The objective was to establish a prediction equation and evaluate the functional capacity test by six-minute walk (6-MWT), identify the presence of abnormal pulmonary system, cardiovascular and autonomic of patients HIV-infected with or without HAART. We evaluated parameters of pulmonary function, maximal static respiratory pressure, autonomic function, 6-MWT, heart rate variability (HRV), and laboratory data related to character disease. Initially, 155 patients were evaluated in the 6-MWT, and 124 participated in the elaboration of a reference equation and 31 comprised the validation of that equation. For the analysis of cardiopulmonary and autonomic functions, 70 patients were divided into 2 groups: 57 under medication (divided into HAART-1 and HAART-2, as recommended drug) and 13 patients formed the group without medication (HIV^{+ve}). From the results of the distances traveled (6-MWD) of the 6MWT, drew up the following equation: (6-MWD) = 610.07 + (gender (0 women, 1 man) * 75.68) - (3.15 * age) + (1.43 * ΔHR); (r^2 0:44). In other reviews there were differences between the time of diagnosis between the HAART and HIV^{+ve} groups (p = 0.006), hemoglobin concentration between the HAART-2 and HIV^{+ve} groups (p = 0.033). There was no difference in the 6-MWD, there was difference between heart rate at the end of the test between HAART-2 and HIV^{+ve} (p = 0.030) and end-diastolic pressure between HAART and HIV^{+ve} (p <0.001). In analyzes of lung function occurred only difference in maximal inspiratory pressure between groups HAART and HIV^{+ve} groups (p = 0.025) and HAART-2 and HIV^{+ve} (p = 0.003). No difference was observed in the HRV variables analyzed. In the assessment of autonomic function, it was found that most patients showed normal classification and HAART patients in group 3 were classified as abnormal. We conclude that a specific of predition equation the 6-MWT in assessing the functional capacity of patients HIV-infected. Furthermore, patients in both groups did not show significant respiratory changes, and there is no difference in HRV between the groups. Most patients have normal classification of autonomic neuropathy. Furthermore, patients with adequate control of the disease have mostly, clinical conditions compatible with healthy subjects.

Key-words: Human immunodeficiency virus, highly active antiretroviral therapy, 6-minute walk test, dysfunction cardiac, dysfunction respiratory, autonomic function.

LISTA DE ABREVIATURAS

AIDS: síndrome da imunodeficiência adquirida.

BMI: body mass index.

BPR: blood pressure response.

CD_4: cluster differentiation.

CVD: cardiovascular disease.

COPD: chronic obstructive pulmonary disease.

CVF: capacidade vital forçada.

DBP: diastolic blood pressure.

DCV: doença cardiovascular.

DM: diabetes mellitus.

DNA: ácido desoxirribonucléico.

DPOC: doença pulmonar obstrutiva crônica.

EUA: Estados Unidos da América.

FC: frequência cardíaca.

FEV_1: forced expiratory volume in one second.

FVC: forced vital capacity.

GE: group equation.

GV: group validation.

HAART: highly active antiretroviral therapy.

HAS: hipertensão arterial sistêmica.

Hb: hemoglobin.

HDL: high-density lipoproteins.

HF: high frequency.

HIV: vírus da imunodeficiência humana.

HR: heat rate.

HRV: heart rate variability.

HTLV: vírus linfotrópico T humano.

IAM: infarto agudo do miocárdio.

IF: inibidores da fusão.

II: inibidores da integrase.

IMC: índice de massa corporal.

IP: inibidores da protease.

iRR: RR intervals.

ITRN: inibidores da transcriptase reversa nucleosídicos.

ITRNN: inibidores da transcriptase reversa não nucleosídicos.

LDL: low-density lipoproteins.

LF: low frequency.

MIP: maximal inspiratory pressure.

MEP: maximal expiratory pressure.

NNRTI: non nucleoside reverse transcriptase inhibitor.

NN50: string heart intervals with greater difference 50 ms.

NRTI: nucleoside reverse transcriptase inhibitor.

OMS: Organização Mundial da Saúde.

PEF: peak flow expiratory.

PI: protease inhibitor.

PI: pulse interval.

PNN50: ratio given by the ratio between NN50 and the total number of intervals NN.

RB: rate breathing.

RHR: heart rate responses.

RMSSD: Root mean square of squares of differences of successive NN intervals.

RNA: ácido ribonucléico.

RV: residual volume.

SBP: systolic blood pressure.

SDPI: standart derivation of the pulse interval.

SpO_2: peripheral oxygen saturation.

TARV: terapia antiretroviral tripla de alta potência.

TLC: total lung capacity.

TNF-α: fator de necrose tumoral alfa.

VARRR: Variability of intervals RR.

VEF_1: volume expiratório forçado no 1º segundo.

VL: viral load.

6-MWT: 6 minute walk test.

6-MWD: 6 minute walk distance.

SUMÁRIO

Capítulo I – Revisão da Literatura

1 INTRODUÇÃO

O vírus da imunodeficiência humana (HIV) foi descrito pela primeira vez em 1981 nos Estados Unidos da América (EUA). Porém, existem registros ocorridos entre as décadas de 40 e 50 de casos na África Central, EUA e Haiti de pacientes que apresentaram sintomas característicos da contaminação por HIV, mas que tiveram a causa de morte descrita como desconhecida (DOURADO *et al*., 2006; BRASIL, 2011; GILBERT *et al.,* 2007).

A partir disso, descobriu-se que a infecção pelo HIV afeta principalmente os linfócitos T, que expressam receptores para o CD_4. Com o aumento da replicação viral associada a fatores externos, ocorre a Síndrome da Imunodeficiência Adquirida (AIDS), cujo primeiro sinal é a redução do número de células CD_4. Sendo que a depleção dessas células reduz as defesas do organismo contra infecções secundárias, também chamadas de oportunistas (SHARP *et al.,* 2010). Esse fato gerou no início da epidemia um grande número de internações hospitalares por esse grupo de pacientes. No Brasil, atualmente, a AIDS encontra-se em processo de estabilização, embora em patamares elevados (DOURADO *et al*., 2006).

Ainda assim, no início da epidemia, as relações homossexuais e o uso de drogas endovenosas eram as principais vias de transmissão da doença. Atualmente, observa-se um aumento da transmissão por relações heterossexuais, sendo essa a maior via de contágio entre as mulheres. Isso ocasionou uma modificação do perfil dos indivíduos infectados, pois houve um aumento crescente do número de mulheres contaminadas e, consequentemente, o número de crianças infectadas pela via vertical (VALENTE *et al*., 2005).

Em vista das complicações decorrentes do HIV resultante da infecção causada pela replicação viral associada a fatores externos, os portadores do vírus apresentavam altos índices de mortalidade. Porém, com o advento da *highly active antiretroviral therapy* (HAART) ou terapia antiretroviral tripla de alta potência (TARV), conhecida popularmente como “coquetel”, observou-se um profundo impacto na história natural da infecção pelo HIV. Apesar disso, observou-se que a terapia antiretroviral é acompanhada de alterações metabólicas como dislipidemia,

resistência insulínica, hiperglicemia e redistribuição de gordura corporal, sendo esses, fatores de risco para doença cardiovascular (HADIGAN *et al*., 2001; VALENTE, *et al*., 2005).

Além disso, ocorrem alterações no sistema respiratório com presença de infecções pulmonares decorrentes de alterações da resposta imune pulmonar (TWIGG *et al*., 2008). Outra modificação ocorre em relação aos músculos ventilatórios, houve relatos de que complicações musculares foram observadas em até 96% dos pacientes infectados pelo HIV, além de uma diminuição de força de 20% a 30% dos músculos inspiratórios e expiratórios decorrente da diminuição de massa muscular (SCHULZ *et al*., 1997; GABBAI *et al*., 1999; LAGHI *et al*., 2003).

Somado a isso, sugere-se uma associação entre HIV e alteração autonômica (COMPOSTELLA *et al*., 2008). Nesse contexto, um estudo com 25 pacientes que avaliou o sistema autônomo demonstrou que pacientes portadores do HIV têm um significativo aumento da frequência cardíaca (FC) em comparação com outros grupos, sugerindo que a infecção por HIV pode estar associada à disfunção do sistema autônomo (ROGSTAD *et al*., 1999).

Nesse sentido, nas próximas sessões, realizaremos uma breve revisão da literatura sobre a AIDS, incluindo o histórico, dados epidemiológicos, fisiopatologia e farmacologia. Dessa forma, haverá uma elucidação sobre as principais complicações causadas pela AIDS e o uso da TARV em alguns sistemas do organismo. Além disso, buscaremos, também, identificar as alterações do sistema respiratório, as respostas do sistema cardiovascular e a presença de neuropatia autonômica de pacientes portadores do HIV com ou sem uso de TARV.

2 Síndrome da Imunodeficiência Adquirida

2.1 HISTÓRICO

A AIDS foi descrita pela primeira vez em 5 de junho de 1981. Gottlieb *et al*., relataram os casos de cinco homens jovens anteriormente saudáveis, homossexuais tratados por *Pneumocystis carinii* em três hospitais de Los Angeles (COCK *et al.,* 2012). No entanto, faz-se ideia, que o vírus iniciou a interação com a espécie humana no início do século vinte, com a transmissão via cruzamento de espécies, sendo que o vírus foi identificado, primeiramente, em chimpanzés do oeste da África Equatorial (SHARP *et al*., 2011). Além disso, exames de sequência genética do HIV-1, sugeriram que a infecção foi da África para o Haiti na década de 60 e mais tarde foi introduzido nos EUA (GILBERT *et al.,* 2007).

O primeiro caso retrospectivo identificado no Brasil foi no estado de São Paulo, no ano de 1980. Em fevereiro de 1983, o *Centers for Disease Control* relatou 1000 pessoas com AIDS nos EUA, com um índice de mortalidade de 39%. Nesse mesmo ano, foram realizadas as primeiras recomendações para prevenção da AIDS, sendo que no ano seguinte o agente causador foi identificado e, em 1985, iniciou-se a avaliação com o teste de anticorpo (COCK *et al.,* 2012).

No ano de 1987, iniciaram-se os testes *in vitro* da Zidovudina, um inibidor da transcriptase reversa, na supressão da replicação viral do HIV, sendo aprovada no mesmo ano nos EUA (VELLA *et al*., 2012). Assim, após 15 anos da primeira descrição da AIDS, foi reportado em 1996, na Conferência Internacional da AIDS em Vancouver, a diminuição da carga viral e o controle da progressão da doença em pacientes sob tratamento medicamentoso com HAART. Nesse mesmo ano, no Brasil, iniciou-se o processo de acesso livre e universal à TARV (COCK *et al.,* 2012).

2.2 EPIDEMIOLOGIA

Estima-se que em meados da década de 90, mais de 20 milhões de pessoas viviam com HIV/AIDS no mundo. Retrospectivamente, soube-se que o pico foi atingido em 1997 (COCK *et al.,* 2012). Segundo a Organização Mundial da Saúde (OMS) (2010), em torno de 34 milhões de pessoas vivem com HIV conforme registros até o ano de 2009, sendo aproximadamente 31 milhões de adultos e a

maioria do sexo feminino. Ainda assim, em 2009, houve mais de 2.6 milhões de novas infecções. Desses dados, aproximadamente 1.4 milhões de pessoas alocadas na América Central e do Sul convivem com o HIV.

Conforme dados divulgados pelo Ministério da Saúde (2011), no Brasil a prevalência do HIV se mantém estável em 0,6%, sendo uma média de 35 mil novos casos diagnosticados a cada ano, com a média/ano de 11 mil óbitos. Estima-se que 630 mil brasileiros convivam com o vírus, acumulados 608.230 mil casos até junho de 2011, sendo mais comum entre os homens.

Ainda conforme o Ministério da Saúde, quando se compara o ano de 2010 com 2009, houve uma queda na taxa de diagnósticos, sendo respectivamente 34.212 contra 35.979. A região sudeste concentra a maioria dos casos (56,4%), porém esse número vem sendo reduzido, pois há um aumento de diagnóstico ao longo dos anos, nas regiões norte, nordeste e sul. Porto Alegre foi a capital com maior incidência de casos em 2010, acumulando 99.8 mil casos, seguida de Florianópolis com 57.9 mil casos. Dentre os 15 municípios com mais casos diagnosticados de HIV/AIDS, 7 são municípios do estado do Rio Grande do Sul.

2.3 FISIOPATOLOGIA

O HIV é da família *Retroviridae*, ou seja, um retrovírus, também composto pelo vírus linfotrópico T humano (HTLV) tipo 1 e 2. O HIV possui dois tipos de vírus: o HIV-1 que é o principal responsável pela infecção humana e o HIV-2 (SHARP *et al*., 2011; VERONESE *et al*., 2002).

O HIV é constituído por um genona de ácido ribonucléico (RNA), sendo esse formado por um capsídeo proteico e um envoltório lipoproteico. No interior do capsídeo, adjacente ao RNA viral, encontra-se uma enzima fundamental para a reprodução do vírus: a transcriptase reversa. O envoltório proteico contém duas glicoproteínas que garantem a ligação do vírus à célula hospedeira: gp120 e gp41. Ambas apresentam um receptor de membrana denominado CD_4, sendo esse o receptor do vírus (VERONESE *et al*., 2002).

Entretanto, para que ocorra a penetração celular do genoma viral após a ligação ao CD_4, são necessários, pelo menos, dois co-receptores: CCR5 e CXCR4. Assim que o RNA viral invade o citoplasma da célula hospedeira, ele sofre a ação da

transcriptase reversa, enzima capaz de transcrever um ácido desoxirribonucléico (DNA) dupla hélice a partir de um RNA hélice única. A partir do momento que o genoma viral passa a ser DNA, ele é capaz de se integrar ao DNA do núcleo da célula (HO *et al*., 1995; SHARP *et al*., 2011; VERONESE *et al*., 2002).

Assim, quando ocorre essa integração, o código genético do vírus consegue produzir as suas próprias proteínas e uma cópia fidedigna do seu RNA. Há então, a formação um de vírus descendente que acaba aumentando o seu número na célula hospedeira durante a replicação viral. A partir do momento do início da replicação viral em grande escala, há um prejuízo na fisiologia celular, culminando na sua morte. Após o início da infecção, ocorre uma depleção massiva das células CD_4, conferindo risco substancial de infecções oportunistas e outras complicações que ocorrem ao longo da infecção pelo HIV (CROTHERS *et al*., 2011).

A transmissão do vírus pode ocorrer por diversas formas: relações sexuais, drogas intravenosas, hemotransfusão, acidentes com materiais perfurocortantes ou semelhantes e transmissão perinatal ou vertical (COCK *et al.,* 2012; VERONESE *et al.,* 2002).

2.3.1 Diagnóstico

O diagnóstico é feito pela sorologia: o teste “anti-HIV”. Esse procedimento é capaz de medir a presença do anticorpo anti-HIV, contra os principais antígenos virais. A maioria dos indivíduos infectados apresenta positividade sorológica anti-HIV entre o período compreendido de 6 a 12 semanas do contágio, porém também pode ocorrer após 6 meses. Durante esses 3 a 6 meses, o paciente já é capaz de transmitir o vírus, mas a sua sorologia ainda se apresenta negativa, constitui o que se chama de “janela imunológica” (VERONESE *et al.,* 2002).

2.3.2 Patogênese

Segundo Veronese *et al.,* (2002), após a contaminação com o vírus, ocorrem 3 fases distintas:

- ***Soroconversão*** (infecção aguda): logo após a entrada do vírus no organismo, ocorre uma replicação viral ocasionando o primeiro pico de viremia, esse

período é acompanhado de uma queda importante na contagem das células CD_4. Essa fase apresenta uma duração de 7 a 14 dias, onde ocorre uma síndrome viral com as seguintes características clínicas: febre, adenopatia, erupções cutâneas no tórax, mialgias e faringite. Ao final de 3 a 6 meses, os pacientes soroconvertem, ou seja, passam a apresentar anticorpos IgG anti-HIV.

- ***Fase assintomática*** (latência clínica): o surgimento da resposta humoral, associada à resposta imune celular citotóxica, contém parcialmente a replicação viral, diminuindo a viremia. Nesse período, as células de CD_4 voltam a assumir valores mais altos, porém, não atingem os valores anteriores à contaminação.

- ***Fase AIDS:*** a classificação se baseia principalmente na contagem de células CD_4 e na presença de sintomas precoces ou doenças oportunistas. Essa fase se caracteriza pela contagem de CD_4 inferior a 200 células por mm^3 de sangue, em decorrência do segundo pico de viremia e pelas doenças oportunistas que só ocorrem após a presença dessa grave imunodeficiência. Pacientes portadores do HIV apresentam baixa imunidade após a infecção, sendo resultado da síndrome de desregulação imune (CROTHERS *et al.,* 2011). O final dessa fase é marcada pelo óbito do paciente.

2.4 TERAPIA ANTIRETROVIRAL

Os índices de mortalidade dos pacientes portadores do HIV reduziram em 1996 e início de 1997, após permanecer constante entre 1994 e 1995, coincidindo com o início do tratamento medicamentoso. A diminuição da mortalidade se correlacionou com a combinação dos regimes antiretrovirais, especialmente com o uso da classe inibidores da protease (IP) (PALELLA *et al*., 1998). Ainda, segundo o referido autor, essa diminuição na mortalidade, correlacionou-se com o uso da combinação de regimes antiretrovirais, especialmente os que contêm a associação com IP.

O acesso livre e universal à TARV no Brasil iniciou em 1996 inaugurando uma nova fase: as pessoas vivendo com a AIDS passaram a ter alternativas às “sentenças de morte” decretadas na década de 80 (ALENCAR *et al*., 2008). A TARV provocou grande redução na mortalidade, diminuição da frequência e duração de internações hospitalares, diminuição nos diagnósticos de AIDS, redução nas

manifestações de doenças oportunistas e também aumento significativo da sobrevida (DOURADO *et al*., 2006; HSUE *et al*., 2012; PALELLA *et al*., 1998; TEIXEIRA, 2009).

Existem 3 grandes classes principais da TARV: inibidores da transcriptase reversa nucleosídicos (ITRN), inibidores da transcriptase reversa não nucleosídicos (ITRNN) e inibidores da protease (IP). Ainda, existem 3 novas classes aprovadas recentemente: inibidores da fusão (IF), inibidores de CCR5 e inibidores da integrase (II) (VELLA *et al.,* 2012). O início da terapia medicamentosa é feito quando a contagem de CD_4 for inferior a 350 cel/mm^3 (OMS, 2010). O uso de TARV suprime a replicação viral e previne complicações causadas pela AIDS na maioria dos pacientes infectados pelo HIV (MOORE *et al.,* 2011). Após 24 semanas do início do uso da TARV, os pacientes apresentam carga viral abaixo do limite detectável. (TWIGG *et al*., 2008).

2.5 ALTERAÇÕES METABÓLICAS

O uso de TARV, fez com que houvesse um declínio nas taxas de morbidade e mortalidade em decorrência da AIDS e apresentou eficiência no controle das infecções dos pacientes portadores de HIV. Porém, houve uma prevalência de condições crônicas, como: doença cardiovascular, pulmonar e hepática, e alguns tipos específicos de tumores. Associa-se a isso, mudanças no perfil lipídico, distribuição anormal de gordura, dislipidemia, resistência insulínica, diabetes, declínio neurocognitivo e osteoporose (GANDHI *et al*., 2012; HSUE *et al*., 2012; MALVESTUTTO *et al*., 2010; PALLELA *et al.,* 2006; STANLEY *et al*., 2012; STEIN *et al.,* 2012).

Os efeitos da dislipidemia, resistência insulínica e lipodistrofia são frequentemente efeitos adversos do uso de TARV (PÉRIARD *et al.,* 1999). O aumento dos níveis lipídicos afetam aproximadamente 50% dos pacientes que utilizam IP, o aumento do colesterol total (CT) afeta 28% dos pacientes e 96% dos pacientes apresentam elevados índices de triglicerídeos, sendo que os aumentos dependem do tempo de tratamento e do tipo de drogas utilizadas (DUBÉ et al., 2000; PRENDERGAST, 2003).

2.5.1 Composição corporal e mudanças metabólicas

A distribuição anormal de gordura afeta em torno de 50% dos pacientes tratados com TARV, esses pacientes apresentam uma lipoatrofia na face, braços e pernas; lipohipertrofia visceral, nas mamas, região cervical, e/ou na região dorsal da coluna cervical. Pode haver ainda uma combinação de lipoatrofia e lipohipertrofia (GRUNFELD *et al*., 2010; STANLEY *et al*., 2012). A lipodistrofia parece ser mediada, mesmo antes do início da TARV, pelo aumento de citocinas inflamatórias decorrentes da própria infecção pelo HIV e, mais tardiamente, também pelos efeitos da TARV (DIEHL *et al*., 2008).

A lipoatrofia ocorre por uma série de fatores que incluem: a inibição da diferenciação de adipócitos pelo IP e a deteriorização da função mitocondrial pelo ITRN, já o mecanismo da lipohipertrofia não se encontra bem descrito, mas parece estar associado aos elevados níveis de citocinas inflamatórias (JOHNSON *et al*., 2004; STANLEY *et al*., 2012). No entanto, pacientes portadores do HIV que apresentam distribuição anormal de gordura, geralmente mantém o índice de massa corporal (IMC) em valores normais ou sobrepeso, porém apresentam complicações cardiovasculares semelhantes a pessoas com obesidade (STANLEY *et al*., 2012).

2.5.2 Resistência insulínica e *diabetes mellitus* (DM)

Um estudo mostrou que marcadores inflamatórios sistêmicos incluindo altos valores da proteína C-reativa (PCR) associada ao fator de necrose tumoral alfa (TNF-α) dos receptores 1 e 2 são associados ao aumento do risco de desenvolvimento de DM em pacientes portadores do HIV, sugerindo que a inflamação crônica prejudica o metabolismo da glicose (BROWN *et al.,* 2010). Além disso, a incidência de DM aumenta com a exposição cumulativa ao uso de TARV após controle de outros fatores de risco (DE WIT *et al*., 2008).

2.5.3 Dislipidemia

Pacientes portadores do HIV já apresentam desde o início da infecção, alterações referentes às modificações lipídicas: das lipoproteínas de alta densidade (HDL), lipoproteínas de baixa densidade (LDL) e níveis aumentados de triglicerídeos. No decorrer do curso da infecção e o tratamento com TARV, há aumento dos níveis de triglicerídeos, os índices de HDL permanecem baixos e o LDL geralmente aumenta (RIDDLER *et al*., 2003). Os índices de triglicerídeos

aumentados frequentemente anunciam anormalidades lipídicas em pacientes portadores do HIV e parecem contribuir independentemente para o aumento de risco cardiovascular nessa população (STANLEY *et al*., 2012).

2.6 ALTERAÇÕES MUSCULARES

Os pacientes portadores do HIV que recebem ou não TARV, apresentam uma fadiga aguda, indicando uma disfunção celular mitocondrial (VOSS, 2005; WANTLAND *et al*., 2011). Além disso, alguns estudos notificaram que ocorrem mudanças da estrutura das fibras musculares dos pacientes com HIV. A biópsia muscular mostrou alterações das fibras musculares estriadas, variações do tamanho da fibra e desnutrição (SCHULZ *et al.,* 1997; GABBAI *et al*., 1999). A fadiga muscular, geralmente é relatada associada a outros sintomas de doenças crônicas, como: fibromialgia, osteoartrite e artite reumatóide (WANTLAND *et al*., 2011).

Apesar do número pequeno de estudos, existem relatos de complicações musculares em 96% dos pacientes portadores do HIV (GABBAI *et al*., 1999; LAGHI *et al*., 2003). Outros relatos de alterações musculares incluem os músculos ventilatórios, onde foi observada uma diminuição de força de 20% à 30% dos músculos inspiratórios e expiratórios. A partir desses achados, sugere-se uma diminuição de massa muscular (SCHULZ *et al*., 1997; GABBAI *et al*., 1999). Além disso, alguns estudos prévios ao início da terapia medicamentosa ou logo após o início dessa terapêutica mostraram um aumento de força muscular após a interrupção do uso da TARV, juntamente com a elevação dos níveis de creatinina quinase, porém não houve recuperação dos valores prévios à infecção (GABBAI *et al*.; 1999; TILL *et al*., 1990).

2.7 ALTERAÇÕES CARDÍACAS

A doença cardiovascular (DCV) é uma das principais complicações que afetam os pacientes portadores do HIV. O aumento do risco de DCV está associada à infecção pelo HIV e por grupos de risco tradicionais, como: tabagismo, efeitos do TARV sobre o perfil lipídico, resistência insulínica, composição corporal e hipertensão arterial sistêmica (HAS) (PRENDERGAST, 2003; STEIN *et al.,* 2012; TRAINT, 2012). A DCV é derivada do processo inflamatório causando durante a ocorrência da replicação viral. Ocorre que as partículas lipídicas infiltram a camada íntima arterial e ativam as células endoteliais (HSUE *et al*., 2012).

Dessa forma, há um aumento na expressão da adesão de moléculas inflamatórias que atraem as células inflamatórias, contribuindo para a formação de ateromas (DAI *et al.,* 2004; HSUE *et al*., 2012; SUBRAMANIAN *et al*., 2012). Nesse contexto, ocorre uma migração de uma variedade de células-T conduzindo um processo não estável de inflamação crônica da placa aterosclerótica. Esse processo inflamatório crônico na artéria promove um depósito de colágeno, fibrose e aumento da espessura da camada arterial (HSUE *et al*., 2012).

Um estudo mostrou elevada taxa de inflamação na artéria aorta quando comparou pacientes portadores do HIV e controles sem HIV (SUBRAMANIAN *et al*., 2012). A interrupção do uso de TARV associa-se com elevados índices de mortalidade e eventos de DCV quando comparado ao uso contínuo das medicações. Além disso, elevados índices de interleucina-6 e D-dímeros foram associados a todas as causas de mortalidade.

Assim, conclui-se que o uso contínuo de TARV reduziu o processo inflamatório resultante da infecção pelo HIV, além da diminuição da replicação viral (EMERY *et al.,* 2008; KULLER *et al*., 2008; STEIN *et al.,* 2012). Estudos sugeriram que a contagem de células CD_4 está fortemente associada ao risco de desenvolvimento de DCV, em virtude do processo inflamatório contínuo decorrente da replicação viral (BACKER *et al*., 2008).

Ao final de 2003, foi publicado um importante estudo para avaliar o risco cardiovascular em indivíduos infectados pelo HIV. Trata-se o *The Data Collection Adverse Events of Anti-HIV Drugs* (DAD) *Study Group*, um estudo prospectivo realizado em países da Europa que acompanhou 23.468 pacientes, dos quais 126 (0,5%) apresentaram infarto agudo do miocárdio (IAM). A incidência de IAM aumentou com o tempo de exposição à terapia antiretroviral, principalmente nos que faziam uso de IP. Não estiveram associados ao IAM: presença de antecedentes familiares para doença cardiovascular, lipodistrofia e HAS.

2.8 ALTERAÇÕES PULMONARES

O trato respiratório tem comunicação direta do organismo com o ambiente externo. As respostas pulmonares imunes são importantes para a manutenção da imunidade do sistema respiratório. A imunidade pulmonar é dividida na resposta

inata e resposta adquirida. A infecção pelo HIV causa mudanças que incluem: ativação de macrófagos e linfócitos, secreção de citocinas pró-inflamatórias e acúmulo de células CD_8 no espaço alveolar, todas essas mudanças têm grande impacto na imunidade pulmonar (TWIGG *et al*., 2007).

Quando um patógeno entra em contato com a mucosa do trato respiratório, é fagocitado pelo macrófago alveolar, sendo essa a principal função da imunidade inata. Na infecção pelo HIV, acontece um grande impacto em todos os componentes pulmonares da resposta imune. Ocorre, então, uma ativação celular generalizada, um acúmulo de células imunes e de mediadores pró-inflamatórios no espaço alveolar (TWIGG *et al*., 2007). Além disso, essas alterações incluem anormalidades da função mucociliar, afetando as defesas com aumento de secreções respiratórias (CROTHERS *et al*., 2011; SHELLITO,2004).

O impacto do uso da TARV no perfil pulmonar ainda não está bem esclarecido. Sistemicamente, o tratamento com TARV diminui a replicação viral, ativação imune e inflamação crônica e aumenta a contagem de células CD_4 (CROTHERS *et al*., 2011; TWIGG *et al*., 2007). Especificamente, dentro do espaço alveolar, os efeitos de TARV levam a uma diminuição da carga viral e da resposta pulmonar inflamatória (TWIGG *et al*., 2008).

O efeito da infecção viral nos pulmões inclui uma série de complicações tanto de origem infecciosa quanto não infecciosa. Dentre as principais alterações, encontram-se: pneumonias, tuberculose, câncer pulmonar, hipertensão pulmonar e doença pulmonar obstrutiva crônica (DPOC) (CROTHERS *et al*., 2011; GINGO *et al*. 2012). Observou-se que pacientes com contagem de CD_4 inferiores a 200 cel/mm^3 apresentam valores mais baixos das variáveis que avaliam a função pulmonar em ambos os sexos (ONYEDUM *et al*., 2010). Assim, infecções pulmonares são provavelmente menores em pacientes com alta contagem de células CD_4, carga viral indetectável e uso de TARV (CROTHERS *et al*., 2010).

Ainda assim, um estudo com espirometria apenas pré-broncodilatador, mostrou que os pacientes portadores do HIV tem 7% de obstrução com a relação volume expiratório forçado no 1º segundo (VEF_1) e a capacidade vital forçada (CVF) mais baixa em pacientes com história prévia de tabagismo e pneumonia ou que utilizavam TARV (GEORGE *et al*., 2009; GINGO *et al.,* 2010; GINGO *et al*. 2012;

ONYEDUM *et al*., 2010). Outro estudo relatou que sintomas respiratórios estão presentes em 2/3 dos pacientes portadores do HIV, mais presente em pacientes tabagistas do que não tabagistas (68,5% *vs* 47,5%). O sintoma mais comum é a dispneia, seguida pela tosse, produção de secreção e dor no peito (GINGO *et al.,* 2010; ONYEDUM *et al*., 2010).

2.9 DISFUNÇÃO AUTONÔMICA

As anormalidades do sistema nervoso autônomo na infecção pelo HIV foram inicialmente trazidas a nossa atenção no ano de 1987, onde reações de síncope foram descritas em quatro de cinco pacientes com AIDS (inclusive com a morte de um deles), como resultado de um aspirado pulmonar com agulha fina. Isso evocou uma atenção à reação vagal e parada cardíaca durante procedimentos invasivos com anestesia geral ou epidural (ROGSTAD *et al*., 1999). O HIV não infecta diretamente os neurônios, ao contrário, pode danificar as células que envolvem os nervos, causando um isolamento neural e retardando, distorcendo ou interrompendo a transmissão de informações dos tecidos para o cérebro (ROGSTAD *et al.,* 1999).

A causa dessas alterações não está bem determinada, as hipóteses são: o vírus destrói diretamente as fibras nervosas ou o nervo é destruído secundariamente por mediadores tóxicos ou radicais livres, sendo consequência anormal dos mecanismos imunológicos (COMPOSTELLA *et al*., 2008; NEILD *et al.,* 2000; WULFF *et al*., 2000).

Os componentes simpático e parassimpático regulam a atividade cardíaca. A atividade cardiovagal é analisada no domínio da frequência e no domínio do tempo que são baseados nas mensurações de variabilidade da frequência cardíaca (VFC). A magnitude da variabilidade traz informações sobre a modulação simpática e parassimpática no coração. Uma alta variabilidade, geralmente reflete grande modulação parassimpática (CHOW *et al.,* 2006). Uma redução da VFC pode implicar em um pior prognóstico com aumento de eventos cardiovasculares (TSUJI *et al*., 1996).

A análise pelo domínio da frequência traz informações sobre o balanço simpatovagal. As modulações simpática e parassimpática são quantificadas pela análise espectral, com um intervalo de variação da frequência de 0.04 a 0.40 Hz

(CHOW *et al.,* 2006). A faixa de baixa frequência (LF) representa tanto o controle simpático quanto parassimpático. Já a faixa de alta frequência (HF), reflete a modulação parassimpática. O aumento da modulação simpática e a diminuição da parassimpática está associado com o aumento da prevalência de doença cardiovascular e DM (CHOW *et al*., 2006).

Pacientes com neuropatia autonômica cardiovascular apresentam redução significativa do número de células CD4 quando comparados com pacientes sem essa neuropatia, sendo significativamente maior sua prevalência em pacientes com infecção avançada (COMPOSTELLA *et al*., 2008; GLÜCK *et al*., 2000). Porém ainda há divergência entre a correlação de CD_4 e parâmetros autonômicos (CHOW *et al.,* 2006) .

A disfunção autonômica pode fornecer uma explicação alternativa para sintomas comumente observados em pacientes infectados pelo HIV tais como: disfunção intestinal e da bexiga, impotência sexual, transtornos de síncope, sudorese e boca seca (ROGSTAD *et al*., 1999). A disfunção autonômica cardiovascular é significativamente associada com a progressão do HIV, o que pode expressar um risco importante de morte súbita nesses pacientes (GLÜCK *et al*., 2000). Dessa forma, a avaliação clínica da função autonômica, ajuda a identificar pacientes com aumento do risco para eventos cardiovasculares como de parada cardiorrespiratória. Sendo essa, considerada atualmente, uma das principais causas de morte dos pacientes portadores do HIV, torna-se importante esclarecer melhor o comprometimento da função simpática e vagal nestes pacientes.

2.10 CAPACIDADE FUNCIONAL

A redução da massa muscular tem significativo impacto no desempenho funcional, independência funcional, além se ser associado à qualidade de vida de pacientes portadores do HIV (SCOTT *et al*., 2007)

A diminuição da atividade física é consequência da infecção crônica e sarcopenia em pacientes portadores do HIV em uso de TARV, tendo como importantes marcadores da atividade física indicadores característicos da infecção, como: CD_4 e carga viral (HIGH *et al.,* 2005; OURSLER *et al*., 2009; RUSCH *et al.,* 2004).

Uma maneira que se pode avaliar a capacidade funcional desses pacientes, é através do teste de caminhada de 6 minutos (TC6min). Esse é um teste prático que avalia de forma global e integrada as respostas de todos os sistemas envolvidos no exercício, como: cardiopulmonar, circulatório, neuromuscular e muscular. É um teste que mede a capacidade submáxima ao exercício, similar ao nível utilizado para realização das atividades de vida diárias (CRAPO *et al.,* 2002; IWAMA *et al*., 2009).

3. JUSTIFICATIVA

O aumento da expectativa de vida dos indivíduos portadores de HIV pela administração da TARV é acompanhado pelo incremento dos riscos das alterações metabólicas, cardiovasculares e pulmonares. Além disso, o desenvolvimento das pesquisas científicas e do conhecimento em torno da AIDS vem acontecendo concomitante com o desenrolar da epidemia (ALENCAR *et al*., 2008). As alterações provocadas por essas medicações estão correlacionadas com o desenvolvimento de alterações fisiológicas. Essas elevam o risco de complicações metabólicas e cardiovasculares destes pacientes, alterando o paradigma da AIDS. Na medida em que aumenta a expectativa de vida destes indivíduos, a comunidade científica se depara com a instalação de outras co-morbidades que, a longo prazo, podem apresentar impacto na qualidade de vida e na mortalidade dos pacientes.

A fisiopatologia das alterações metabólicas, bem como seus agentes causais, ainda não foram totalmente identificados. Assim sendo, somente uma adequada elucidação dos mecanismos fisiopatológicos da síndrome propiciará a escolha de medidas terapêuticas mais eficazes, reduzindo-se os riscos cardiovasculares (VALENTE *et al*., 2005; GUIMARÃES, *et al*., 2007).

Além disso, a disfunção autonômica cardiovascular está significativamente associada com a progressão do HIV, o que pode identificar um risco importante de morte súbita nesses pacientes (GLÜCK *et al*., 2000). Soma-se a isso o fato de que a disfunção autonômica pode fornecer uma explicação alternativa para os sintomas comumente observados em indivíduos infectados pelo HIV (ROGSTAD *et al.,* 1999; VERMA, 2001).

Portanto, com base nestas informações e também devido à falta de uma avaliação precisa de algumas variáveis importantes para o adequado funcionamento do sistema respiratório e cardiovascular, bem como para a capacidade funcional que podem se apresentar alteradas, torna-se relevante uma avaliação detalhada destes parâmetros nos pacientes portadores do HIV, com e sem uso de TARV. Este detalhamento irá proporcionar um melhor entendimento das alterações apresentadas por esse grupo de pacientes. Por fim, os resultados obtidos poderão

servir para identificar as alterações cardiopulmonares dos pacientes portadores do HIV e revelar qual a influência da TARV nessas alterações.

4. OBJETIVOS

4.1 Objetivo Geral

Estabelecer a equação de predição e avaliar a capacidade funcional submáxima pela distância percorrida no teste de caminhada de seis minutos, investigar a presença de alterações dos sistemas respiratório, as respostas do sistema cardiovascular e da função autonômica de pacientes portadores do HIV com ou sem uso de TARV.

4.2 Objetivos Específicos

- Descrever uma equação de predição e avaliar a capacidade funcional pela distância percorrida durante o teste de caminhada dos seis minutos;
- Verificar volumes e capacidades pulmonares;
- Verificar a força dos músculos ventilatórios;
- Verificar as respostas da FC e pressão arterial (PA) durante mudanças de decúbito, respiração profunda e manobra de Valsalva;
- Avaliar o comportamento da variabilidade da FC (VFC);
- Correlacionar os resultados dos testes com os exames laboratoriais, tempo de diagnóstico e aos grupos utilizados no tratamento medicamentoso.

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Capítulo II – Artigos Científicos

ARTIGO I

The six-minute walk test in HIV-infected patients: validity, reproducibility and reference equation

(Artigo formatado conforme *Respiratory Medicine*)

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THE SIX-MINUTE WALK TEST IN HIV-INFECTED PATIENTS: VALIDITY, REPRODUCIBILITY AND REFERENCE EQUATION

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Running title: REFERENCE EQUATIONS FOR THE 6-MINUTE WALK TEST IN HIV-INFECTED PATIENTS

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Summary The measured distance in the 6-minute walk test (6-MWT) was investigated 155 patients infected with human immunodeficiency virus (HIV) by reference equation in healthy subjects, and these underestimated or overestimated measured the distance percored (6-MWD) in patients HIV-infected. From this, 124 patients were allocated to the group equation (GE) and 31 patients in the group validation (GV) to create the equation for predicting the 6-MWD in patients with HIV. The measured 6-MWT was 503.6 ± 70.4 m women and 590.8 ± 76.2 m men ($p < 0.001$) in GE and 509.4 ± 76.4 m women and 613.4 ± 85.6 m men ($p < 0.001$) in GV. For the multiple regression analysis data,the vital signs measured before and after the 6-MWT were considered: heart rate (HR), rate breathing (RB), oxygen saturation (SpO_2), blood pressure (BP) and the perception of dyspnea Borg scale. In addition, the values of lipid profile, blood glucose, creatinine, CD_4, viral load, smoking, time of diagnosis and medication use and anthropometric variables were analyzed. The variables were correlated gender ($r = 0.51$, $p < 0.05$), age ($r = -0.37$, $p < 0.05$), ΔHR ($r = 0.31$, $p < 0.05$). The equation was developed: 6-MWD = 610.07 + (gender (0 women, 1 men) * 75.68) - (age * 3.15) + (ΔHR * 1.43) ($r^2 = 0.44$). From these results, it was found that HIV-infected patients presented longer measured 6-MWD that the predicted value obtained by this reference equation.

Keyworks: Human immunodeficiency vírus. 6-minute walk test. Physical activity. Reference equation. Functional capacity. Validity.

Introduction

The 6-min walk test (6-MWT) is a practical test that evaluates the global and integrated responses of all components involved in the exercise, such as cardiopulmonary, circulatory, muscular and neuromuscular systems.[1-2] It is a test that measures the endurance to submaximal exercise, similar to the level used to perform daily life activities[2]. The result is obtained through predicted distance equations (6-MWD), which reference values are now validated for healthy subjects of different age groups in different countries, as well as for patients with different chronic diseases. [3-6] In addition, the 6-MWT has been considered a good predictor of mortality in patients with chronic obstructive pulmonary disease (COPD)[7] and after liver transplantation[1]. It also presents high reproducibility and effectiveness in the determination of physical activity in patients with heart failure.[8-9]

Patients with human immunodeficiency virus (HIV) have cardiopulmonary changes associated with the natural evolution of the virus in the body, as well as due to cardiotoxicity of medications. Lung diseases are responsible for most part of morbidity and mortality in patients with HIV[10-11]. Other less common lung diseases may affect these patients, such as lung cancer, pulmonary hypertension and fibrosis[11]. Still, respiratory symptoms are present in approximately 2/3 of patients with HIV, being dyspnea and cough the main primary symptons.[12]

The population with HIV needs constant medical monitoring due to its peculiarities and clinical control of the disease. It is known that these patients have high rates of physical inactivity.[13] The 6-MWT, has been used in the assessment of patients with HIV, but the test's accuracy is not well validated in this population.[14] Furthermore, the use of reference equations derived from populations that do not meet the specificities of these patients may overestimate the 6-MWD for this population.[15] Thus, patients may have a poor performance in the 6-MWT and, by mistake, could be considered individuals with lower functional capacity than real.

Therefore, the aim of the present study was to evaluate the performance of a group of HIV patients compared to reference equations used in healthy subjects, and

to develop and validate a reference equation for the 6-MWD in the 6-MWT in patients with HIV .

Methods

Subject

For the present cross-sectional study were recruited 450 patients of both genders, aged over 18 at 75 years, HIV infected, from the Department of Infectious Diseases of the Irmandade da Santa Casa de Misericórdia de Porto Alegre (ISCMPA), Rio Grande do Sul, Brazil from October 2011 to October 2012. The study was approved by the ethics committee under protocol number 075/05 of the Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA). Prior to data collection, the patients signed an informed consent form.

Two hundred ninety-five patients were excluded **(Figure 1)** and two hundred fity-four did were not eligible according exclusion [co-infection with hepatitis C virus (113), hepatitis B virus (16), human T-lymphotropic virus (10), Chagas disease (6), refused to participate in the study (41), lack of adherence to therapy (22), motor sequelae that hindered ambulation (14), diagnosed with psychological disorders (9), cardiac abnormalities (7), pregnant (7), use of orthoses in lower limb (7), body mass index (BMI) less than 18 kg/cm^2 (7), BMI greater than 35 kg/cm^2 (6), illicit drug use (5), visually impaired (4), hypertension (3), cognitive impairment (3), severe anemia (3), age over 75 years (2), lung cancer (1) and association of one or more exclusion criteria (9), and 41 have withdrawn from the study. Therefore, a hundred fifty-five patients (74 women) were eligible to perform the 6-MWT and subsequent comparison of the measured 6-MWD with the predicted 6-MWD by reference equations used in healthy subjects, respecting the track age group. [3, 6, 16-18] Additionally, the 155 subjects who underwent the 6-MWT were distributed into two groups, group equation (GE), which results were used in the preparation of the referece equation, and group validation (GV), which results were used to validate the reference equation. This group was formed after a random sample of 20% of each gender (15 women and 16 men) **(Figure 1)**.

6-min walk test

Two 6-MWT were done, however only the distance covered in the second test was considered for the analysis. The 6-MWT was performed according to the

guidelines of the American Thoracic Society[2] by two trained researchers, in a corridor of 30 meters marked every 5 meters. Prior to the start of the tests and shortly after completion, heart rate (HR), rate breathing (RB), systolic blood pressure (SBP), diastolic blood pressure (DBP), peripheral oxygen saturation (SpO_2) and feeling perceived exertion (dyspnea) by the Borg scale (17) were mensuared. The second test was performed at 30 minute intervals or when the patients had the measured variables values similar to those observed at the time prior to the first testing.

Patients were instructed to walk in a corridor as fast as they could during six minutes and to continue walking. When necessary, the patient was allowed to rest during the tests, and continued walking when he/she felt in conditions to complete the task. During the tests, patients received standardized phrases of encouragement every minute by the evaluator.

Anthropometric and clinical data

Besides the anthropometric variables (height, body mass and BMI), the following variables were used in the preparation of the reference equation: gender, age, time of medication use, and the results of some routine tests performed by patients with HIV (triglycerides, total cholesterol, high-density lipoproteins (HDL) and low-density lipoproteins (LDL) cholesterol, cluster of differentiation (CD_4) and glucose). Finally, the resting HR (HR_{rest}), HR observed in the sixth minute test (HR_{end}) and HR variation occurred during the test (ΔHR) were included in the analysis.

Statistical Analysis

Statistical analysis was performed using Statistica software version 10 (StatSoft, USA). Data are presented as mean ± standard deviation. A descriptive analysis of the variables, analysis of the distribution (Shapiro-Wilk test) and correlation analysis (Pearson coefficient) between the 6 minute walk distance (6-MWD) and other variables were performed. The comparison of variables between groups or genders was achieved using the unpaired t test or Fisher's exact test. To determine which variables were contributing in distance walked during 6-MWT and thus influenced in the reference equation for HIV-infected patients, we used a backward stepwise multiple regression was used. They were considered statistically significant at $p < 0.05$.

Results

The characterization data of the sample are presented in **Table 1**, according to both the allocation group and gender of patients. GE presented statistically significant differences in weight and height, but not in BMI. Statistical differences present in routine laboratory tests (CD_4, triglycerides, glucose and creatinine) have no clinical significance, because patients are within the standard reference range. The women of the GE had longer time of medication use than men, but this difference did not occur in GV. The viral load (VL) was calculated only between patients that had values above the detection limit, that is, higher than 50 copies/cell. Patients who did not use medication had significant viral load values. Patients that took the medication presented undetectable viral load, which demonstrates better adherence to drug treatment. The GV had few statistically significant differences and these were related to weight, height and creatinine. In both groups the men presented better performance characterized by longer 6-MWD. However, only the GE showed significant difference in the ΔHR. The comparison between the groups showed differences only in height and time of medication use between women. This result demonstrates the homogeneity of the GV compared to GE, which allows a better assessment of the results of the equation validation.

The **table 2** shows the comparisons of 6-MWD for patients in both groups with the reference equations used in healthy subjects. It is observed that among the equations developed in other countries, those proposed by Enrigth and Sherril[6], and Gibbons and colleagues,[17] overestimate the 6-MWD, while that proposed by Chetta and colleagues,[18] overestimates just for women. Moreover, the equations proposed from studies in Brazil[3, 16] present good prediction for men, but irregular prediction for women. The reference equation proposed in this study, based on data from GE was able to adequately predict 6-MWD patients of GV.

In all subjects, the 6-MWD was significantly correlated ($p<0.05$) related to gender ($r = 0.51$), height ($r = 0.41$), age ($r = -0.37$), HRpeak ($r = 0.31$), ∆HR ($r = 0.31$) and CD_4 ($r = -0.19$). The reference equation generated by backward stepwise multiple regression analysis for the 6-MWD included gender, age and ∆HR **(Table 3)**.

Discussion

The present study provides important information about the need for developing a reference equation for the 6-MWD in HIV-infected patients. When the performance of these patients was compared with the 6-MWT reference equations developed from data of healthy subjects, none of the equations was able to adequately predict the 6-MWD, especially for women. Thus, from a broad set of common data (gender, age, weight, height, BMI e HR), and specific data for patients with chronic conditions (time of medications use, CD_4, VL, glucose, total cholesterol, HDL and LDL), a reference equation for the 6-MWD was elaborated. Afterward this reference equation was validated in another HIV-infected patients group.

In this study, we employed the guidelines of the ATS to perform the 6-MWT, including the use of a 30 meters corridor, standardized incentive and a prior test for knowledge of the procedures. These recommendations were used in some studies[3, 16, 18], where they were employed to compare the 6-MWD results, but not in others.[6, 17] Not following some of these recommendations may have contributed to the overestimation of the 6-MWD as well as the fact that these studies have been conducted in countries in which the habits related to physical activities may differ from those observed in our country. Indeed the studies developed in Brazil[3, 16] have shown good prediction of 6-MWD for men, which confirm this statement.

The reference values obtained from the equation proposed may be useful to assess the functional capacity of the HIV-infected patients. Furthermore, the 6-MWD can also be used as a resource to assist in a safe prescription of an aerobic training program[19], as it has often been employed in non-advanced functional class heart failure patients.[8-9] For the determination of 6-MWD we performed two 6-MWT. They were performed because most studies have reported an increase in 6-MWD during the second test[9, 20], probably due to the familiarity and learning effect during the second 6-MWT. The 6-MWD is higher in young patients. According to Scholten and collegues[21], the age-related functional decline in HIV-infected patients is similar to other chronic disease populations. Nevertheless, in our study, the 6-MWD of older men (≥50 years) from GE (549 ± 80 m) and GV (544 ± 51 m), was higher than the 6-MWD observed in another study (514 ± 91 m), that also evaluated a middle-aged HIV-infected men group.[19] Furthermore, the performance of HIV-infected patients in 6-MWT is also related to its lower peripheral muscle mass[20]. Additionally, the

presence of CD_4 < 200 cells/mm^3 and low concentrations of hemoglobin are associated with lower physical performance of HIV-infected patients.[15]

Although a number of changes are commonly found in patients with HIV, such as abnormal fat distribution, dyslipidemia, insulin resistance, diabetes[13, 22-23], decreased CD_4 and increased VL, these findings did not correlate significantly with the 6-MWD, as well as the time of diagnosis and the time of medication use. Besides, although gender has a high correlation with the 6-MWD, we found lower values of the variation of 6-MWD when we analyzed the data by gender (men: r^2 0.27, p <0.001; women: r^2 0.16, p <0.001). Thus, the reference equation that includes the variables gender, age and ΔHR was chosen (r^2 0.44, p <0.001). Other reference equations have used gender and age among their variables.[3, 16] However, although there is a physiological significance in the HR variation and even a suggestion that it should be considered in relation to 6-MWD [18], to the best of our knowledge there are no studies that employed this variable in reference equations for the 6-MWD .

Nevertheless, future studies should investigate the relationship of this variable with the possible presence of autonomic modulation impairment, common in patients with chronic diseases.[24-25]

The results of the study of Paton and colleagues[13] allow us to infer that HIV-infected patients have low levels of physical activity and, consequently, reduced functional capacity[19]. One of the probable reasons is the symptom of acute fatigue in such patients. It is due to the presence of mitochondrial dysfunction, regardless of the use of highly active antiretroviral therapy (HAART).[26-27] In addition, HIV-infected patients may have other comorbidities that limit the functional capacity, such as fibromyalgia, osteoarthritis and rheumatoid arthritis.[27] Unfortunately it was not possible to evaluate the aerobic capacity by cardiopulmonary exercise testing, which would allow us to determine the degree of functional impairment and their possible causes. Another important limitation of the study was the inability to determine the time of infection.

The results of this study indicate that the use of non-specific reference equations for the studied population may under or overestimate the 6-MWT performance, measured by 6-MWD. Also, it accords with the results of other studies regarding the factors that relate positively (male gender and higher height) and negatively (older age) with 6-MWD. Although the CD_4, which is associated with better clinical condition of HIV-infected patients, showed no statistical significance to be

included in the reference equation, it also appears to positively influence their functional capacity. Finally, higher HR variation is associated with better performance on the 6-MWT.

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Figure 1: Flow diagram of patients for the development and validation for the 6-min walk test (6-MWT)

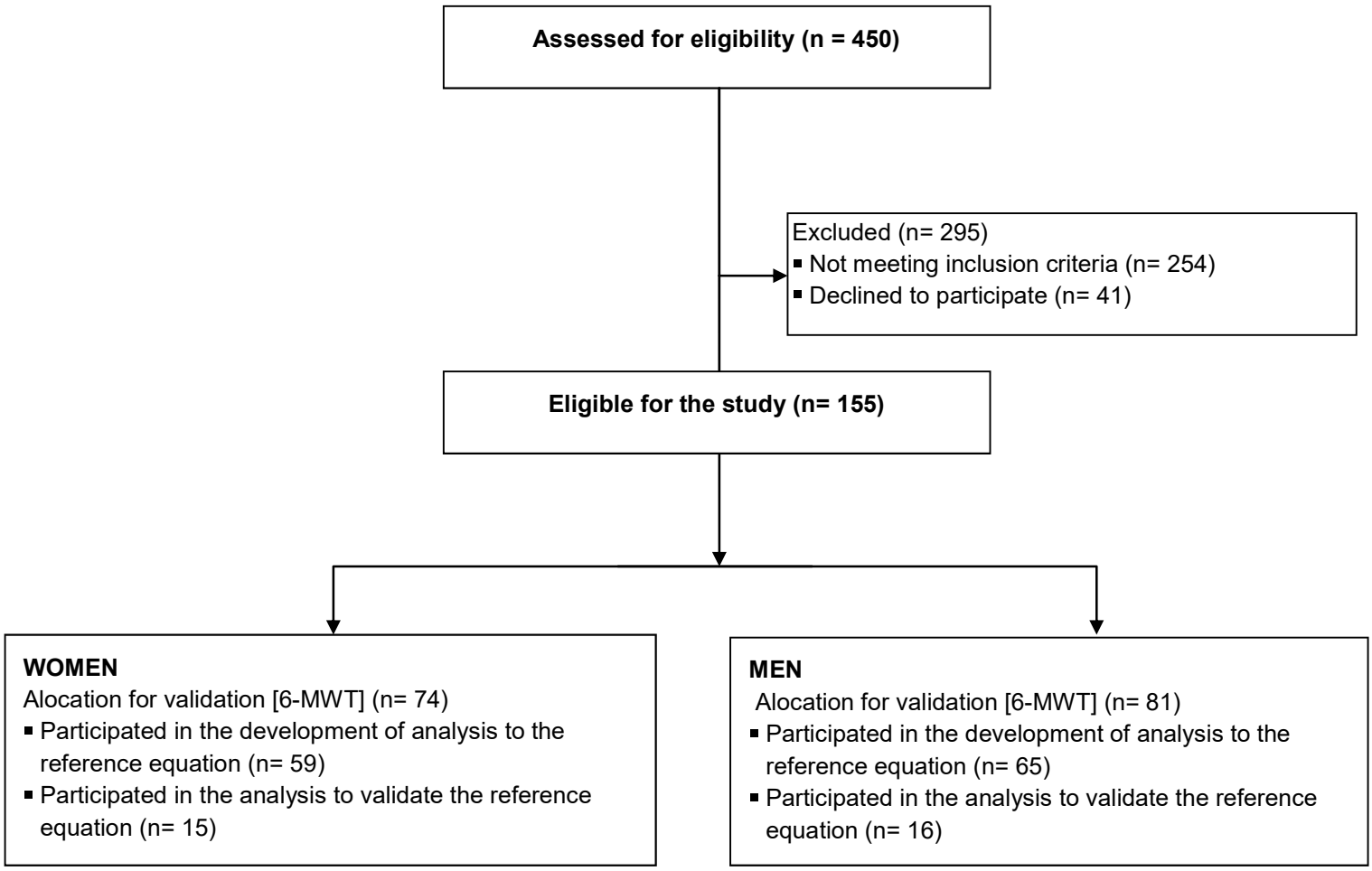

Table 1: Personal details and 6-min walk test measures of the study patients

	GE			GV			GE *vs* GV	
	Women (*n* = 59)	**Men** (*n* = 65)	***p*#**	**Women** (*n* = 15)	**Men** (*n* = 16)	***p*#**	**Women** ***p***	**Men** ***p***
Age (years)	43.9 ± 10.9	42.9 ± 9.2	0.604	44.1 ± 12.0	39.0 ± 10.3	0.217	0.954	0.140
Weight (kg)	63.7 ± 10.8	74.9 ± 11.8	<0.001	65.8 ± 11.6	77.0 ± 12.7	0.015	0.523	0.535
Height (meters)	1.60 ± 0.1	1.73 ± 0.1	<0.001	1.65 ± 0.1	1.75 ± 0.1	<0.001	0.012	0.359
BMI (kg/m^2)	25.0 ± 3.9	24.9 ± 3.4	0.895	24.2 ± 3.3	25.0 ± 3.0	0.450	0.445	0.890
Smoking (%)*	17.0 (28.9%)	13.0 (19.7%)	0.297	2.0 (13.3%)	4.0 (25%)	0.653	1.000	0.724
Time of HIV infection (months)	111.3 ± 58.2	89.6 ± 64.7	0.052	87.2 ± 45.9	67.2 ± 54.4	0.278	0.198	0.206
Use the medications*	48 (81.4%)	58.0 (89.2%)	0.307	13.0 (86.7%)	13.0 (81.3%)	1.000	1.000	0.405
Time of medications (months)**	102.8 ± 49.0	78.2 ± 53.5	0.015	71.8 ± 40.2	64.9 ± 48.0	0.697	0.027	0.388
CD_4 (cél/mm^3)	716.3 ± 301.4	590.5 ± 236.6	0.011	773.7 ± 375.3	579.7 ± 212.5	0.084	0.533	0.868
Viral load (cél/mm^3)***	1923.8 ± 2036.9	15765.6 ± 34647.2	0.238	4318.5 ± 5981.4	6194.3 ± 6888.8	0.771	0.671	0.429
Triglycerides (mg/dL)	156.2 ± 84.4	196.1 ± 120.3	0.037	151.5 ± 68.2	163.9 ± 83.7	0.655	0.839	0.316
Glucose (mg/dL)	94.0 ± 12.7	101.8 ± 19.1	0.009	96.3 ± 10.2	98.9 ± 11.9	0.531	0.515	0.565
HDL (mg/dL)	47.7 ± 12.4	45.8 ± 8.9	0.331	54.5 ± 16.8	45.7 ± 7.2	0.067	0.082	0.988
LDL (mg/dL)	131.7 ± 44.1	133.6 ± 43.2	0.809	136.3 ± 52.1	125.8 ± 36.2	0.520	0.733	0.506
Total Cholesterol (mg/dL)	210.7 ± 52.1	218.76 ± 53.9	0.404	221.0 ± 53.9	204.3 ± 53.9	0.362	0.496	0.332
Creatinine (mg/dL)	0.8 ± 0.2	1.0 ± 0.3	<0.001	0.8 ± 0.1	1.0 ± 0.1	<0.001	0.901	0.862
ΔHR (beats/min)	22 ± 15	28 ± 15	0.029	28 ± 23	25 ± 17	0.728	0.245	0.513
6-MWD (meters)	503.6 ± 70.4	590.8 ± 76.2	<0.001	509.4 ± 76.4	613.4 ± 85.6	0.001	0.780	0.304

Dates as mean ± standart deviation. Group equation (GE). Group validation (GV). Body mass index (BMI). Cluster of differentation (CD_4). Subtraction of heart rate (HR), resting HR after the test (ΔHR). * Fisher's exact test. ** Time of medications applied only in patients with values below the detection limit < 50 copies/cel (45 women and 55 men in GE; 13 women and 13 men in GV). *** Viral load applied only in patients with values above the detection limit < 50 copies/cel (14 women and 10 men in GE; 2 women and 3 men in GV). #Test applied within groups. ¨Test intergroup applied. Applied Student t test, statistical significance $p < 0.05$.

Table 2: Comparison between the predicted distance traveled by different reference equation and distance observed in 6-min walk test by patients in the equation and validation

		GE				GV			
		Women (n=59)		Men (n=65)		Women (n=15)		Men (n=16)	
Studies	**Age (years)**	**Predicted (m)**	**Observed/ Predicted (%)**	**Predicted (m)**	**Observed/ Predicted (%)**	**Predicted (m)**	**Observed/ Predicted (%)**	**Predicted (m)**	**Observed/ Predicted (%)**
Enright and Sherril (1998)*	40 – 80	561.3 ± 50.8	88 ± 10	654.9 ± 67.3	91 ± 12	609.0 ± 67.8	84± 13	684.9 ± 67.8	90 ± 11
Gibbons et al. (2001)	20 – 80	662.9 ± 32.7	76 ± 10	740.4 ± 27.7	80 ± 9	662.3 ± 36.0	77± 10	752.2 ± 30.8	81 ± 11
Iwama et al. (2009)	13 – 84	541.5 ± 20.2	93 ± 12	604.7 ± 17.1	98 ± 12	641.1 ± 22.2	94 ± 13	612.0 ± 19.0	100 ± 13
Soares et al. (2011)	20 – 80	445.4 ± 30.4	113 ± 14	608.6 ± 33.6	97 ± 12	587.4 ± 35.1	87 ± 11	621.9 ± 33.0	98 ± 12
Chetta et al. (2006)**	20 – 50	586.4 ± 23.8	89 ± 12	623.4 ± 19.2	96 ± 11	581.2 ± 25.1	91 ± 10	639.7 ± 16.4	98. ± 13
Proposed equation	20 – 75	---	---	---	---	511.4 ± 44.5	100 ± 13	614.4 ± 67.8	100 ± 14

Data presented as mean ± standard deviation. Group equation (GE). Group validation (GV). Age = age of the subjects in their studies. *GE, 36 women and 41 men; GV, 9 women, 6 men. ** GE, 43 women and 54 men; GV, 10 women, 13 men.

Table 3: Predicting model for walk distance the 6-min walk test

	Coefficient	Standard error	*p*	95% confidence intervals	
r^2 = 0.44					
Constant	610.07	27.36	< 0.001	556.45	663.69
Gender	75.68 (0 women; 1 men)	11.83	< 0.001	52.51	98.85
Age	-3.15	0.58	< 0.001	-2.01	-4.29
ΔHR	1.43	0.38	< 0.001	0.69	2.17

6-MWD = 610.07 + (gender (0 women; 1 men)*75.68) – (age*3.15) + (ΔHR*1.43), standart error of estimate = 64.40.

ARTIGO II

Changes in respiratory and cardiovascular function in HIV-infected patients using highly active antiretroviral therapy

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CHANGES IN RESPIRATORY AND CARDIOVASCULAR FUNCTION IN HIV-INFECTED PATIENTS USING HIGHLY ACTIVE ANTIRETROVIRAL THERAPY

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Running title: ASSESSMENT OF CARDIOPULMONARY SYSTEM IN HIV-INFECTED PATIENTS

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Background. The use of highly active antiretroviral therapy (HAART) suppresses viral replication and prevents complications caused by acquired immunodeficiency syndrome in most patients infected with human immunodeficiency virus (HIV). However, this condition has been associated to chronic conditions, such as cardiovascular and lung diseases and autonomic dysfunction. Therefore the aim of the present study was to evaluate of the effect of the HAART on respiratory and cardiovascular functions, as well as distance covered in 6-minute walk test in patients HIV-infected. **Methods.** For the present cross-sectional study were included patients of both sexes, aged 21 to 71 years were inclued. These patients were undergone combinations of two drugs of the class of nucleoside reverse transcriptase inhibitors (NRTIs) associated with a drug of the class of inhibitor of nucleoside reverse transcriptase drug – HAART-1 group with 36 patients or two NRTIs associated with a drug of the class of inhibitor protease – HAART-2 group with 21 patients. Patients who had never used HAART were included in naive group (HIV^{+ve}) with 13 patients. All patients underwent two 6-MWT on the first day. At the second day they performed the pulmonary function test, maximal static respiratory muscle pressure test, heart rate variability (HRV) and functional autonomic tests. **Results.** We found difference between the time of diagnosis between HAART and HIV^{+ve} groups ($p = 0.006$). There was statistically significant difference in hemoglobin between HAART-2 when compared to the HIV^{+ve} group ($p = 0.033$). There was no difference in distance walked during 6-MWT in groups. There was difference between HIV-2 and HIV^{+ve} groups in heart rate at end ($p = 0.030$) and the diastolic blood pressure at end was different between HIV^{+ve} and HAART groups ($p < 0.001$). In lung function tests, the only difference found was in the muscle inspiratory presseure between the group HAART-2 and HIV^{+ve} ($p = 0.025$). There was no difference between groups in the heart rate variability. It was noted that the assessment of autonomic function were normal in most patients of the studied groups, except three patients in the HAART group exhibit abnormal classification. **Conclusion.** It can ne conclued, that there was no difference in the majority of variables from the HAART and HIV^{+ve} groups. This demonstrates that patients with a rigorous control HIV infection are able to maintain a good respiratory, cardiovascular and autonomics condition.

Keyworks: Human immunodeficiency vírus. Highly active antiretroviral therapy. Functional capacity. Cardiopulmonary system. Autonomic function.

BACKGROUND

The use of highly active antiretroviral therapy (HAART) suppresses viral replication and prevents complications caused by acquired immunodeficiency syndrome (AIDS) in most patients infected with human immunodeficiency virus (HIV)(1). Systemically, treatment with HAART decreases viral replication, chronic immune activation and inflammation and increased CD_4 cell count (2-3). Therewith, there was a large reduction in mortality, decreased frequency and duration of hospitalization, decrease in AIDS diagnoses, reduction in the manifestations of opportunistic diseases and also significant increase in survival of these patients (4-6). However, there was a prevalence of chronic conditions, such as cardiovascular disease, lung and liver, and some specific types of tumors have arisen. Associated with this, changes in the lipid profile, abnormal fat distribution, dyslipidemia, diabetes, osteoporosis, and neurocognitive decline were also observed (5, 7-11).

Another change found in HIV patients independent of use the HAART is acute fatigue, indicating a cellular mitochondrial dysfunction (12-13). Muscle fatigue is usually associated with other reported symptoms of chronic symptoms, such as fibromyalgia, osteoarthritis and rheumatoid arthiritis (13). In addition, HIV patients have low levels of physical activity which results in a decrease in functional capacity (14).

Furthermore, the cardiovascular disease (CVD) is a major complication that affects these patients. The increased risk of CVD associated with HIV infection is due to the inflammatory process that occurs during viral replication, besides the association with traditional risk groups: smoking, effects of HAART on lipid profile, insulin resistance, body composition and hypertension (11, 15-17).

Moreover, HIV-infected patients with cardiovascular autonomic neuropathy showed a significant reduction in the number of CD_4 cells when compared with patients without neuropathy. Additionally, neuropathy prevalence was significantly higher in patients with advanced HIV infection (18-19). Cardiovascular autonomic dysfunction was significantly associated with progression of HIV, suggesting a significant risk of sudden death for these patients (19).

The respiratory system is often affected by HIV infection. The major changes are the activation of macrophages and lymphocytes, cytokine secretion and CD_8 cell

accumulation in the alveolar space. All these changes have high impact on lung immunity (3). The impact of the use of HAART in lung profile is not yet well understood. The effect of the viral infection in the lungs includes a number of complications both infectious and non-infectious. Among the major changes are: pneumonia, tuberculosis, lung cancer, pulmonary hypertension and chronic obstructive pulmonary disease (COPD) (2, 20).

However there are no definitive studies evaluating changes in respiratory muscle strength, pulmonary function, functional capacity, heart rate variability and autonomic neuropathy in HIV patients using HAART in comparison with HIV patients without HAART. Therefore the aim of the present study was to evaluate the effects of the HAART use on the functional capacity, respiratory, cardiovascular and autonomics fuctions in HIV patients.

METHODS

Subjects and study design. For the present cross-sectional study patients of both sexes, HIV-infected, aged from twenty-one to seventy-one years, under regular use of HAART for at least six months and with undetectable viral load were included, being named group HAART. Patients who had never used HAART were included in naive group (HIV^{+ve}). Patients co-infected were excluded with hepatitis C virus (HCV), hepatitis B virus (HBV), human T-lymphotropic virus (HTLV) and/or Chagas disease were excluded. Furthermore, patients with conditions that could influence the results were also exclude, such as: motor sequelae that harmed ambulation, diagnosed psychological disorders, cardiac disorders, pregnancy, the use of lower limb orthoses, illicit drug use, visually impaired, hypertension, cognitive impairment, severe anemia, age greater than seventy-five years and lung cancer (Figure 1). We studied HIV patients from the Department of Infectious Diseases of the Irmandade da Santa Casa de Misericórdia de Porto Alegre (ISCMPA) took part in the study. The study was approved by the ethics committees of the Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) and ISCMPA. All patients signed an informed consent form prior to data collection.

Procedures. All patients underwent a six-minute walk test (6-MWT) in the afternoon of the first day of the assessment protocol. In the morning of the second day they performed the pulmonary function test, maximal static respiratory muscle pressure test, heart rate variability (HRV) and functional autonomic tests in the physiology laboratory at UFCSPA. Throughout the course of data collection, the tests were conducted in the same environment at controlled temperature of 23ºC by two blinded researchers, previously trained. Blood samples were obtained from each patient after a twelve-hour fast. Glucose, total cholesterol and triglycerides were determined enzymatically. The CD_4 T-lymphocyte count was performed by flow cytometry system BD FACSCount ® (Becton Dckinson). All laboratory tests were analyzed in the ISCMPA clinical laboratory. Anthropometric data were measured using a stadiometer and a scale previously calibrated. For these measurements the patients were oriented to wear light clothing and without foot wear. The body mass index (BMI) was calculated using the equation weight in kilograms divided by height in meters squared. After completion of the tests, the patients' charts were checked to consult

the diagnostic time, time of medication use, associated diseases and type of drug treatment schema used. The patients that received HAART used combinations of two drugs of the class of nucleoside reverse transcriptase inhibitors (NRTIs) associated with a drug of the class of inhibitor of nucleoside reverse transcriptase (NNRTI) drug – HAART-1 group or two NRTIs associated with a drug of the class of inhibitor protease (IP) – HAART-2 group.

Maximal Static Respiratory Pressure. The respiratory muscle function was tested by using a pressure transducer (MVD-300, Globalmed, Porto Alegre, Brazil) connected to a system containing two check valves (DHD Inspiratory Muscle Trainer, Chicago, Illinois). Maximal inspiratory pressure was determined through a deep inspiration from residual volume (RV). The maximal expiratory pressure was measured by a forced expiration from total lung capacity (TLC). These measurements were performed while the patient was sitting with his/her elbows on a counter, with nostrils occluded with a nose clip and circuit fitted with a safety valve. There were carried out at least six maximum maneuvers, with a one-minute rest interval between efforts. Three acceptable and reproducible maneuvers (difference of ten percent or less between efforts) were selected, and the highest value of maximal static inspiratory and expiratory pressure (MIP and MEP) were recorded (21). The predicted values were calculated according to Neder *et al*. (21).

Pulmonary function test. Pulmonary function was assessed by a spirometer (Mininspir, MIR, USA), according to guidelines of the American Thoracic Society (ATS). Patients were instructed into the sitting position, with nostrils occluded by a noseclip. We analyzed the forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), and FEV_1/FVC ratio were measured. The procedures were explained and demonstrated by researchers to all patients. Patients were encouraged to perform a maximal inspiration, followed by a forced expiration for the minimum of six seconds. At least three and at most eight readings were held. The highest values of the variables were recorded.

Functional capacity. It was measured by the 6-MWT according to guidelines of the ATS (22) by two trained assessors. The patients performed the tests in a thirty feet corridor marked every five meters. Such hallway was located in the Infectology Department of ISCMPA. Prior to the beginning of the tests and shortly after

completion, heart rate (HR), rate breathing (RB), systolic blood pressure (SBP) and diastolic blood pressure (DBP) and peripheral oxygen saturation (SpO_2) were measured, in addition the dyspnea, evaluated by means of the Borg scale (23). After thirty minutes of the first test and / or when the patients reached the resting values previously measured, the second test was performed. To determine the distance walked data from the second test was considered (24-25).

Patients were instructed to walk in a corridor as fast as they could in the time of six minutes. The patient was allowed to rest when needed and returned to walk when he/she felt in conditions to complete the task. During the tests, patients received standardized incentive phrases stated every minute by the same researcher.

Heart rate variability. The acquisition signal of the RR intervals was verified with the patient standing comfortably in the supine position with the head elevated to thirty degrees during spontaneous breathing. For HRV analysis, time series of RR intervals (iRR) were obtained by recording continuous ECG signal (sampling frequency = 1 kHz) in the MP150 system (Biopac, California, USA). These were decimated, interpolated and obtained in equal intervals of time series. Subsequently, they were subjected to a Fast Fourier Transform using an algorithm developed in Matlab language (Matlab 6.0, Mathworks Inc., USA). The time series of iRR was analyzed by a blinded investigator in the time and frequency domains.

Autonomic neuropathy. This evaluation was performed with use of the Ewing autonomic function tests (26). It was requested that the patient did not use alcohol, tobacco and caffeine for at least twelve hours before the test. To obtain the resting HR record, patients stayed in a supine position for fifteen minutes. The heart rate responses (RHR) were measured on three conditions, a) deep breathing RHR - the individual breathing deeply and slowly at a rate of six cycles per minute (a ringtone indicated five seconds for inspiration and five seconds for expiration). The mean HR from three successive cycles was used as data in this study; b) RHR to the supine position: the individual assumed the supine position without any assistance. The response was the quotient between the largest iRR and the smallest iRR; and c) RHR to Valsalva maneuver: the individual blowing into a silicone tube attached to an aneroid manometer against a pressure of forty millimeters of mercury during fifteen

seconds. The response, known as Valsalva ratio is the quotient between the largest iRR immediately after the maneuver and the smallest iRR during the maneuver. The blood pressure response (BPR) to the supine position was measured by auscultation concurrently with the implementation of the RHR. The variation in systolic AP between supine position and orthostatic position, one minute after assuming the supine position, was recorded as a measure of postural hypotension. For analysis, the classification was performed using a score obtained with values determined according to the response for each test: normal response (0); borderline (1) and abnormal (2). The end classification is the sum of the classification values: ≤ 2 normal;> 2 <6 borderline and ≥ 6 abnormal (26-27).

Statistical analysis. The data presented are comparisons between the HAART *vs* HIV^{+ve} groups; HAART-1 *vs* HIV^{+ve} groups; HAART-2 *vs* HIV^{+ve} groups; HAART-1 and HAART-2 groups. Statistical analyses were performed using Statistic software version 10 (StatSoft, USA). Data presented are the mean ± standard deviation or median (minimum - maximum) or frequency (%). A descriptive analysis of the variables and analysis in the distribution (Shapiro-Wilk test) were performed. For comparisons of normal distribution data between HAART *vs* HIV^{+ve} groups was applied t test, for comparisons between HAART-1 *vs* HIV^{+ve} groups; HAART-2 *vs* HIV^{+ve} groups; and HAART-1 *vs* HAART-2 one way ANOVA with Tukey as post-hoc were applied. For comparisons of no normal distribution data between HAART *vs* HIV^{+ve} groups the Mann-Whitney test was applied, for comparisons between HAART-1 *vs* HIV^{+ve} groups; HAART-2 *vs* HIV^{+ve} groups; and HAART-1 *vs* HAART-2 the Kruskal-Wallis with Dunn as post-hoc was applied. In comparisons of frequencies the Fisher's exact test was used. The correlation analysis was performed using Pearson coefficient. The level of statistical significance was $p < 0.05$.

RESULTS

Patient characteristics. Fifty-seven patients were allocated in the HAART group. This group was divided in other two groups according to the medication profile: HAART-1 with thirty-six patients under use of the association of two NRTIs one NNRTI and HAART-2 with twenty-one patients under use of one class of drugs combination of two NRTIs and one drug from the class of PI. Thirteen patients that did not receive drug intervention were allocated in the HIV^{+ve} group. The characterization data of the sample are presented in (Table 1). Comparing HAART to HIV^{+ve} group, there was statistically significant difference in the variable time of diagnosis (98 [9 – 251] vs 24 [1 – 156]; p = 0.006). The anthropometric variables and lipid profile were not statistical different among groups. Despite this fact, the HAART group presents a tendency towards regular values. There was statistically significant difference in hemoglobin (Hb) in HAART-2 when compared to the HIV^{+ve} group (13.7 ± 1.2 vs 14.7 ± 0.1, p = 0.033), with the highest value in the group without medication.

Functional capacity. The (Table 2) shows the cardiorespiratory variables measured during the 6-MWT and the covered distance (6-MWD). Statistically significant difference was observed only between the HR_{rest} HAART-2 and HIV^{+ve} groups (72 ± 8 *vs* 83 ± 12, p = 0.030), there was a higher resting HR in HIV^{+ve} group. Also, the diastolic blood pressure at rest (DBP_{rest}) was different between HIV^{+ve} and HAART groups (80 [60 – 90] vs 70 [60 – 110]; p <0.001).

Pulmonary function tests. The respiratory muscle strength and pulmonary function data are shown in (Table 3). There was statistically significant difference in MIP between HAART and HIV^{+ve} groups (75 ± 32 vs 98 ± 38; p = 0.025). The difference remained when HAART-2 was compared to HIV^{+ve} group (64 ± 25 vs 98 ± 38; p = 0.003). There were no statistically significant differences from MEP and spirometric data among the groups.

Heart rate variability. Table 4 presents the data of HRV in the frequency and time domains. There was no statistically significant difference between the HIV^{+ve} and HAART groups, including its subgroups. For these analyses, five patients we excluded, two from HAART-1 (one defective record of artifact and one cardiac

arrhythmia), two from HAART-2 (defective record of artifact), and one from HIV^{+ve} group (defective record of artifact).

Autonomic function. The autonomic function data are expressed as percentage of total patients (Table 5). There was no statistically significant difference between the HAART group and its subgroups when compared with the HIV^{+ve} group. However, it is observed that only the medications group presented patients with abnormal autonomic function. It is observed that the majority of patients, regardless of group, presented normal autonomic function.

Correlations. There was a moderate negative correlation between age and CD_4 count of patients in the HAART group ($r = -0.69$, $p < 0.05$). There was a moderate correlation between 6-MWD and MIP in the HAART group ($r = 0.64$, $p < 0.05$). In the HIV^{+ve} group there was a moderate correlation with PEF ($r = 0.63$, $p < 0.05$). The HR_{rest} correlated with the PI variable the HRV in the HAART group ($r = -0.47$, $p < 0.05$) in the HIV^{+ve} group, the correlation ($r = 0.64$, $p < 0.05$). This difference remained when HIV^{+ve} group, HAART-1 and HAART-2 groups were compared. In the HIV^{+ve} group, there was a moderate negative correlation between DBP at rest and end of 6-MWT and autonomic function score, respectively ($r = -0.66$, $r = -0.75$, $p < 0.05$). In HIV^{+ve} group there was correlation between LF and LF% with Hb ($r = 0.60$; 0.65, $p < 0.05$); and RMSSD was correlated with both VL and Hb ($r = -0.62$; 0.59, $p < 0.05$); the VARRR presented strong correlation with Hb ($r = 0.85$, $p < 0.05$).

DISCUSSION

In the present study we found no differences in anthropometric variables between the groups. There was a difference between the time of diagnosis between HAART and HIV^{+ve} groups, which value was higher in the HAART group. Another variable that presented significant difference was Hb concentration, showing higher in the HIV^{+ve} group. There was no difference in distance walked during 6-MWT between among groups. There was only a difference in variables HR_{rest} and DBP_{rest} between HIV-2 and HIV^{+ve} groups. In lung function tests, the only difference was found in the variable MIP between HAART-2 and HIV^{+ve} groups. There was no difference between groups in the HRV. We observed that, in the assessment of autonomic function, most patients rated normal, disregarding the study group. Only three patients, from the HAART group, exhibited abnormal classification.

Patients in both groups had values within the normal range of Hb concentration, since patients with severe anemia, with Hb concentration below 10 g / dL (28) were excluded. Furthermore, this cohort revealed that before the start of HAART, the Hb concentration was low, returning to normal after one year of treatment with HAART and remained within this range the remaining nine years of follow-up. In our study, patients in the HAART group and the HIV^{+ve} group were within the normal values of Hb concentration, with no statistical difference between these groups. However, comparing the HAART-2 with HIV^{+ve}, there was statistically significant difference. The results of present report and others (29) suggest that the concentration of Hb is associated with the effectiveness of treatment with HAART. On the other hand, the difference in Hb concentration may be related to other factors, such as development of the country conducting the study, socio-economic status of patients, nutritional management, among other factors. In addition, another variable that represents the clinical condition of these patients is CD_4 count. Thus, a study performed in Brazil found better results for the CD_4 count, and even higher in the group of patients who did not use HAART, although no statistical significance was found (462.1 ± 280.5 *vs* 405.0 ± 223.9) (30). This can be explained by the difference in time between diagnosis (29). There was a shorter diagnosis time among of patients without medication, which presents itself as a relevant factor in the progression of the disease and in the consequences of a chronic disease. Another possibility is that the small number of patients included in the group without

medication could be associted with the results showed here. Indeed the people with HIV without drug treatment are scarce and can make a difference within groups (31).

Regarding the lipidic profile, we found no difference between groups. Furthermore, the groups evaluated are within normal ranges for the variables of triglycerides, glucose, LDL, HDL and total cholesterol. In fact, changes in lipid concentration are not only related to the disease or pharmacological treatment, but are also dependent of genetic factors (32). In the present study, patients in the HIV^{+ve} group presented no normal distribution of data, which by itself demonstrates the heterogeneity of the data in subjects of the sample. Likely, the time of blood collection to measure total cholesterol, LDL and HDL may affect the analysis of data from clinical trials. Indeed, since the first analysis is done before initiating HAART it seems that the treatment increases, for example, the values of LDL (32). To reduce the possibility of wrongful interpretation, the samples should be collected prior to seroconversion, to see if the changes are not resulting from their metabolism, regardless of HIV infection. However, it is known that this kind of procedure is uncommon in most studies.

Related to the functional capacity evaluated by the distance covered in the 6-MWT, there are no diferences between groups. The good performance of our patients in the 6-MWT can be related to the optimized clinical treatment observed. Other studies have similar results to ours regarding the distance covered during 6-MWT (33). Also, there was an increase in HR, RR and SBP during the 6-MWT. However, there is no difference among the groups in these variables. Regarding DBP behavior during 6-MWT, this variable was reduced in HAART groups, whereas the HIV^{+ve} group did not show the same reduction. This results suggested that HIV patients without pharmacological treatment could exhibit diastolic dysfunction as previously reported (34).

Refering to respiratory muscle strength, we found that MIP was higher in HIV^{+ve} when compared to HAART group. In the subgroups analysis, we found that the difference between the HIV^{+ve} group and HAART-2 ($p = 0.003$) was more evident. As previously described, the difference between these groups was also observed in the Hb concentration, which could be associated with a reduced respiratory muscle

performance. In accordance to this, there are studies that describe best global physical performance in patients with higher concentrations of Hb (35-36).

Additionally, HIV infection is related to abnormalities of the pulmonary function (3, 37). In the present report we did not find differences in spirometric variables, demonstrating that pulmonary function was normal in the studied population. In contrast, another study showed that airflow obstruction is present in HIV-infected patients and is associated with changes in CD_4 count as well viral load (37). In fact, we found that our patients on HAART treatment had higher mean CD_4 count and undetectable viral load, which confirms this relation and demonstrates a better clinical condition of HIV-infected patients who participated in our study, that contributed to the decrease of risk of pulmonary complications (38). Still, the decrease in viral load in the alveolar space can significantly reduce the incidence of HIV associated complications like emphysema and lung infections (2-3, 39). In fact, the incidence of COPD and asthma is lower in HIV-infected patients using HAART (2).

In our study, we conducted a spirometry, as well as all other tests, in a pre-bronchodilator state. The respiratory maneuvers were measured in a single session. Thus, the use of a bronchodilator would interfere with the results of other tests. Still, a study (37) of pre-bronchodilator spirometry showed that just 7% of HIV-infected patients presented obstruction. Also, there is a relation between reduction in FEV_1 and FVC ratio reduction in HIV-infected patients with history of smoking or pneumonia and HAART use (37, 40-41).

The autonomic nervous system is a regulatory structure that helps in adapting the change of subject in the environment. This system consists of the sympathetic and parasympathetic nervous system, responsible for homeostasis and physiological functions (42). HIV infection does not directly infect neurons. In contrast, it can damage the cells surrounding the nerves, causing a neural isolation and delaying, distorting or disrupting the transmission of information to the brain tissue (43). The cause of these changes is not well determined, and we speculate whether the changes are directly caused by the virus destroying the nerve fibers or by the nerve being destroyed by toxic mediators or secondary free radicals as a consequence of an abnormal immunological mechanism (18, 44-45). Hence the importance of

assessing HRV to check the balance of both sympathetic and parasympathetic systems. In HRV analysis, we decided to exclude five patients due to record interferences which could affect results in the overall analysis. We found that there was no statistically significant difference in the comparison between groups. The evaluated patients in our study showed these variables as in the time domain as frequency domain are at values below the ones in healthy subjects, indicating a shift in the sympathetic/parasympathetic balance. The low values found in RMSSD indicate a loss of parasympathetic influence on HR. Also, patients submited to HAART treatment showed increased resting HR and decreased HRV indicating parasympathetic dysfunction (46). Moreover, the correlations between Hb concentration and SDNN, RMSSD and PNN50 have shown that the Hb seems to be an important marker of the clinical condition of these patients (47).

In our study, there are no differences between the ratings of autonomic dysfunction in normal, borderline and abnormal. However, it was noted that three patients, classified to the HAART group are abnormal, although the majority of patients in both groups exhibit normal classification. These results are similar to those found in HIV patients in Africa, where patients with abnormal ratings were the minority, and the majority of patients met the classification between normal and borderline (27). Our findings are similar to those found in other studies, where most HIV patients showed normal distribution. Only 3 patients from the HAART group displayed abnormal classification (18). Despite the similarities of results, many factors influence in the results of autonomic dysfunction tests, such as nutritional status of the patient, time of diagnosis, medication use, illicit drug use, among other factors (19).

Our study has limitations, such as the time of HIV infection, once this information was unknownand and some patients may remain asymptomatic even after a few years, which may hinder early diagnosis of the disease. In addition, our patients had a good clinical condition and had the disease controled. Patients in the HIV^{+ve} group are at constant clinic visits. In addition, monitored patients tend to be more mindful of their physical health. However, we pointed out that the Hb concentration is an important variable in the studied population and correlates with many of the variables analyzed. Still, there were no statistically significant differences between the HAART group and the HIV^{+ve} group.

In conclusion, we consider concentration of Hb and CD_4 count are important factors to be considered in assessing the clinical condition of HIV-infected patients. Furthermore, we observed differences in the analyses of HR_{rest} and DBP_{rest} in the 6-MWT of the HAART-2 and HIV^{+ve} groups. We only found difference in MIP and Hb between HAART-2 and HIV^{+ve} groups, where the highest values of both variables were observed in the HIV^{+ve} group, which also had shorter time of diagnosis. As for HRV, despite the fact that our groups presented lower values than the evaluated references, there was no difference between the groups. Referring to the autonomic function, there were only three patients classified as abnormal, and these were using medication. Therefore, there was no difference in the majority of variables from the HAART and HIV^{+ve} groups, showing that the patients' disease was well-controlled.

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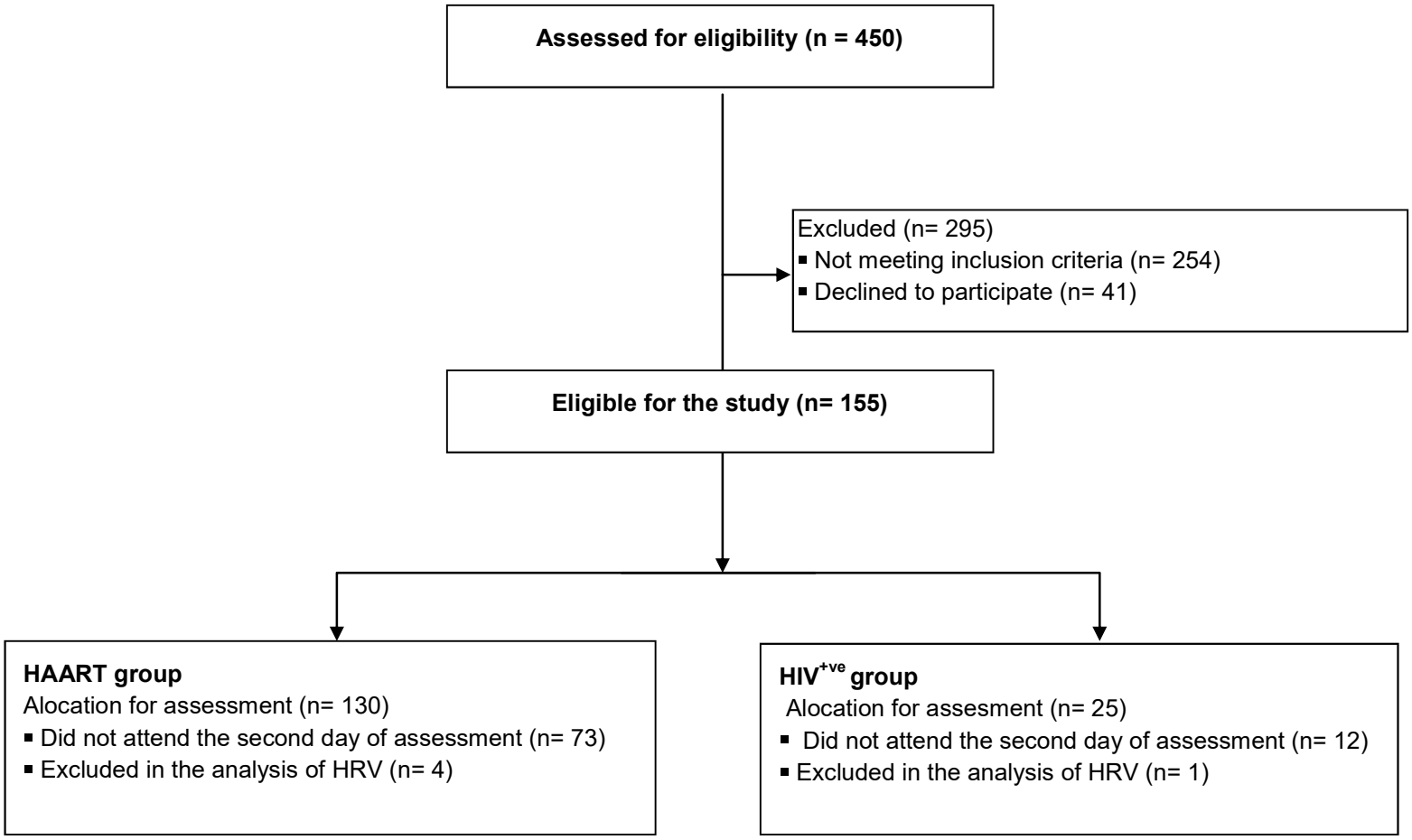
Figure 1: Flow diagram of patients for the assessment the cardiorespiratory system, functional capacity and autonomic function.



NOTE: HAART: Highly active antiretroviral therapy. HIV^{+ve}: Human immunodeficiency vírus no medication. HRV: heart rate variability.

Table 1: Demographic, anthropometric and clinical data of medications and no medication group.

	Medications Group			No medication group	p values	
	HAART (n = 57)	**HAART-1 (n = 36)**	**HAART-2 (n = 21)**	**HIV^{+ve} (n = 13)**	*	**
Gender (women/men)$^{\#}$	(29/28)	(16/20)	(13/8)	(4/9)	0.230	NA
Age (years)	46.1 ± 9.7	45.6 ± 10.0	46.9 ± 9.4	44.0 ± 13.8	0.518	0.740
Weight (kg)	68.6 ± 14.5	69.1 ± 14.7	67.9 ± 14.6	71.6 ± 13.0	0.494	0.756
Height (m)	1.66 ± 0.1	1.68 ± 0.1	1.64 ± 0.1	1.72 ± 0.1	0.097	0.069
BMI (kg/m^2)	24.7 ± 4.6	24.4 ± 4.5	25.3 ± 4.8	24.3 ± 3.7	0.761	0.719
Smoker n (%)$^{\#}$	8 (14)	4 (11)	4 (19)	5 (39)	0.111	NA
Time of diagnosis (months)	98 (9 – 251)	88 (9 – 216)	124 (40 – 251)	24 (1 – 156)	0.006	0.008$^{£}$
Time of medication (months)	90.6 ± 52.6	87.4 ± 56.4	96.2 ± 46.4	0	-	0.548$^{\mu}$
CD_4 (cell/mm^3)	652.4 ± 286.8	647.0 ± 255.1	661.6 ± 341.1	554.7 ± 134.5	0.246	0.504
Viral load (cell/mm^3)$^{\$}$	-	-	-	980 (56 – 13487)	NA	NA
Triglycerides (mg/dL)	148.0 (64.0 - 451.0)	150.0 (64.0 – 451.0)	148.0 (97.0 – 354.0)	117.0 (55 – 592)	0.066	0.172
Glucose (mg/dL)	101.0 ± 33.7	102.9 ± 39.1	97.8 ± 22.3	93.0 ± 16.0	0.407	0.604
HDL (mg/dL)	47.1 ± 9.3	49.2 ± 8.7	43.5 ± 9.5	48.1 ± 7.5	0.718	0.063
LDL (mg/dL)	127.9 ± 39.6	131.0 ± 39.7	122.6 ± 39.9	127.6 ± 32.1	0.982	0.730
Total cholesterol (mg/dL)	212.5 ± 51.1	218.0 ± 51.3	203.2 ± 50.6	211.6 ± 56.4	0.953	0.585
Hemoglobin (mg/dL)	14.1 ± 1.2	14.2 ± 1.2	13.7 ± 1.2	14.7 ± 0.1	0.143	0.019$^{£}$

NOTE. Date as mean ± standart deviation or median (minimum – maximum) or frequency (%) or frequency. HAART: highly active antiretroviral therapy. HAART-1: 2NRTI+1NNRTI. HAART-2: 2NRTI+1IP. NNRTI: non-nucleoside reverse transcriptase inhibitor. NRTI: nucleosides reverse transcriptase inhibitor. PI: protease inhibitor. HIV^{+ve}: human immunodeficiency virus no medication. BMI: body mass índex. CD_4: cluster of differentation. $^{\$}$Viral load calculed only patients of HIV^{+ve} group with values above the detection limit. *Unpaired t test or Mann-Whitney test were applied for HAART vs HIV^{+ve} comparison. ** ANOVA one way (Tukey test as post-hoc) or Kruskal-Wallis (Dunn test as post-hoc) were applied to compare HAART-1, HAART-2 and HIV^{+ve}. $^{\#}$Fisher's exact test. Statistical significance difference = $p<0.05$. $^{\mu}$ Unpaired t test were applied for HAART-1 vs HAART-2. $^{£}p<0.05$ between HAART-2 *vs* HIV^{+ve}. NA: not applied.

Table 2: Cardiovascular and respiratory variables of medication and no medication groups, observed during the 6-minute walk test.

	Medications group			No medication group	p	
	HAART (n = 57)	**HAART-1 (n = 36)**	**HAART-2 (n = 21)**	**HIV^{+ve} (n = 13)**	*	**
6-MWD (m)	539.9 ± 88.1	546.8 ± 100.2	528.1 ± 63.0	568 ± 93.1	0.308	0.448
HR rest (bpm)	76 ± 10	79 ± 11	72 ± 8[!]	83 ± 12	0.063	0.014
HR end (bpm)	99 ± 18	100 ± 17	98 ± 20	107 ± 17	0.150	0.315
ΔHR (bpm)	23 ± 15	21 ± 14	25 ± 16	24 ± 17	0.693	0.592
RB rest (cpm)	16 (12 – 28)	16 (12 – 28)	16 (12 – 24)	16 (12 – 28)	0.069	0.249
RB end (cpm)	20 (14 - 40)	20 (16 – 40)	22 (14 - 28)	24 (14 - 36)	0.082	0.234
ΔRB (cpm)	4 (0 – 24)	4 (0 – 24)	4 (0 – 14)	8 (-8 – 14)	0.729	0.497
SpO_2 rest (%)	98 (94 – 99)	98 (94 – 99)	98 (95 – 99)	98 (97 – 99)	0.214	0.697
SpO_2 end (%)	98 (94 – 100)	97 (94 – 100)	97 (94 – 99)	98 (95 – 99)	0.921	0.903
ΔSpO_2 %	0 (-5 – 5)	0 (-5 – 5)	1 (2 – 5)	-1 (-3 – 1)	0.448	0.527
SBP rest (mmHg)	120 (85 – 170)	115 (85 – 170)	120 (90 – 170)	130 (100 – 150)	0.595	0.800
SBP end (mmHg)	120 (90 – 170)	120 (90 – 170)	130 (90 – 170)	130 (100 – 170)	0.292	0.377
ΔSBP (mmHg)	10 (-25 – 40)	5 (-20 – 40)	10 (25 – 30)	10 (-15 – 30)	0.516	0.646
DBP rest (mmHg)	70 (60 – 110)	80 (60 – 110)	80 (60 - 100)	80 (60 – 90)	<0.001	0.006
DBP end(mmHg)	80 (60 – 110)	80 (60 – 110)	90 (70 – 110)	90 (70 – 100)	0.142	0.119
ΔDBP (mmHg)	10 (-10 – 140)	0 (-30 - 20)	10 (-10 - 30)	10 (0 – 30)	0.336	0.583

NOTE. Date as mean ± standart or median (minimum – maximum). 6-MWT: 6-minute walk test. HR: Heart rate. RB: Rate breathing. SpO_2: Peripheral oxygen saturation. SBP: Systolic blood pressure. DBP: Diastolic blood pressure. Δ = end – rest values. HAART: Highly active antiretroviral therapy. HIV^{+ve}: Human immunodeficiency vírus no medication. *Applied Student t test or Mann-Whitney for group HAART vs HIV^{+ve}. HAART-1: 2NRTI+1NNRTI. HAART-2: 2NRTI+1IP. **Applied ANOVA one way wtih post-hoc Tukey for dates ordinarys, apllied Kruskal-Wallis with post-hoc Dunn for dates no-ordinarys. [!]Difference between HAART-2 *vs* HIV$^{+vê}$, p = 0.030. Statistical significance p < 0.05. NNRTI: Non-nucleoside reverse transcriptase inhibitor. NRTI: Nucleosides reverse transcriptase inhibitor. PI: Protease inhibitor.

Table 3: Data of pulmonary function tests of medication and no medication groups.

	Medications group			No medication group	p	
	HAART (n = 57)	**HAART-1 (n = 36)**	**HAART-2 (n = 21)**	**HIV^{+ve} (n = 13)**	*	**
MIP (cmH_2O)	75 ± 32	81 ± 34	64 ± 25$^{!}$	98 ± 38	0.025	0.013
Predicted (%)	71 ± 25	73 ± 27	64 ± 19$^{€}$	90 ± 33	0.032	0.022
MEP (cmH_2O)	99 ± 35	102 ± 35	94 ± 35	114 ± 37	0.167	0.280
Predicted (%)	92 ± 27	91 ± 21	92 ± 34	99 ± 33	0.353	0.652
FVC (L)	3.1 ± 0.7	3.2 ± 0.8	3.0 ± 0.6	3.5 ± 1.2	0.112	0.187
Predicted (%)	78 ± 12	78 ± 13	82 ± 9	84 ± 14	0.239	0.217
FEV_1 (L)	3.0 ± 0.7	3.1 ± 0.8	2.9 ± 0.6	3.4 ± 1.2	0.119	0.195
Predicted (%)	94 ± 14	92 ± 15	97 ± 12	99 ± 15	0.119	0.260
PEF (L/s)	6.1 ± 2.0	6.2 ± 2.0	6.0 ± 2.0	6.3 ± 2.9	0.684	0.849
Predicted (%)	77 ± 20	76 ± 21	80 ± 18	75 ± 24	0.684	0.759
FEV_1/FVC	0.97 ± 0.1	0.97 ± 0.1	0.96 ± 0.1	0.96 ± 0.1	0.815	0.465
Predicted (%)	96 ± 0.1	96 ± 0.1	94 ± 0.1	0.95 ± 0.1	0.858	0.798

NOTE. Date as mean ± standart or median (minimum – maximum). MIP: muscle inspiratory pressure. MEP: muscle expiratory pressure. HAART: Highly active antiretroviral therapy. HIV^{+ve}: Human immunodeficiency vírus no medication.). FVC: Forced vital capacity. FEV_1: Expiratory volume in one second. PEF: Peak flow expiratory. *Applied Student t test group HAART vs HIV^{+ve}. HAART-1: 2NRTI+1NNRTI. HAART-2: 2NRTI+1IP. **Applied ANOVA one way wtih post-hoc Tukey. Statistical significance $p < 0.05$. $^{!}$Difference between HAART-2 vs HIV^{+ve}, $p = 0.003$. $^{€}$ Difference between HAART-2 vs HIV^{+ve}, $p = 0.008$. NNRTI: Non-nucleoside reverse transcriptase inhibitor. NRTI: Nucleosides reverse transcriptase inhibitor. PI: Protease inhibitor.

Table 4: Data of heart rate variability (HRV) in the frequency domain and time domain of medications and no medication groups.

	Medications group			No medication group	p	
	HAART (*n* = 53)	**HAART-1 (*n* = 34)**	**HAART-2 (*n* = 19)**	**HIV^{+ve} (*n* = 12)**	*	**
LF	126.4 (3.8 – 849.3)	130.0 (3.8 – 849.3)	126.4 (19.9 – 366.1)	122.1 (6.3 – 2314.3)	0.509	0.792
HF	115.7 (17.2 – 1702.6)	115.7 (17.2 – 1702.6)	125.0 (19.3 – 859.1)	164.3 (8.3 – 2498.9)	0.520	0.808
%LF	30.2 (6.7 – 107.0)	32.7 (7.1 – 71.7)	25.5 (6.7 – 107.0)	25.4 (6.4 – 80.8)	0.973	0.836
%HF	28.8 (4.4 – 72.6)	26.9 (7.8 – 72.6)	33.3 (4.4 – 67.6)	25.5 (5.6 – 72.7)	0.697	0.911
LF/HF	0.9 (0.1 – 8.5)	0.9 (0.1 – 7.0)	0.9 (0.1 – 8.5)	0.8 (0.1 – 7.7)	0.786	0.892
Pulse interval	800.7 (608.2 – 1122.7)	783.7 (608.2 – 949.9)	862.9 (647.1 – 1122.7)	753.2 (648.8 – 1148.0)	0.548	0.122
SDPI	29.2 (8.5 – 58.6)	28.3 (8.5 – 58.6)	33.4 (12.0 – 46.0)	53.4 (10.8 – 69.4)	0.092	0.231
NN50	3.5 (0 – 355.0)	3.0 (0 – 355.0)	14.0 (0 – 273.0)	92.0 (0 – 301.0)	0.451	0.485
PNN50	0.5 (0 - 56.6)	0.4 (0 – 56.6)	2.4 (0 – 46.8)	10.9 (0 – 58.1)	0.441	0.476
RMSSD	16.6 (4.6 – 71.2)	16.6 (7.1 – 71.2)	23.1 (5.2 – 54.6)	34.6 (4.3 – 81.1)	0.148	0.297
VARRR	852.6 (71.7 – 3439.9)	799.1 (71.9 – 3440.0)	116.9 (145.0 – 2118.7)	2851.2 (117.7 – 4818.5)	0.092	0.234

NOTE. Data expressed as median (minimum – maximun). : Highly active antiretroviral therapy. HIV^{+ve}: Human immunodeficiency vírus no medication . HAART-1: 2NRTI+1NNRTI. HAART-2: 2NRTI+1IP . NRTI: Nucleoside reverse transcriptase inhibitor. NNRTI: Non-nucleoside reverse transcriptase reverse. PI: Protease inhibitor. LF: Low frequency. HF: High frequency. PI*: Pulse interval. SDPI: Standard deviation of the pulse interval. NN50: Number of differences NN consecutive intervals exceeding 50 ms. PNN50: Ratio given by the ratio between NN50 and the total number of intervals NN. RMSSD: Root mean square of squares of differences of successive NN intervals. VARRR: Variability of intervals RR. *Applied MannWhitney test between group HAART vs HIV^{+ve}. ** Applied Kruskal-Wallis. Statistical significance $p<0.05$.

Table 5: The frequency distribution of autonomic function in normal, borderline and abnormal of medications and no medications groups.

	Medications group			**No medication group**	***p***			
	HAART (n = 57)	**HAART-1 (n = 36)**	**HAART-2 (n = 21)**	**HIV^{+ve} (n = 13)**	*	**	***	****
Normal (≤ 2)	36 (63)	22 (61)	14 (67)	8 (61)	1.000	1.000	1.000	0.779
Borderline (> 2 < 6)	18 (32)	12 (33)	6 (28)	5 (39)				
Abnormal (≥ 6)	3 (5)	2 (6)	1 (5)	0 (0)				

NOTE. Values expressed as n (%). Classification according score Ewing-Clarke. HAART: Highly active antiretroviral therapy. HIV^{+ve}: Human immunodeficiency vírus no medication. HAART-1: 2NRTI+1NNRTI. HAART-2: 2NRTI+1IP. *Applied Fisher exact test for group HAART *vs* HIV^{+ve}. **HAART-1 *vs* HIV^{+ve}.. *** HAART-2 *vs* HIV^{+ve} . ****HAART-1 *vs* HAART-2. Applied Fischer exact test, difference significative $p < 0.05$.

6. CONCLUSÕES

Conclui-se que o uso de uma fórmula de predição para pacientes portadores do HIV no TC6min é capaz de predizer a distância percorrida da avaliação da capacidade funcional submáxima. Ainda assim, o TC6min por ser um teste prático e de fácil execução pode ser utilizado como uma ferramenta no acompanhamento desses pacientes para avaliação da influência da doença na capacidade funcional dessa população.

Além disso, verificou-se que os pacientes portadores de HIV com ou sem uso de TARV não apresentam alterações respiratórias significativas na avaliação da capacidade pulmonar. Quanto à avaliação da VFC não foram observadas diferenças significativas nas variáveis analisadas no domínio do tempo e no domínio da frequência entre os pacientes portadores do HIV em uso de TARV ou sem tratamento medicamentoso. No entanto, verificou-se que essas variáveis apresentam valores inferiores aos valores de referência. Isso demostra que pacientes portadores do HIV com ou sem uso de TARV apresentam uma baixa VFC, sugerindo um prognóstico de alterações cardíacas decorrentes da infecção viral nessa população. Soma-se a isso, que grande parte dos pacientes portadores do HIV apresenta classificação normal de neuropatia autonômica. Porém, observou-se que considerável número desses pacientes foram classificados como limítrofes, sugerindo que com a evolução da doença e/ou uso cumulativo de TARV, esses pacientes podem vir a evoluir para a classificação anormal.

No entanto, percebeu-se que pacientes portadores do HIV com controle adequado da doença apresentam, em sua maioria, condições clínicas compatíveis com sujeitos saudáveis. Assim, percebe-se a necessidade de um acompanhamento dessa população para se verificar os efeitos da evolução da doença e do uso cumulativo de TARV e suas influências nas variáveis avaliadas, além de um teste para verificar a capacidade funcional máxima desses pacientes. Além disso, percebe-se a importância de uma avaliação de pacientes portadores do HIV coinfectados e com presença de anemia.

ANEXOS

ANEXO A – CARTA DE APROVAÇÃO DO CEP DA UFCSPA

UFCSPA

COMISSÃO CIENTÍFICA E COMISSÃO DE PESQUISA E ÉTICA EM SAÚDE

COMITÊ DE ÉTICA EM PESQUISA - CEP
UFCSPA

O Comitê de Ética em Pesquisa da UFCSPA, registrado na Comissão Nacional de Ética em Pesquisa (CONEP) sob o nº 075/05 em 23/07/04, analisou o Projeto:

Projeto: 11-752 **Versão do Projeto:** **Versão do TCLE:**

Pesquisadores:
PEDRO DALL'AGO
CANDISSA SILVA DA SILVA

Título: AVALIAÇÃO DAS ALTERAÇÕES DO SISTEMA RESPIRATÓRIO E RESPOSTA DO SISTEMA CARDIOVASCULAR DE PACIENTES HIV POSITIVO U USO DE TARV.

Esse projeto foi aprovado em seus aspectos éticos e metodológicos conforme as Resoluções 196/09 e demais Resoluções complementares. Toda e qualquer alteração do projeto, assim como eventos adversos graves, deverão ser comunicados a este CEP. Os TCLE, quando necessários, somente poderão ser utilizados após prévia e explicita aprovação (carimbo) de sua redação por este CEP".

Porto Alegre, 06 de maio de 2011.

José Geraldo Vernet Taborda
Coordenador do CEP/UFCSPA

ANEXO B – CARTA DE APROVAÇÃO DO CEP DA ISCMPA

Irmandade da Santa Casa de Misericórdia de Porto Alegre
Rua Prof. Annes Dias, 295 – Telefone: (51) 3214.8080 – Fax: (51) 3214.8585
CEP 90020-090 – Porto Alegre – Rio Grande do Sul – CNPJ: 92815000/0001-68
Site: www.santacasa.org.br – E-mail: marketing@santacasa.tche.br

PARECER CONSUBSTANCIADO

Parecer nº 262/11

Protocolo nº 3569/11

Título: *"Avaliação das alterações do sistema respiratório e resposta do sistema cardiovascular de pacientes HIV positivo e uso de TARV".*

Pesquisador Responsável: Pedro Dal Lago

Instituição onde se realizará – Irmandade da Santa Casa de Misericórdia de Porto Alegre.

Data de Entrada: 13/06/2011

II – Objetivos – Objetivo Geral: Avaliar a presença de alterações do sistema respiratório e de neuropatia autonômica em pacientes infectados pelo HIV em uso de TARV.
Objetivos Específicos: • Verificar volumes e capacidades pulmonares;
• Verificar a força dos músculos respiratórios;
• Verificar a endurance muscular respiratória;
• Verificar as respostas da FC e pressão arterial (PA) durante mudanças de decúbito;
Avaliar o comportamento da variabilidade da FC.

III - Sumário do Projeto

Descrição e caracterização da amostra: Estudo quantitativo do tipo transversal. Os grupos de estudo serão compostos por indivíduos HIV+ que realizam controle de carga viral no ambulatório de infectologia do Hospital Santa Casa da cidade de Porto Alegre.

Critérios de inclusão: • Ter diagnóstico de HIV+;
• Realizar o controle da carga viral no Hospital Santa Casa;
• Ser adulto, sem delimitação de faixa etária;
• Fazer uso de TARV por pelo menos 6 meses ininterruptos ou que nunca tenha feito uso dessas medicações.

Critérios de exclusão: • Apresentar alterações metabólicas (dislipidemia, DM ou lipodistrofia) antes do diagnóstico de infecção pelo HIV;
• Uso de glicocorticóides ou outros esteróides, hormônio do crescimento, beta-bloqueadores e/ou tiazídicos, ou outros fármacos que induzam alterações metabólicas e/ou distribuição da gordura corporal;
• Pacientes desidratados ou anêmicos;
• Portadores de afecções clinicamente relevantes que possa dificultar a implementação do estudo ou interpretação dos resultados;
• Apresentar alterações cognitivas e/ou mental que impeça a realização dos testes.

Adequação das condições - Hospital escola com infra-estrutura adequada para a realização do estudo descrito

Irmandade da Santa Casa de Misericórdia de Porto Alegre

Rua Prof. Annes Dias, 295 – Telefone: (51) 3214.8080 – Fax: (51) 3214.8585
CEP 90020-090 – Porto Alegre – Rio Grande do Sul – CNPJ: 92815000/0001-68
Site: www.santacasa.org.br – E-mail: marketing@santacasa.tche.br

IV - Comentários:

- **Justificativa do uso de placebo** – Não aplica.

- **Análise de riscos e benefícios** – Adequado.

- **Adequação do termo de consentimento e forma de obtê-lo** – Adequado.

- **Informação adequada quanto ao financiamento** – Com financiamento adequado por órgão de fomento a pesquisa.

- **Outros centros no caso de estudos multicêntricos** – Não se aplica.

V - Parecer do Relator —*"Após avaliação do protocolo acima descrito, o presente comitê não encontrou óbices quanto ao desenvolvimento do estudo em nossa Instituição e poderá ser iniciado a partir da data deste parecer".*

VI - Data da Reunião: 05/07/2010.

"Projeto e Termo de Consentimento, Aprovados".

Obs.: 1 - O pesquisador responsável deve encaminhar à este CEP, Relatórios de Andamento dos Projetos desenvolvidos na ISCMPA. Relatórios Parciais (pesquisas com duração superior à 6 meses). Relatórios Finais (ao término da pesquisa) e os Resultados Obtidos (cópia da publicação).

2 – Para o início do projeto de pesquisa, o investigador deverá apresentar a chefia do serviço (onde será realizada a pesquisa), o Parecer Consubstanciado de aprovação do protocolo pelo Comitê de Ética.

Porto Alegre, 12 de Julho de 2011.

Prof. Dr. Cláudio Telöken
Coordenador do CEP/ISCMPA

ANEXO C – INSTRUÇÕES (Respiratory Medicine)

Guide for Authors

- *Respiratory Medicine* is an internationally-renowned, clinically-oriented journal, combining cutting-edge original research with state-of-the-art reviews dealing with all aspects of respiratory diseases and therapeutic interventions, but with a clear clinical relevance. The journal is an established forum for the publication of phased clinical trial work at the forefront of interventive research. As well as full-length original research papers, the journal publishes reviews, correspondence, and short reports. The Journal also publishes regular supplements on areas of special interest.

Online Submission of Manuscripts

http://ees.elsevier.com/yrmed

Submission and peer review of all papers is now conducted entirely online, increasing efficiency for editors, authors, and reviewers, and enhancing publication speed. Authors are guided stepwise through the entire process, and are kept abreast of the progress of their paper at each stage.The system creates PDF version of the submitted manuscript for peer review, revision and proofing. All correspondence, including the Editor's decision and request for revisions, is conducted by e-mail. Authors requesting further information on online submission are strongly encouraged to view the system, including a tutorial, at http://ees.elsevier.com/yrmed.Authors are asked to bear in mind the guideline peer review and publication times available at the Respiratory Medicine journal homepage: click here for Journal News.

Peer Review

Submissions are allocated to a handling editor, typically an Associate Editor. Should the paper be considered suitable for peer review, appropriate reviewers will be recruited. Authors are required to provide the name and full contact details of 2 potential reviewers, though choice of reviewers is at the discretion of the handling editor. The final decision-making responsibility lies with the handling editor, who reserves the right to reject the paper despite favourable reviews depending on the

priorities of the journal. For full details on the peer review process and current peer review decision times please click here for Journal News.

Cover letter

Corresponding authors must provide a cover letter which includes statements answering the following questions:

- Has the work been seen and approved by all co-authors?
- How is the work clinically relevant, and how does it add to existing research?
- Have papers closely related to the submitted manuscript been published or submitted for publication elsewhere? If so please provide details.

Failure to provide a cover letter addressing each of the questions above will result in the paper being returned to the author. The cover letter must be uploaded as a separate submission item.For queries, please contact the journal editorial office directly: respiratorymedicine@elsevier.com

Authorship

All authors should have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

Acknowledgements

All contributors who do not meet the criteria for authorship as defined above should be listed in an acknowledgements section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, or a department chair who provided only general support. Authors should disclose whether they had any writing assistance and identify the entity that paid for this assistance.

Conflict of interest

The potential for conflict of interest exists when an author (or the author's institution), reviewer or editor has financial or personal relationships that may inappropriately influence his or her actions. Editors and reviewers for the journal are responsible for disclosing to the Chief Editor any personal or financial relationship that may bias their work during the peer review process. Authors are specifically asked to reflect on financial conflicts of interest (such as employment, consultancy, stock ownership, honoraria and paid expert testimony) as well as other forms of conflict of interest, including personal, academic and intellectual issues.For the conflict of interest statement all authors must disclose any financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. The Conflict of Interest Statement is a mandatory submission item: authors are required to submit the statement to proceed with their submission. Conflict of Interest statements will typically be published within the body of the article, and will appear preceding the references section. All sources of funding should be declared as an acknowledgement at the end of the text. Authors should declare the role of study sponsors, if any, in the study design, in the collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication. If the study sponsors had no such involvement, the authors should so state.

Randomised controlled trials

All randomised controlled trials submitted for publication in Respiratory Medicine should include a completed Consolidated Standards of Reporting Trials (CONSORT) flow chart. Please refer to the CONSORT statement website at http://www.consort-statement.org for more information. Respiratory Medicine has adopted the proposal from the International Committee of Medical Journal Editors (ICMJE) which require, as a condition of consideration for publication of clinical trials, registration in a public trials registry. Trials must register at or before the onset of patient enrolment. The clinical trial registration number should be included at the end of the abstract of the article. For this purpose, a clinical trial is defined as any research study that

prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects of health outcomes. Health-related interventions include any intervention used to modify a biomedical or health-related outcome (for example drugs, surgical procedures, devices, behavioural treatments, dietary interventions, and process-of-care changes). Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. Purely observational studies (those in which the assignment of the medical intervention is not at the discretion of the investigator) will not require registration. Further information can be found at http://www.icmje.org.

Disclosure of Clinical Trial Results

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In line with the position of the International Committee of Medical Journal Editors , the journal will not consider results posted in the same clinical trials registry in which primary registration resides to be prior publication if the results posted are presented in the form of a brief structured (less than 500 words) abstract or table. However, divulging results in other circumstances (eg, investors' meetings) is discouraged and may jeopardise consideration of the manuscript. Authors should fully disclose all posting in registries of results of the same or closely related work.

Supplementary material

Respiratory Medicine accepts electronic supplementary material to support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, movies, animation sequences, high-resolution images, background datasets, sound clips and more. Supplementary files supplied will be published online alongside the electronic version of your article including ScienceDirect:http://www.sciencedirect.com. In order to ensure that your submitted material is directly usable, please ensure that data is provided in one of our recommended file formats. Authors should submit the material in electronic format together with the article and supply a concise and descriptive caption for each

file. For more detailed instructions please visit our artwork instruction pages at http://www.elsevier.com/artworkinstructions.

Ethics

Work on human beings that is submitted to *Respiratory Medicine* should comply with the principles laid down in the Declaration of Helsinki; Recommendations guiding physicians in biomedical research involving human subjects. Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964, amended by the 29th World Medical Assembly, Tokyo, Japan, October 1975, the 35th World Medical Assembly, Venice, Italy, October 1983, and the 41st World Medical Assembly, Hong Kong, September 1989. The manuscript should contain a statement that the work has been approved by the appropriate ethical committees related to the institution(s) in which it was performed and that subjects gave informed consent to the work. Studies involving experiments with animals must state that their care was in accordance with institution guidelines. Patients' and volunteers' names, initials, and hospital numbers should not be used.

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- Cover letter (mandatory)
- Abstract (including clinical trial registration number where appropriate) (mandatory)
- Conflict of Interest Statement (mandatory) Manuscript including ethics statement as appropriate (mandatory)
- Artwork (optional)
- Supplementary files eg. datasets, video files (optional)
- Permissions letters (As necessary, see below)
- Consolidated Standards of Reporting Trials (CONSORT) flow chart as appropriate

Reviews

The journal welcomes submission of state-of-the-art reviews on important topics with a clinical relevance. Potential review authors are encouraged to contact the Deputy Editor Dr N. Hanania hanania@bcm.tmc.edu in advance with their review proposals.

Case Reports (Respiratory Medicine Case Reports)

All case reports will henceforth no longer be considered for publication in Respiratory Medicine, but instead for the sister publication **Respiratory Medicine Case Reports.** *Please note that this is a separate publication to the regular journal. Case reports may be submitted for consideration for Respiratory Medicine Case Reports via the same online submission site as the regular journal, as described below. Respiratory Medicine Case Reports is an open access journal and all authors will be required to pay a £250 processing fee to cover the costs of publishing the article, which authors will be required to pay once an article has passed peer review.*

Preparing your manuscript

Authors are asked to bear in mind the following additional points before entering the submission process.

Format and Structure

Most text formats can be accommodated, but Microsoft Word is preferable. In general, articles should conform to the conventional structure of Summary, Introduction, Methods, Results, Discussion and References.

Title

Your title page, should give the title in capital letters (not exceeding 100 letters), a running title (not exceeding 50 letters) and the authors' names (as they are to appear), affiliations and complete addresses, including postal (zip) codes. The author and address to whom correspondence should be sent must be clearly indicated. Please supply telephone, fax and e-mail numbers for the corresponding author.

Abstract

An abstract of your manuscript summarizing the content, at a maximum of 250 words, should be provided as a separate submission item.

Reference Format

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Text: Indicate references by superscript numbers in the text. The actual authors can be referred to, but the reference number(s) must always be given.
List: Number the references in the list in the order in which they appear in the text.
Examples:
Reference to a journal publication:

1. Van der Geer J, Hanraads JAJ, Lupton RA. The art of writing a scientific article. J Sci Commun 2000; **163**:51–9.
Reference to a book:
2. Strunk Jr W, White EB. The elements of style. 3rd ed. New York: Macmillan; 1979.
Reference to a chapter in an edited book:
3. Mettam GR, Adams LB. How to prepare an electronic version of your article. In: Jones BS, Smith RZ, editors. Introduction to the electronic age. New York: E-Publishing Inc; 1999, p. 281–304. Note shortened form for last page number. e.g.,

51–9, and that for more than 6 authors the first 6 should be listed followed by 'et al.' For further details you are referred to "Uniform Requirements for Manuscripts submitted to Biomedical Journals" (*J Am Med Assoc* 1997;277:927–34), see also http://www.nlm.nih.gov/tsd/serials/terms_cond.html.

Figures

Figures of good quality should be submitted online as a separate file. For detailed instructions on the preparation of electronic artwork, consult: http://www.elsevier.com/authors. Permission to reproduce illustrations should always be obtained before submission and details included with the captions.

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Tables should be submitted online as a separate file, bear a short descriptive title, and be numbered in Arabic numbers. Tables should be cited in the text.

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A list of three to six keywords should be supplied: full instructions are provided when submitting the article online.

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These should be given in SI units with the traditional equivalent in parentheses where appropriate. Conventions for abbreviations should be those detailed in Units, Symbols, and Abbreviations, available from the Royal Society of Medicine.

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Editorial Office

To contact the Respiratory Medicine editorial office, please email respiratorymedicine@elsevier.com. We aim to respond to all queries rapidly.

ANEXO D– INSTRUÇÕES (The Journal of Infectious Disease)

Submission

Please read these instructions carefully and follow them closely to ensure that the review and publication of your paper is as efficient and quick as possible. The Editors reserve the right to return manuscripts that are not in accordance with these instructions. All material to be considered for publication in the *Journal of Infectious Diseases* (*JID*) should be submitted in electronic form via the journal's online submission system. Once you have prepared your manuscript according to the instructions below, instructions on how to submit your manuscript online can be found by clicking here.

Language Editing Pre-Submission

Language editing, if your first language is not English, to ensure that the academic content of your paper is fully understood by journal editors and reviewers, is available but optional. Language editing does not guarantee that your manuscript will be accepted for publication. For further information on this service, please seehttp://www.oxfordjournals.org/for_authors/language_services.html. Several specialist language editing companies offer similar services and you can also use any of these. Authors are liable for all costs associated with such services.

Your manuscript will be returned if you do not do the following:

1. Specify the type of article and adhere to the following limits:

- Major Article: 3500 words, 50 references, 7 figures or tables in print, 3 figures or tables online
- Brief Report: 2000 words, 15 references, 2 figures or tables in print, 3 figures or tables online

2. Include a cover letter with the following information:

- A statement that the manuscript has not been submitted or accepted elsewhere
- A statement that all authors fulfill the criteria given in the Authorship paragraph (see below)

- A statement indicating whether any writing assistance other than copy editing was provided in the preparation of the manuscript
- A list of 5 potential reviewers, with their e-mail addresses

3. All file names—for manuscript, cover letter, figures, tables—should contain no spaces between numbers or letters. File names may be run together (eg, authornamearticletitleversion1) or contain punctuation such as underscores, hyphens, or both (eg, author-name-article-title-version-1 or author_name_article_title_version_1).

4. Ensure that the references are appropriately formatted in JID style

5. Ensure that all text, including tables and references, is double spaced

6. Use a title of no more than 160 characters and spaces and a running title of no more than 40 characters and spaces

7. Include the word count of the abstract and of the text

8. Include a footnote page with the following items:

- A conflict of interest statement
- A funding statement
- Mention of any meeting(s) where the information has previously been presented
- Corresponding author contact information

9. Include 3–10 key words at the end of the abstract

10. Include, in the Methods section, a statement regarding informed consent and human and/or animal experimentation guidelines, when indicated

11. Include the registry number for a report of a clinical trial

12. Provide written permission for all personal communications

13. Provide accession numbers for nucleotide sequences

14. Use only approved human genetic nomenclature and notation (see the relevant subsections of the "Manuscript Preparation" section, below)

15. Submit newly identified single-nucleotide polymorphisms (SNPs) to the appropriate database; include previously recognized or recently submitted SNP numbers

JID complies with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (for the complete text, see http://www.icmje.org), except that reference citations should appear in the text in square brackets (not parentheses). Text, tables, references, and legends must be double spaced. Italics should be used for genus and species names and for genes, but not for in vivo, in vitro, in situ, et al., or other Latin-derived expressions listed in Webster's Collegiate Dictionary; see a recent issue of *JID* for appropriate style.

All manuscripts—Major Articles, Brief Reports, Correspondence, Perspectives, Editorials, Reviews, and Supplement Articles—must have conflict of interest and funding statements (see the below sections for further details).

Major Articles describe original investigations that are an important advance in the field and that have been brought to an acceptable degree of completion. Major Articles must be no longer than 3500 words of text (from the beginning of the introduction to the end of the discussion; do not count the abstract or the references), and illustrations must be limited to the minimum necessary for clear and concise presentation. For Major Articles describing results of clinical trials (see "Clinical trials registration," below), the abstract must be structured with the headings *Background, Methods, Results*, and *Conclusions* and must be no more than 200 words; for other Major Article manuscripts, the abstract may be structured (200-word limit) or unstructured (150-word limit). Major Articles are limited to a maximum of 7 inserts (tables and figures combined) in print, plus 3 online, and 50 references.

Brief Reports present complete studies that are narrower in scope than those described in Major Articles or that represent new developments. Manuscripts that are descriptive or primarily methodologic in nature, that report results of phase I and II vaccine trials (see "Clinical trials registration," below), or that describe in vitro chemotherapeutic studies should, in general, be submitted as Brief Reports. Brief Reports include an abstract (no more than 100 words) and are limited to no more than 2000 words of text, a total of 2 inserts (tables or figures) in print, plus 3 online, and 15 references.

Correspondence (letters) must be submitted in reference to a previous publication in *JID* (within the preceding 12 months); otherwise they will not be considered. Please prepare the letter in manuscript format, including a title page. The letter cannot exceed 750 words of text, 1 insert (table or figure), and 10 references.

Editorials are invited by the Editor and are overviews or critiques of articles appearing in *JID*.

Perspectives are brief (<1500 words, limit 2 inserts, 25 references) individual viewpoints on controversial topics in infectious diseases. Unsolicited perspectives are considered, and authors should contact the Editor in advance of submission to determine whether the topic is deemed appropriate.

Reviews that are research oriented will be considered. They should be no longer than 3500 words (from the beginning of the introduction to the end of the discussion) and have no more than 7 inserts (tables and figures combined) in print, 3 online, and 50 references. Authors should contact the Editor in advance of submission to determine whether a specific topic is deemed appropriate and timely.

Supplements

Supplements are published by *JID*. Requirements for supplement manuscripts follow those for *JID* manuscripts (e.g., cover letter, conflict of interest, and funding statements). Inquiries related to suitability of topic, program organization, and production should be made in writing to the Editor.

Related Manuscripts

If there appears to be significant overlap between a manuscript submitted to *JID* and another manuscript*submitted* (to *JID* or another journal) by the same authors or if there is an overlap between a manuscript submitted to *JID* and one published by *JID* or another journal, the editors will ask the corresponding author to respond to the question of overlap. On the basis of the response, the editors may choose to consider the manuscript further, ask for modification of the manuscript, or reject the manuscript; in the case of a rejected manuscript, the editors will provide the author(s) with a detailed explanation. The editors may also choose to take further action, which could include contacting the appropriate superior at the home institution and/or suspending publishing privileges in *JID* for a designated period of time. If it is found

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Title Page

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Uherova P, Connick E, MaWhinney S, Schlichtemeier R, Schooley RT, Kuritzkes DR. In vitro effect of interleukin-12 on antigen-specific lymphocyte proliferative responses

from persons infected with human immunodeficiency virus type 1. J Infect Dis **1996**; 174:483-9.

Book chapter

McIntosh K. Diagnostic virology. In: Fields BN, Knipe DM, Chanock RM, et al., eds. Fields virology. 2nd ed. Vol 1. New York: Raven Press, **1990**:411-40.

Internet site

Public Health Service Task Force. Recommendations for the use of antiretroviral drugs in pregnant HIV-1 infected women for maternal health and interventions to reduce perinatal HIV-1 transmission in the United States. Available at: http://www.hivatis.org. Accessed 24 April 2002.

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