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GUILHERME AMARAL CALVET

MENOPAUSA EM UMA COORTE DE MULHERES COM HIV/AIDS NO RIO DE JANEIRO

Rio de Janeiro
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Menopausa em uma coorte de mulheres com HIV/AIDS no Rio de Janeiro

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Tese apresentada ao curso de Pós-Graduação *Stricto Sensu* do Instituto de Pesquisa Clínica Evandro Chagas para obtenção do grau de Doutor em Pesquisa Clínica em Doenças Infecciosas.

Orientadoras:

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RESUMO

Introdução: É esperado um aumento global de mulheres mais velhas que irão conviver com a infecção pelo HIV e que alcançarão a menopausa durante o curso da doença, em função principalmente do aumento da sobrevida após a expansão e acesso à terapia antirretroviral combinada (TARV) e do crescente número de mulheres mais velhas sendo diagnosticadas. **Artigo 1**. Objetivo: Investigar a idade e as taxas de incidência de menopausa natural e menopausa natural precoce e seus preditores em uma coorte de mulheres HIV-positivo no Rio de Janeiro, Brasil. Métodos: Foram incluídas mulheres HIV-positivo, com 30 anos ou mais de idade. Menopausa foi definida como última menstruação ocorrida há mais de um ano. Modelos de riscos proporcionais de Cox foram utilizados para identificar preditores de idade da menopausa natural e menopausa natural precoce. Resultados: 667 mulheres foram incluídas. A idade mediana no início do estudo foi de 34,9 [intervalo interquartil (IQR): 30,9-40,5] anos, 507 (76%) eram pré-menopausadas e 160 (24%) alcançaram a menopausa no final do acompanhamento. A idade mediana da menopausa natural foi de 48 (IQR: 45-50) anos; 36 (27%) tiveram menopausa natural precoce (≤ 45 anos). Menarca < 11 anos [*Hazard Ratio* (HR) 1,79, intervalo de confiança (IC) 95% 1,08-2,98], hepatite C crônica (HR 2,77, IC95% 1,39-5,50), contagem de CD4 < 50 células/mm^3 (HR 3,41; IC95% 1,17-9,94), presença de doença definidora de aids (HR 1,68, IC95% 1,15-2,45) e exposição <10 anos à TARV (HR 2,97, IC95% 1,76-5,01) permaneceram significativamente associados com a idade da menopausa natural no modelo final. Tabagismo também foi associado com idade da menopausa natural precoce (HR 2,78, IC95% 1,29-5,99). Conclusões: Estes resultados têm implicações clínicas e de saúde pública significativos porque o início precoce da menopausa tem sido associado com aumento da morbidade e mortalidade. Mulheres pós-menopáusicas HIV-positivo representam um grupo em expansão e o manejo adequado dessa população visando uma melhor qualidade de vida é fundamental. **Artigo 2.** Objetivo: Comparar a eficácia da TARV de primeira linha em mulheres pré e pós-menopausadas. Foram estudadas mulheres virgens de TARV que iniciaram o esquema antirretroviral entre janeiro 2000 e junho de 2010, no Instituto de Pesquisa Clínica Evandro Chagas. Métodos. As mulheres eram consideradas como pós-menopausadas após 12 meses consecutivos de amenorreia. Contagem de células CD4 e carga viral (CV) para o HIV foram comparadas entre pré e pós-menopausadas, aos 6, 12 e 24 meses após o início da TARV. Mulheres que modificaram ou descontinuaram uma classe de drogas ou que morreram devido a uma doença oportunista foram classificadas como falhas. As variáveis foram comparadas pelos testes de Wilcoxon, $\chi 2$ ou teste exato de Fisher. As chances de eficácia da TARV (CV < 400 cópias/mL e ou não modificação do esquema antirretroviral) foram comparadas por meio de regressão logística. Modelo linear foi utilizado para acessar a relação entre CD4 e menopausa. Resultados: Entre 383 mulheres, 328 (85%) estavam na pré-menopausa e 55 (15%) na pós-menopausa. As medianas das contagens de CD4 antes do início da TARV foram de 231 e 208 células/mm^3 (p = 0,14) em mulheres pré e pós-menopausadas, respectivamente. Nenhuma diferença na mediana de CV foi encontrada antes do inicio da terapia (ambas 4,8 cópias/mL). As medianas nas contagens de CD4 foram semelhantes aos 6 e 12 meses. Aos 24 meses após o início da TARV, a mediana de CD4 entre as mulheres na pós-menopausa foi significativamente menor do que entre as mulheres na pré-menopausa (p = 0,01). No entanto, quando a análise foi restrita às mulheres com CV indetectável, esta diferença não foi observada. Não houve diferença significativa entre os grupos em relação à efetividade da TARV aos 6, 12 e 24

meses. Efetividade da TARV foi observada em 63,7 % das mulheres em 24 meses. Conclusão: Estar na menopausa no momento do início da TARV de primeira linha não afeta as contagens de células CD4 em até 24 meses entre mulheres com resposta virológica. Não foi observada relação entre menopausa e resposta virológica.

Palavras-chave: 1. Aids 2. Efetividade de tratamento 3. Estudos de coorte 4. Hepatite C crônica 5. HIV 6. Idade da menopausa 7. Menopausa 8. Menopausa precoce 9. Mulheres, 10. Terapia antirretroviral combinada.

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ABSTRACT

Introduction: As a result of the expansion of combination antiretroviral therapy (cART) coverage leading to reduction in morbidity and mortality and an increasing number of older individuals being diagnosed with HIV infection, an increased number of HIV-infected women entering menopause is expected. **Article 1.** Objective: To investigate the age and incidence rates of natural and earlier natural menopause and their predictors in a cohort of HIV-infected women in Rio de Janeiro, Brazil. Methods: HIV-infected women with 30 years or older were included. Menopause was defined as having more than one year since the last menstrual period. Multivariate Cox proportional hazard regression analysis was used to identify predictors of age at natural menopause and early age at natural menopause. Results: 667 women were included, median age at baseline was 34.9 [interquartile interval (IQR): 30.9-40.5 years], 507 (76%) were premenopausal and 160 (24%) reached menopause by the end of follow-up. Median age at natural menopause was 48 (IQR: 45–50) years; 36 (27%) of them had early menopause (≤45 years). Menarche <11 years [Hazard Ratio (HR) 1.79, 95% Confidence Interval (CI) 1.08-2.98], chronic hepatitis C (HR 2.77, 95% CI 1.39-5.50), CD4 count <50 cells/mm3 (HR 3.41, 95% CI 1.17-9.94), AIDS defining illness (HR 1.68, 95% CI 1.15-2.45) and combination antiretroviral therapy exposure <10 years (HR 2.97, 95% CI 1.76-5.01) remained significantly associated with age at natural menopause in the final model. Cigarette smoking was also associated with early age at natural menopause (HR 2.78, 95% CI 1.29-5.99). Conclusions: These results have significant clinical and public health implications as early onset of menopause has been associated with increased morbidity and mortality. HIV-infected postmenopausal women are expanding and adequate management of this population aiming a better quality of life is critical. **Article 2**. Objective: To compare the effectiveness of first-line cART between premenopausal and postmenopausal women. Methods: ART-naïve women initiating cART between January 2000/June 2010 at the Instituto de Pesquisa Clínica Evandro Chagas Cohort were studied. Women were considered as postmenopausal after 12 consecutive months of amenorrhea. CD4 cell counts and HIV-1RNA viral load (VL) measurements were compared between pre- and postmenopausal at 6, 12 and 24 months after cART initiation. Women who modified/discontinued a drug class or died due to an AIDS defining illness were classified as ART-failures. Variables were compared using Wilcoxon test, $\chi 2$ or Fisher's exact test. The odds of cART effectiveness (VL<400 copies/mL and/or no need to change cART) were compared using logistic regression. Linear model was used to access relationship between CD4 change and menopause. Results: Among 383 women, 328 (85%) were premenopausal and 55 (15%) postmenopausal. Median pre cART CD4 counts were 231 and 208 cells/mm3 (p=0.14) in pre- and postmenopausal women, respectively. No difference in the median pre cART VL was found (both 4.8 copies/mL). Median CD4 changes were similar at 6 and 12 months. At 24 months after cART initiation, CD4 changes among postmenopausal women were significantly lower than among premenopausal women (p=0.01). When the analysis was restricted to women with VL<400 copies/mL, no statistical difference was observed. Overall, 63.7% achieved cART effectiveness at 24 months without differences between groups at 6, 12 and 24 months. Conclusion: Menopause status at the time of first-line cART initiation does not impact CD4 cell changes at 24 months among women with a virologic response. No relationship between menopause status and virologic response was observed.

Keywords: 1.Age at menopause 2.Early menopause 3.HIV 4.AIDS 5.Chronic hepatitis C 6.Cohort studies 7.Women 8.Menopause 9.Combination antiretroviral therapy 10.Effectiveness, treatment.

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LISTA DE ABREVIATURAS

ADI	*AIDS Defining Illnesses*/Doenças defininoras de aids
AIDS/aids	*Acquired Immunodeficiency Syndrome*/Síndrome da imunodeficiência adquirida
AM	*Age at natural menopause*/Idade da menopausa natural
ART	*Antiretroviral Therapy*/Terapia Antiretroviral
BMI/IMC	*Body Mass Index*/ Índice de Massa Corporal
cART/TARV	*Combination Antiretroviral Therapy*/Terapia Antiretroviral Combinada
EH	*Exogenous Hormone*/Hormônio exógeno
FSH	Hormônio folículo-estimulante
FMP	*Final Menstrual Period*/ Período menstrual final
HCV	Vírus da Hepatite C
HIV	*Human Immunodeficiency Virus*/Vírus da Imunodeficiência Humana
HR	*Hazard Ratio*/Razão de riscos
IOP	Insuficiência Ovariana Prematura
IQR	*Interquartile Interval*/Intervalo Interquartil
LSD	*Lysergic acid diethylamide*/ Dietilamida do Ácido Lisérgico
NRTI/ITRN	*Nucleoside Reverse Transcriptase Inhibitor*/Inibidores da Transcriptase Reversa Análogos de Nucleosídeos
NNRTI/ITRNN	*Non-Nucleoside Reverse Transcriptase Inhibitor*/Inibidores da Transcriptase Reversa Não Análogos de Nucleosídeos

OC/ACO	*Oral contraceptive/*Anticoncepcional
OR	*Odds Ratio/*Razão de chance
PI/IP	*Protease inhibitors/*Inibidores da Protease
RNA	*Ribonucleic Acid*/Ácido Ribonucleico
SPSS	*Statistical Package for Social Sciences*
TSH	Hormônio estimulante da tireoide
VL/CV	*Viral Load*/Carga Viral

LISTA DE SIGLAS

ACTG	*AIDS Clinical Trials Group/* Grupo de Ensaios Clínicos em AIDS
CDC	*Centers for Disease Control and Prevention*/Centro de Controle e Prevenção de Doenças
CEP	Comitê de Ética em Pesquisa
DIDI	*Donne con Infezione Da HIV/* Mulheres com infecção por HIV
EUA	Estados Unidos da América
Fiocruz	Fundação Oswaldo Cruz
IBGE	Instituto Brasileiro de Geografia e Estatística
IeDEA	*International epidemiological Database to Evaluate AIDS*
IPEC	Instituto de Pesquisa Clinica Evandro Chagas
NA-ACCORD	*North American AIDS Cohort Collaboration on Research and Design*
UNAIDS	*United Nations Programme on HIV/AIDS* – Programa das Nações Unidas em HIV/AIDS
WHO/OMS	*World Health Organization/* Organização Mundial de Saúde
WIHS	*Women's Interagency HIV Study*

SUMÁRIO

1 INTRODUÇÃO

1.1 IDADE DA MENOPAUSA E FATORES ASSOCIADOS

As mulheres representam metade das pessoas que vivem com o HIV no mundo; a maioria reside em países de baixa ou média renda (UNAIDS, 2012). Em 2011, a taxa de incidência de casos de aids em mulheres brasileiras foi de 14,7/100.000 habitantes, com aumento progressivo da mesma entre 2002 e 2011 nas faixas etárias acima de 50 anos (Brasil, boletim epidemiológico HIV/aids, 2012). A introdução da terapia antirretroviral combinada (TARV) associada ao aumento da sobrevida das pessoas com diagnóstico da infecção pelo HIV pode ser responsável em parte por esse aumento (Hacker *et al*., 2004). Por outro lado, alguns estudos identificaram prática de sexo desprotegido, baixa percepção do risco de infecção pelo HIV e dificuldade de negociação do uso do preservativo, aliados à manutenção da atividade sexual no climatério, como possíveis fatores de risco associados à infecção pelo HIV em mulheres nessa fase da vida (Valadares *et al*., 2010). Portanto, é esperado um aumento global de mulheres mais velhas que irão conviver com a infecção e que alcançarão a menopausa durante o curso da doença pelo HIV (Cejtin, 2012).

A menopausa é um processo natural que ocorre na vida das mulheres como parte do processo de envelhecimento normal (Harlow *et al*., 2012). Menopausa natural é definida como a cessação da menstruação, resultante da perda de atividade folicular ovariana, reconhecida após 12 meses consecutivos sem períodos menstruais (WHO, 1996).

A transição para menopausa, ou perimenopausa, começa em média quatro anos antes do último período menstrual, e inclui uma série de alterações fisiológicas que pode afetar a qualidade de vida da mulher (Utian & Woods, 2013). É caracterizada por ciclos menstruais

irregulares e flutuações hormonais; muitas vezes acompanhadas de ondas de calor, transtornos do sono, alterações de humor e ressecamento vaginal (Edwards, 2013).

A idade da menopausa varia substancialmente entre populações (Thomas *et al*., 2001). Uma série de fatores têm sido estudados como preditores da idade da menopausa na população em geral, incluindo fatores genéticos, sociodemográficos, história reprodutiva, estilo de vida e condições de saúde da mulher na infância e na idade adulta (Henderson *et al.*, 2008; Mishra *et al*., 2009; Gold, 2011; Gold *et al.*, 2013). Entre estes, muito são comuns entre mulheres HIV-positivo, como tabagismo, raça negra, baixo nível de escolaridade, abuso de substâncias ilícitas e baixo índice de massa corporal (Kanapathipillai *et al*., 2013).

O conceito de menopausa antes dos 45 anos de idade é controverso. Menopausa ocorre em 5% das mulheres entre 40 e 45 anos e em 1% daquelas com idade inferior a 40 anos, sendo considerada, respectivamente, como precoce e prematura (Shuster *et al*., 2010).

Muitas vezes a terminologia na literatura sobre menopausa precoce ou prematura se confunde com a definição de insuficiência ovariana prematura (IOP), comumente denominada de falência ovariana prematura. A IOP é caracterizada por amenorreia, hipoestrogenismo e níveis elevados de gonadotrofinas, sendo causa comum de infertilidade em mulheres com menos de 40 anos. IOP não é sinônimo de menopausa natural precoce porque é caracterizada pela função ovariana intermitente em metade das mulheres afetadas. As mulheres com IOP podem produzir estrógenos intermitentemente e podem ovular e consequentemente engravidar em 5-10% dos casos após o diagnóstico. A etiologia da IOP permanece desconhecida na maioria das vezes, mas pode estar associada a diversos fatores, como: genéticos, destruição autoimune dos ovários, quadros infeciosos e metabólicos ou lesão iatrogência dos ovários (Shelling, 2010; Jim *et al*., 2012).

Na população geral, a menopausa precoce tem sido associada a um aumento do risco de aterosclerose (Lee *et al*., 2013; Ramezani Tehrani *et al*., 2013), doenças cardiovasculares

(Archer, 2009; Wellons *et al*., 2012), acidente vascular cerebral (Rocca *et al*., 2012; Wellons *et al*., 2012; Fukuda *et al*., 2013), osteoporose e fraturas ósseas (Cauley *et al*., 2012, Svejme *et al*., 2012; Nedergaard *et al*., 2013). Alguns estudos também sugerem que a taxa específica de mortalidade por todas as causas e por causa específica é mais elevada em mulheres com menopausa precoce (Mondul *et al*., 2005; Shuster *et al*., 2010; Svejme *et al*., 2012; Li *et al*., 2013). Por outro lado, alguns trabalhos indicam que a menopausa natural mais tardia é associada com um risco aumentado de câncer de mama, endométrio e ovário (*Collaborative Group on Hormonal Factors in Breast Cancer*, 2012; Cramer, 2012). Também existem evidências de que mulheres menopausadas vivendo com o HIV/aids sejam mais vulneráveis às comorbidades observadas na população geral (Triant *et al*., 2007; Maki *et al*., 2009; Yin *et al*., 2010; Yin *et al*., 2012; Kanapathipillai *et al*., 2013).

Muitas mulheres HIV-positivo, além de ficarem expostas às complicações metabólicas comuns à fase de pós-menopausa decorrentes da perda de estrogênio, podem possuir previamente outros fatores de risco ou complicações relacionadas à infecção pelo HIV e ao uso prolongado da TARV, como resistência à insulina, dislipidemia, lipodistrofia e osteopenia (Nagy, 2003; Alvarez *et al*., 2010; Libois *et al*., 2010; Giannarelli *et al*., 2011). Além disso, com início mais precoce da menopausa, estas complicações podem ser agravadas.

Em países desenvolvidos (Dratval *et al*., 2009; Yasui *et al*., 2012; Gold *et al*., 2013), estudos sugerem que a idade média da menopausa na população geral é mais elevada do que nos países em desenvolvimento (Sidibe, 2005, Castelo- Branco *et al*., 2006; Palacios *et al*., 2010). No Brasil, quase todos os estudos epidemiológicos relacionados à idade da menopausa natural na população geral não são de base populacional (Aldrighi *et al*., 2005; Otero *et al*., 2010). Apenas um estudo transversal, de base populacional entre 456 mulheres brasileiras, selecionadas através de amostragem por conglomerados, apresentou uma idade média de menopausa natural de 51,2 anos (Pedro *et al*., 2003), similar ao observado em países

desenvolvidos.

Estudos sobre idade na menopausa na população de mulheres HIV-positivo são escassos. A tabela 1 sumariza os principais resultados dos estudos que avaliaram a idade e os fatores relacionados à menopausa em mulheres com diagnóstico da infecção pelo HIV. Alguns estudos internacionais sugerem que a idade da menopausa ocorre mais cedo em mulheres HIV-positivo, com idades medianas relatadas entre 46 e 49 anos (Clark *et al*., 2000; Schoenbaum *et al*., 2005; de Pommerol *et al*., 2011; Boonyanurak *et al*., 2012).

Foram associados com um risco maior de menopausa precoce: contagens de células CD4 inferiores a 200 células/mm^3 (Schoenbaum *et al*., 2005; de Pommerol *et al*., 2011), ter sido classificada como categorias B e C (Boonyanurak *et al*., 2012) ou ter apresentado uma condição indicadora de aids (categoria C) (Cicconi *et al*., 2012), segundo os critérios do Centro de Controle e Prevenção de Doenças (CDC) (53) (CDC, 1993). De Pommerol e colaboradores também sugeriram que a ocorrência de menopausa precoce foi associada ao uso de drogas injetáveis e à descendência africana (de Pommerol *et al*., 2011). Ferreira e colaboradores, em um estudo transversal incluindo 96 mulheres brasileiras HIV-positivo, mostraram que a idade média da menopausa foi de 47,5 anos (Ferreira *et al*., 2007), no entanto, este estudo brasileiro não avaliou os fatores relacionados à idade da menopausa.

Fantry e colaboradores não observaram início precoce da menopausa natural na população estudada e o status de pós-menopausa estava associado apenas ao uso de metadona nos seis meses anteriores à aplicação do questionário do estudo (Fantry *et al*., 2005). De fato, estudo de Harlow e colaboradores sugere que mulheres HIV-positivo usárias de drogas injetáveis que estavam em programas de manutenção com metadona eram mais propensas a apresentar amenorreia do que mulheres HIV-positivo que não usavam essas substâncias (Harlow *et al*., 2003).

Cejtin e colaboradores (Cejtin *et al*., 2004; Cejtin *et al*., 2006) estudaram a idade da menopausa, fatores associados à menopausa e amenorreia prolongada superior a 12 meses por outras causas não definidas como menopausa em mulheres HIV-positivo e HIV-negativo no *Women's Interagency HIV Study (*WIHS). Os autores não encontraram diferença significativa na mediana da idade da menopausa entre mulheres HIV-positivo e HIV-negativo da coorte (47,7 e 48,0 anos, respectivamente) (Cejtin *et al*., 2004). Os dados também sugeriram que a amenorreia prolongada, na presença de níveis normais de hormônio folículo estimulante (FSH) em mulheres vivendo com HIV, estava relacionada com uso de opiáceos, baixos níveis séricos de albumina, história de doença definidora de aids (categoria C), baixa renda e etnia não hispânica/outras. (Cejtin *et al*., 2006). Em outro estudo com mulheres desta mesma coorte que ainda estavam menstruando, os níveis de FSH da fase folicular precoce, estradiol, inibina B, e do hormônio anti-mulleriano foram semelhantes nas mulheres HIV-positivo e HIV-negativo (Seifer et., 2007). No entanto, alguns estudos não encontraram associação de amenorreia com a infecção pelo HIV (Shah, *et al*., 1994; Ellerbrock *et al*., 1996).

Tabela 1. Principais estudos sobre idade e fatores associados à menopausa natural, precoce e prematura em mulheres vivendo com HIV.

Autores/ano/ revista	**Local**	**Período**	**Objetivos**	**Desenho do estudo**	**N Grupo do estudo**	**Faixa etária**	**Análise estatística**	**População do estudo**	**Idade média ou mediana de menopausa natural**	**Prevalência de menopausa precoce/ prematura**	**Fatores associados à menopausa**	**HR ou OR ajustada (IC 95%)**
Clark *et al*., 2000 (JAIDS)	Centros ACTG dos EUA.	Não disponível	Definir população de mulheres HIV+ que podem se beneficiar de reposição hormonal e determinar prevalência de sintomas na pós-menopausa.	Transversal	101	≥40 anos (média de 47 anos)	Qui-quadrado e teste t.	Mulheres HIV+	Média: 47 (35-57) anos (n=26)	Não disponível	Não disponível	Não disponível
Clark *et al*., 2001 (J Infect Dis)	EUA	Não disponível	Obter informações sobre prevalência de anovulação, menopausa precoce e função do eixo hipófise-gonadal.	Transversal	Amostras estocadas de 52 mulheres	20 a 42 anos	Teste exato de Fisher e teste exato de Kruskal-Wallis.	Mulheres HIV+	Não disponível	Precoce: 8% (n=2/24)	Frequência elevada de anovulação com tendência de associação com baixas contagens de CD4.	Não disponível
Cetjin *et al*., 2004 (poster, XV International AIDS Conference)	EUA	Não disponível	Caracterizar menopausa no estudo "WIHS" e comparar menopausa em mulheres HIV+ e HIV- da coorte	Coorte	1335 (1063 HIV+ e 272 (HIV-)	<55 anos	Qui-quadrado e teste t, regressão logística	Mulheres HIV+ e HIV-	HIV+: Mediana de 47,7 anos Vs HIV-: mediana de 48 anos (n=não reportado)	Não disponível	Em pacientes HIV+ sem associação com CD4, carga viral par o HIV, CDC categoria C e uso de antirretrovirais	Não disponível
Fantry *et al*., 2005 (AIDS Patient Care and STD)	EUA	Julho de 2001 a Março de 2002	Determinar idade da menopausa, fatores associados ao status de pós-menopausa e prevalência de sintomas da menopausa.	Transversal	120	40 a 57 anos (média: 45,5 anos)	Kaplan-Meir e modelo de regressão de Cox	Mulheres HIV+	Mediana: 50 anos (IQR: 49-53) (n=30)	Precoce: 20% (n=6) Prematura: 23,3% (n=7)	Associação de uso de metadona nos últimos 6 meses com status menopausa.	HR univariada (metadona): 3,3 (1,6-6,8)
Schoenbaum *et al*., 2005 (Clin Infect Dis)	EUA	Setembro de 2001 a Setembro de 2003	Estudar a relação da infecção pelo HIV e uso de drogas com início da menopausa natural ("Ms Study").	Coorte	571 (52,9% HIV+)	Mediana: 43 anos (IQR: 40-46)	Regressão Logística	Mulheres HIV+ e HIV-	HIV+: Mediana de 46 (IQR: 39-49) anos (n=62) Vs HIV-: mediana de 47 (IQR: 44,5-48) anos (n=40)	Prematura (<40): 26% (n=16/62)	Contagem de CD4 <200 células/mm^3 associado à menopausa natural em pacientes HIV+.	OR ajustada: CD4>500: 0,191 (0,076-0,484) p<0,0001 CD4 200-500: 0,346 (0,147-0,813) p=0,015 CD4<200= Ref.

Autores/ano/ revista	Local	Período	Objetivos	Desenho do estudo	N Grupo do estudo	Faixa etária	Análise estatística	População do estudo	Idade média ou mediana de menopausa natural	Prevalência de menopausa precoce/ prematura	Fatores associados à menopausa	HR ou OR ajustada (IC 95%)
Ferreira *et al*., 2007 (Gynecol Endocrinol)	Brasil	Junho de 2005 a Maio de 2006	Estudar prevalência e fatores associados aos sintomas da menopausa.	Transversal	251 (96 HIV+ e 155 HIV-)	≥40 anos HIV+: Média de 48,9 ± 7,4 anos	Equação de estimação generalizada (GEE) para sintomas	Mulheres HIV+ e HIV-	Mediana: 47,5 anos	Não estudado	Não estudado	Não estudado
de Pommerol *et al*., 2011 (Int J STD AIDS)	França	Abril de 2007 a Fevereiro de 2008	Descrever características de mulheres pós-menopausadas e investigar fatores associados à menopausa precoce	Coorte	404	19 a 79 anos	Kaplan-Meir e modelo de regressão de Cox	Mulheres HIV+	Mediana: 49 anos (IQR: 40-50) (n=69)	Prematura (<40 anos): 12% Precoce (40-45): 22%	Preditores de menopausa precoce: (1) Afrodescendente (2) uso de drogas injetáveis (3) contagem de CD4 <200 células/mm^3.	(1) Afrodescendente: 8,16 (2,23-29,89) (2) UDI: 2,46 (1,03-5,85) (3) CD4<200: 2,25 (0,94-5,39)
Boonyanurak, *et al*., 2012 (Menopause)	Tailândia	Junho de 2010 a Março de 2011	Investigar idade da menopausa e os sintomas relacionados à menopausa	Transversal	268	≥40 anos mediana de 44,6 anos (41,8-48,7)	Modelo de regressão de Cox	Mulheres HIV+	HIV+: Média: 47,3 ± 5,1 anos (n=55) População geral: Média: 49,5 ±3,6 anos	Não estudado	(1) Categorias B+C (CDC) (2) ausência de atividade sexual no último mês estavam associadas à menopausa.	(1) CDC B+C: 1,7 (1,0-3,03), p=0,04 (2) ausência de atividade sexual no último mês: 4,9 (1,5-16,0), p=0,01
Cicconi *et al*., 2012 (JAIDS)	Itália	Novembro de 2010 a Fevereiro de 2011	Estudar prevalência e fatores associados à menopausa precoce e prematura (Estudo DIDI)	Transversal	352	<46 anos mediana de 40 anos (34-43)	Regressão Logística	Mulheres HIV+	Não disponível	Precoce (<46 anos): 7,7% (n=27/352) vs 7,1% (população geral) Prematura (≤40 anos): 5,2% (n=9/173) vs 1,8% (população geral)	Doença definidora de aids (categoria C do CDC) como preditor de menopausa precoce.	OR ajustada: 3,33 (1,14-8,69)

ACTG- "Aids Clinical Trials Group"; CDC- "Centers for Disease Control and Prevention"; DIDI- "Donne con Infezione Da HIV"; EUA- Estados Unidos; IQR –Intervalo interquartil; WIHS- "Women's Interagency HIV Study"

1.2 RESPOSTA IMUNOLÓGICA E VIROLÓGICA À TERAPIA ANTIRRETROVIRAL

Estudos têm demostrado que o envelhecimento e a infecção pelo HIV têm efeitos muito específicos na função e no número células T CD4+ (Malaguarnera *et al*., 2001; Nguyen & Holodniy, 2008; Haynes & Maue, 2009; Hearps *et al*., 2011; Cardoso *et al*., 2013; Pirrone *et al*., 2013). A imunossenescência é caracterizada pela deterioração natural do sistema imunológico produzido pelo envelhecimento (Malaguarnera *et al*., 2001). Em indivíduos idosos soronegativos é observada uma diminuição na contagem de células T CD4+ (Maue & Haynes, 2009). Com o envelhecimento ocorre involução do timo, levando a uma redução da capacidade do organismo em substituir as células T CD4+ que estão diminuídas na infecção pelo HIV (Douek *et al*., 1998; Li *et al*., 2011; Kolte, 2013).

A infecção pelo HIV também está associada a uma diminuição da capacidade para o crescimento de células percursoras de linfócitos T (Ballon *et al*., 2001). Embora o número de células T CD4+ de memória aumente com a idade, a capacidade de responder a patógenos primários diminui (Haynes *et al*., 2003; Ferrando-Martinez *et al*., 2011; Moro-Garcia *et al*., 2012).

Esteróides sexuais naturais podem mediar alterações no sistema imunológico e estrógenos podem regular as respostas imune, humoral e celular, com diminuição nas subpopulações de células T CD4 + e linfócitos B em mulheres pós-menopausadas (Yao & Hou 2004; Gameiro Romão *et al*., 2010; Oertelt-Prigione, 2012).

Estudos têm demonstrado que pacientes HIV-positivo mais velhos apresentam menores contagens de células TCD4+ no momento do diagnóstico (Cardoso *et al*., 2013). Outros trabalhos também sugerem que mulheres mais jovens com o diagnóstico da infecção

pelo HIV têm maiores contagens de células T CD4+ e níveis mais baixos de RNA do HIV antes de iniciarem a TARV, quando comparadas com homens HIV-positivo (Gandhi *et al*., 2002; Napravnik *et al*., 2002; Grinsztejn *et al*., 2011). Assim, a recuperação imunológica pode ser menos eficaz em indivíduos mais velhos, quando comparados aos mais jovens.

Estudos avaliando o impacto da idade na resposta imunológica à TARV são controversos; alguns deles evidenciaram diminuição da resposta (Manfrediet *et al*., 2000; Viard *et al*., 2001; Grabar *et al*., 2004; Althoff *et al*., 2010; Mutevedzi *et al*., 2011; Balestre *et al*., 2012), enquanto outros sugerem que pessoas mais velhas podem alcançar resposta imunológica semelhante aos indivíduos mais jovens (Wellons *et al*., 2002; Tumbarello *et al*., 2004; Patterson *et al*., 2007).

Diversos estudos também avaliaram a influência do gênero sobre a resposta antirretroviral com resultados divergentes (Raboud *et al*., 2010; Barber *et al*., 2011; Perez-Molina *et al*., 2012; Soon *et al*., 2012; Thorsteinssond *et al*., 2012). Uma recente meta-análise incluindo 20.328 homens e mulheres HIV-positivo de 40 ensaios clínicos randomizados, não observou nenhuma diferença em relação à resposta virológica em 48 semanas de tratamento antirretroviral, em pacientes que eram virgens de tratamento ou que estavam em uso de TARV de resgate (Soon *et al*., 2012). No entanto, Kwakwa e colaboradores, em outra meta-análise envolvendo sete estudos clínicos controlados, em pacientes masculinos e femininos previamente virgens de TARV, observaram que as mulheres eram 28% menos propensas a alcançar supressão virológica do que os homens (Kwakwa *et al*., 2012).

Em uma coorte suíça, mulheres pós-menopausadas apresentaram menores contagens de linfócitos T CD4+ três anos após a soroconversão para o HIV, em comparação com mulheres pré-menopausadas, embora a diferença não fosse estatisticamente significativa (van Benthem *et al*., 2002). Patterson e colaboradores não encontraram diferença significativa sobre a resposta imunológica e virológica de 6 a 24 meses após o início de TARV em um

grupo de mulheres pré e pós-menopausadas, previamente virgens de tratamento antirretroviral (Patterson *et al*., 2009). Por outro lado, uma meta-análise envolvendo 4.414 mulheres virgens de TARV, recrutadas em 32 estudos randomizados entre 2000 e 2010, concluiu que mulheres com 50 anos ou mais de idade tinham significativamente maiores chances de supressão viral após 24 e 48 semanas do início de TARV de primeira linha do que mulheres com 35 anos ou menos de idade. A idade não influenciou significativamente as respostas imunológicas 24 ou 48 semanas após o inicio de TARV, embora mulheres mais jovens, em uso de TARV contendo inibidores da transcriptase reversa não análogos de nucleosídeos (ITRNN), tivessem significativamente melhores respostas de células T CD4+ do que o grupo mais velho ao longo de 48 semanas de tratamento (Yan *et al*., 2013).

Portanto, a eficácia da TARV pode ser diferente de acordo com o status da mulher em relação à menopausa (97-99) (van Benthem *et al*., 2002; Patterson *et al*., 2009; Yan *et al*., 2013).

2 JUSTIFICATIVA

Apesar do acesso universal ao tratamento antirretroviral e aos efeitos positivos observados com a terapia em diminuir a morbidade e mortalidade relacionadas à aids, tem se observado no Brasil um aumento da taxa de incidência de casos de aids nas faixas etárias acima de 50 anos (Brasil, boletim epidemiológico HIV/aids, 2012). Portanto, mulheres mais velhas irão conviver com a doença e alcançar a idade da menopausa durante o curso da mesma (Cejtin, 2012).

Existem inúmeros estudos publicados em países desenvolvidos e subdesenvolvidos acerca da idade natural e fatores associados ao início da menopausa na população geral.

(Henderson *et al.*, 2008; Mishra *et al*., 2009; Gold, 2011; Gold *et al.*, 2013). No entanto, dados sobre a menopausa na população de mulheres HIV-positivo são escassos (Clark *et al*., 2000; Schoenbaum *et al*., 2005; Fantry *et al*., 2005; de Pommerol *et al*., 2011; Boonyanurak *et al*., 2012; Cicconi *et al*.,2012). A maioria é de estudos retrospectivos, transversais, sendo a informação da idade da menopausa sujeita a viés de memória.

A maioria dos artigos sobre resposta imunológica e virológica ao tratamento antirretroviral compara faixas etárias em grupos de homens e mulheres (Wellons *et al*., 2002; Tumbarello *et al*., 2004; Greenbaum *et al*., 2008; Raboud *et al*., 2010; Barber *et al*., 2011; Balestre *et al*., 2012). Estudos têm demonstrado uma relação inversa entre contagem de células T CD4 + e a idade do paciente; no entanto, este achado é controverso, pois alguns deles não demonstraram relação entre idade e resposta à TARV no que concerne às contagens de células T CD4+ (Viard *et al*., 2001; Wellons *et al*., 2002; Grabar *et al*., 2004; Tumbarello *et al*., 2004; Patterson *et al*., 2007; Althoff *et al*., 2010; Mutevedzi *et al*., 2011; Balestre *et al*., 2012). Em relação à resposta virológica ao tratamento antirretroviral, os estudos apresentam dados conflitantes, pois enquanto alguns não encontraram diferença entre pacientes jovens e mais velhos, outros encontraram uma melhor resposta em pacientes idosos (Raboud *et al*., 2010; Barber *et al*., 2011; Kwakwa *et al*., 2012; Perez-Molina *et al*., 2012; Soon *et al*., 2012; Thorsteinssond *et al*., 2012).

Além disso, estudos comparando especificamente populações de mulheres pré e pós-menopausadas são escassos e também apresentam resultados controversos (van Benthem *et al*., 2002; Patterson *et al*., 2009; Yan *et al*., 2013).

Desde 1996, o Instituto de Pesquisa Clínica Evandro Chagas (IPEC), através de sua equipe multidisciplinar, acompanha uma coorte de mulheres HIV-positivo, considerada uma das principais e maiores coortes urbanas e abertas do Brasil. O objetivo desta coorte é estudar a história natural de infecção pelo HIV. São convidadas a integrar a coorte todas as mulheres

com idade igual ou superior a 18 anos, com diagnóstico confirmado de infecção pelo HIV e matriculadas no IPEC. Portanto, essa coorte nos permite estudar os fatores associados à idade da menopausa e efeitos da menopausa sobre a resposta imunológica e virológica, no contexto de um estudo longitudinal, podendo ajudar a preencher lacunas no manejo clínico e contribuir para o delineamento de decisões terapêuticas nesta população.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Estudar fatores associados à idade da menopausa e eficácia da terapia antirretroviral em uma coorte de mulheres HIV-positivo em acompanhamento no Instituto de Pesquisa Clínica Evandro Chagas/Fiocruz, no período de 1996 a 2011.

3.2 OBJETIVOS ESPECÍFICOS

- Conhecer a idade de ocorrência natural da menopausa.
- Determinar os fatores associados à ocorrência natural da menopausa.
- Determinar os fatores associados à ocorrência precoce da menopausa.
- Comparar a resposta imunológica e virológica da terapia antirretroviral de primeira linha entre mulheres pré e pós-menopausadas.

4 ESTRUTURA DA TESE

Os capítulos de metodologia, resultados e discussão referentes aos objetivos foram apresentados na forma de dois artigos científicos:

Artigo 1. *Factors associated with earlier age at natural menopause in HIV-infected women in Brazil.*

Artigo 2. *Absence of Effect of Menopause Status at Initiation of First-Line Antiretroviral Therapy on Immunologic or Virologic Responses: A Cohort Study from Rio de Janeiro, Brazil.*

5 ASPECTOS ÉTICOS RELACIONADOS AOS ESTUDOS

O presente estudo foi desenvolvido dentro dos principais objetivos dos projetos de pesquisa: "História Natural da Infecção pelo Papilomavírus Humano em uma Coorte de Mulheres Infectadas pelo HIV no Rio de Janeiro" e "Estudo longitudinal da História Natural da Infecção pelo HIV em pacientes acompanhados no IPEC-FIOCRUZ", ambos aprovados pelo Comitê de Ética em Pesquisa em Seres Humanos do IPEC sob os números: 020/2001 e 0032.0.009.000-10. O pesquisador se comprometeu a seguir todas as normas de boas práticas clínicas e de proteção aos sujeitos de pesquisa, assim como toda a legislação brasileira.

Todas as pacientes passaram pelo processo de consentimento informado, assinando o termo ao concordarem em participar do estudo. Os termos de consentimento livre e esclarecidos foram elaborados de acordo com as normas estabelecidas pela Resolução 196 do Conselho Nacional de Saúde, descrevendo todos os procedimentos, riscos, custos e aspectos relacionados à confidencialidade e direito à recusa, sem prejuízos ao seu tratamento rotineiro.

6 ARTIGOS CIENTÍFICOS

6.1 ARTIGO 1

O Artigo 1 será submetido à revista *Menopause.*

TITLE: Factors associated with earlier age of natural menopause in HIV-infected women in Brazil

RUNNING TITLE: Predictors of menopause in HIV-infected women

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ABSTRACT

OBJECTIVE: To investigate the age of natural menopause and its predictors in a cohort of HIV-infected women in Rio de Janeiro, Brazil. **METHODS:** HIV-infected women with ≥30 years of age were included. Menopause was defined as having ≥ one year since the last menstrual period. Early age of natural menopause was defined as the onset of menopause at ≤45 years. Multivariate Cox proportional analysis was applied. **RESULTS:** 667 women were included in the analysis, median age at baseline was 34.9 [interquartile interval (IQR): 30.9-40.5 years], 507 (76%) were premenopausal and 160 (24%) reached menopause by the end of follow-up. Median age at natural menopause was 48 (IQR: 45-50) years; 36 (27%) of them had an early menopause. Menarche <11 years [Hazard Ratio (HR), 1.79; 95% Confidence Interval (CI), 1.08-2.98], chronic hepatitis C (HR, 2.77; 95% CI, 1.39-5.50), CD4 count <50 cells/mm^3 (HR, 3.41; 95% CI, 1.17-9.94), AIDS defining illness (HR, 1.68; 95% CI, 1.15-2.45) and antiretroviral therapy exposure <10 years (HR, 2.97; 95% CI, 1.76-5.01) were significantly associated with age at natural menopause. Menarche <11 years (HR, 2.31; 95% CI, 1.04-5.17), cigarette smoking during follow-up (HR, 2.78; 95% CI, 1.29-5.99), chronic hepatitis C (HR, 4.12; 95% CI, 1.37-12.34), CD4 count <50 cells/mm^3 (HR, 5.30; 95% CI, 1.40-20.04) and antiretroviral therapy exposure <10 years (HR, 8.58; 95% CI, 2.01-36.71) remained significantly associated with earlier age at natural menopause. **CONCLUSIONS:** HIV-infected postmenopausal women are an expanding group. Adequate management of this population is critical because early onset of menopause has been associated with increased morbidity and mortality.

Key words: Age at menopause, early menopause, human immunodeficiency virus, chronic hepatitis C, cohort studies.

INTRODUCTION

In Brazil, between 1998 and 2010 an increase in AIDS cases was reported among individuals with an age ranging from 50 to59 years (from 9.5 to 16.3/100.000 inhabitants) and those 60 years of age or older (from 2.8 to 5.1/100,000 inhabitants age group)[1] as a result of the expansion of combination antiretroviral therapy (cART) coverage leading to reduction in morbidity and mortality[2] and also an increasing number of older individuals being diagnosed with HIV infection.[1, 3, 4] As a consequence, increasing numbers of HIV-infected women entering menopause are expected.[3-5]

Natural menopause is defined as the permanent cessation of menstruation resulting from the loss of ovarian follicular activity, recognized after 12 consecutive months without menstrual periods.[6] Early natural menopause is defined as permanent cessation of menstruation between 40 and 45 years of age. This condition affects 5% of women in the general population, whereas premature natural menopause occurring under 40 years of age affects 1% of women.[7]

Age at natural menopause (AM) varies substantially within and across populations.[8] In the developed world[9-11] the mean age at AM in the general population is usually higher than that observed in the developing world.[12-14] A cross-sectional, population-based study among 456 Brazilian women selected through area cluster sampling showed a mean age at AM of 51.2 years,[15] similar to that observed in developed countries.[9-11]

Several studies have suggested that AM occur earlier in HIV-infected women, with age reported between 46 and 49 years.[16-19] A cross-sectional study among 96 HIV-infected Brazilian women showed that median AM was 47.5 years.[20] A number of factors have been studied as predictors of woman's AM in the general population, such as genetics, socio-demographics, lifestyle, smoking, reproductive history, adult and early childhood health conditions.[9, 21-23] Among these factors, smoking, African ethnicity, lower educational level,

substance abuse, low body weight are common among HIV-infected women.[24] Moreover, in this population, CD4 cell count <200 cells/mm^3 [16,17] and Centers for Disease Control and Prevention clinical classification B/C[18] or C[25] were associated with an increased risk of earlier menopause.

Identifying factors associated with AM is crucial because earlier natural menopause has been associated with increased risk of negative outcomes such as atherosclerosis,[26, 27] cardiovascular disease,[28,29] stroke,[28,30,31] osteoporosis and fracture,[32-34] . In addition, postmenopausal women living with HIV/AIDS are more vulnerable to co-morbidities observed in the general population.[24,35-38]

The purpose of this study was to investigate the age and incidence rate of natural and earlier natural menopause and potential predictors in a cohort of HIV-infected women in Rio de Janeiro, Brazil.

METHODS

Ethical Statement

The study protocol was reviewed and approved by the Evandro Chagas Clinical Research Institute, Oswaldo Cruz Foundation (CAE 020/2001) ethics committee. Written informed consent was obtained from all women.

The HIV/AIDS Women`s Cohort and the study population

This study was conducted within the HIV/AIDS Women's Cohort, which has been established in 1996 at the Evandro Chagas Clinical Research Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil. Data from this cohort has been published elsewhere.[39, 40] Briefly, study visits occur every 6 months; socio-demographic information, behavior, reproductive, gynecologic and laboratory data are collected using structured questionnaires

and specimen collections for Pap smears, colposcopy and STD diagnosis are processed. A total of 1002 women with HIV/AIDS were enrolled in the cohort between May 20, 1996 and December 31, 2010.

Eligibility criteria for the present study included a premenopausal state at age 30 years or older at cohort entry (To), as these subjects were considered to be at risk for natural menopause and the completion of two or more visits after To. Women less than 30 years of at cohort entry contributed to this analysis if they turned 30 during the study inclusion period (until December 31, 2010). Follow-up included data collected up to December 31, 2011.

Postmenopausal women before To (n=163; 16.3%) and those with less than 30 years of age up until the end of inclusion period (n=111; 11.1%) were not eligible for this analysis. Seven hundred twenty eight women met the initial inclusion criteria: 31 (4.3%) were lost to follow-up before completing 30 years of age (e.g., before To) and 30 (4.1%) completed only one visit after To and were excluded from this analysis. Six hundred and sixty seven premenopausal women were considered for the analysis of AM (Figure 1). Women aged more than 45 years at cohort enrolment (n=59) were not eligible for the early AM analysis as they were not at risk for this outcome; thus, 608 premenopausal women were included in the second analysis.

Study definitions

Outcomes:

Our study outcomes were AM and early AM. Menopausal status was prospectively captured at the bi-annual cohort interviews.

AM was defined according to the World Health Organization (WHO) as the permanent cessation of menstruation resulting from the loss of ovarian follicular activity.[6] It

was clinically recognized after at least12 months of amenorrhea when final menstrual period (FMP) was characterized with certainty.[6]

Induced menopause followed either by surgical removal of both ovaries (with or without hysterectomy) or by iatrogenic ablation of ovarian function (e.g. by chemotherapy or radiation) were not considered as natural menopause.

Early AM was defined as the natural onset of menopause at an age less than or equal to 45 years.

Covariates

Socio-demographic factors:

Race/ethnicity, schooling, and monthly family income (in Brazilian minimum wages) were self-reported at cohort entry and evaluated as fixed-effect covariates.

Reproductive factors:

Age at menarche: self-reported at cohort entry.

Parity: assessed through the number of children born before To and at the end of the observational period; analyzed as a time-dependent variable for which women were allocated into 4 categories (0, 1, 2, and ≥ 3).

Oral contraceptive (OC) or other exogenous hormone (EH) exposure: self-reported and analyzed as a time-dependent variable with two levels ("Yes": a report of OC or other EH use at To or during the observational period; and “No”: women who had never been exposed to OC or other EH during the observational period).

Lifestyle factors:

Alcohol consumption: self-reported at cohort entry and assessed through the question “When you drink, how much distilled or fermented drinks do you ingest?”

Cigarette smoking: was assessed through questions on the date of the first and last cigarette exposure and the number of smoked cigarettes per day. Using the birth date, we computed the age at the first and the last cigarette exposure. Cigarette smoking was analyzed as two time-dependent covariates allowing a unique point change: a- cigarette smoking during study follow-up: women who reported cigarette smoking at To or during study follow-up were considered "exposed" since that date and until the date of the last cigarette exposure; b- Pack-years: the average number of cigarettes smoked per day was multiplied by length of smoking time, and divided by 20. Time (in years) from smoking initiation to the outcome or censorship was calculated. Ever smokers were classified into three groups: <10 pack-years, 10-19 pack/years, and ≥ 20 pack/years.

Lifetime illicit drug use: self-reported at cohort entry and considered as "Yes" if the woman used marijuana, cocaine, crack, glue or Lysergic acid diethylamide (LSD). Use of intravenous and snorted cocaine was assessed in the risk models.

Health related factors:

All health related covariates were assessed using the IPEC HIV/AIDS clinical database.[41,42]

Body Mass Index (BMI): Anthropometric data were obtained from the patient's medical charts. BMI was calculated as the weight (kilogram) divided by the square of the height (meters) and was reported as Kg/m^2 using all available data. BMI was categorized according to WHO standards for adults[43,44] as underweight (<18.5 Kg/m^2), normal weight (18.5-24.9 Kg/m^2) and overweight/obese (≥ 25.0 Kg/m^2). BMI was analyzed as time-dependent covariate allowing multiple points change. BMI for To was defined as the value obtained within six months from To.

Comorbidities: were analyzed as time-dependent variables with a unique point change at the date of the diagnosis and defined as follows:

Type II Diabetes: a fasting plasma glucose greater than or equal to 126 mg/dL in two samples collected in different days or by 2-hour plasma glucose greater than or equal to 200 mg/dL during an oral glucose tolerance test.

Chronic hepatitis C (HCV): diagnosed using HCV ELISA and or HCV RNA assays.

Hypothyroidism: the presence of at least one of the following: goiter, fatigue, cold intolerance, dry skin, constipation, bradycardia, weight gain, changes in menstrual pattern and decreased levels of thyroxine and tri-iodothyronine or increased thyroid-stimulating hormone.

HIV/AIDS related factors:

CD4+ T-cell counts: available data were categorized into ≥350, 200-349, 100-199, 50-99 and <50 cells/mm^3 and were analyzed as a time-dependent covariate allowing multiple points change. CD4+ T-cell counts at To was described and defined as the value obtained within six months from To.

Nadir CD4+ T-cell count: the lowest CD4+ T-cell count available from HIV diagnosis up to the end of study follow-up.

AIDS defining illnesses (ADI): the presence of any 1993 Centers for Disease Control and Prevention (CDC)-defined ADI[45] at any time during the course of HIV infection up to the end of study follow-up. ADI was assessed as a time-dependent variable.

Combination Antiretroviral Therapy (cART): two or more nucleoside reverse transcriptase inhibitors with a non-nucleoside reverse transcriptase inhibitor or at least one protease inhibitor. Time (in years) from cART initiation to the outcome or censorship was calculated and further categorized into ≥ 10 years and <10 years.

Statistical analysis

Median [interquartile range (IQR)] and frequency (%) were used to describe participant demographic characteristics for continuous and categorical data, respectively.

A Kaplan-Meier plot of natural menopause and early natural menopause was performed to estimate the survival function between each of the outcomes and age and was reported as a probability to be at natural menopause at age 45 and 50 years.

Incidence rates were estimated for both outcomes and reported per 100 person-years. Cox proportional hazards regression analysis using age as a time scale was used to assess the role of selected covariates on both outcomes. Women who presented secondary menopause (hysterectomy, bilateral oophorectomy, chemotherapy and/or radiotherapy) as well those who were lost to follow-up before December 31, 2011 were censored at the time of its occurrence and in the last gynecological visit, respectively. Women were also censored at the age of 45 years for the early natural menopause outcome, as they would not be any more at risk for this outcome. Collinearity between variables was assessed. We fitted the unadjusted models and selected all covariates statistically significant at 20% for natural menopause and at 10% for early natural menopause as thresholds for the multivariate analysis. A backward procedure was used to remove sequentially covariates with the highest p-value. Covariates with statistical significance at 5% ($p<0.05$) and those that were considered as confounders (e.g., when removed, a change equal or higher than 10% in the hazard ratio of any other variable of the model was observed) remained in the final model.[46] The assumption of the proportionality of risks was tested using the Schoenfeld residuals analysis.[47]

For all statistical analyses we used the software R, version 3.0.2 (The R Foundation for Statistical Computing, Vienna, Austria; http://www.r-project.org).

RESULTS

Sample characteristics

Six hundred sixty seven women were followed for a total of 3,814.0 person-years with a median follow-up of 5.0 (IQR: 2.7-8.3) years. Of the 667 women, 142 (21.3%) were censored: 41 (28.9%) deaths, 23 (16.2%) had surgically-induced menopause, five (3.5%) had chemotherapy or radiation-induced menopause, four (2.8%) transferred to another facility and 69 (48.6%) missed their scheduled follow-up gynecological visit for more than one year.

General characteristics are shown in Table 1. Median baseline age was 34.9 (IQR: 30.9-40.5) years. The majority of women were non-white (60.4%), with up to eight years of schooling (56.2%) and with a family income equal or less than five minimum wages (80.8%). Median age at menarche was 13 (IQR: 12-14) years, 60.9% women were multiparous. Forty percent (n=314) of women had reported lifetime exposure to cigarette smoking, but only 26% (n=173) were exposed during the follow-up period. Overall, women had quit smoking 11.7 (5.5-19.0) years prior to natural menopause or censorship. Among those 173 women exposed to cigarette smoking during the study period, 20.2% (n=35) reported smoking cessation; of these, 20% had natural menopause and the median time since smoking cessation until onset of natural menopause was 11.5 (IQR: 5.8-18.6) years.

Clinical co-morbidities assessed for this study were present in 114 (17.1%) women: type II diabetes in 78 (11.7%), chronic hepatitis C in 34 (5.1%) and hypothyroidism in 13 (1.9%). AIDS defining illnesses occurred in 44.1% women. At the initiation of follow-up, 22.6% of the women had a CD4 count less than 200 cells/mm^3; 4.2% had more severe immunodeficiency (CD4 < 50 cells/mm^3). The median nadir CD4 count was 182 (IQR: 74-278) cells/mm^3, 53.9% had a nadir CD4 less than 200 cells/mm^3. Most women were under cART (88.5%) for a median time of 4.9 years (IQR: 2.4-9.0) at the end of follow-up or censorship.

Natural menopause

Natural menopause was observed in 132 women, which corresponded to an incidence rate of 3.46 [95% Confidence Interval (CI), 2.90-4.09] per 100 person-years. The probability of reaching menopause at age ≤ 50 years was 0.5 (95% CI, 0.40-0.57) (Figure 2). The median age at natural menopause was 48 (IQR: 45-50) years.

The results from univariate and multivariate analysis for AM are presented in Table 2. Collinearity between "cigarette smoking during follow-up" and "pack-years" covariates was observed and the first covariate was chosen to multivariate analysis. The same was observed to "time-dependent CD4 cell count" and "CD4 cell count nadir" covariates and the first was entered in the initial multivariate model.

Early menarche (HR, 1.79; 95% CI, 1.08-2.98), chronic hepatitis C (HR, 2.77; 95% CI, 1.39-5.50), CD4 count less than 50 cells/mm^3 (HR, 3.41; 95% CI, 1.17-9.94), AIDS defining illness (HR, 1.68; 95% CI, 1.15-2.45) and time under cART exposure less than 10 years (HR, 2.97; 95% CI, 1.76-5.01) were found to be significantly associated to AM in the final multivariate model.

Having given birth to more than three babies showed a borderline association with later age of menopause (HR, 0.51; 95% CI, 0.26-1.03, p=0.059) and was also retained in the final model. Cigarette smoking during follow-up and body mass index remained as confounder variables and were kept in the final multivariate model.

Early Natural Menopause

Six hundred and eight women were evaluated for the early menopause outcome for a total of 3,299.1 person-years with a median follow-up of 4.6 (IQR: 2.5-7.7) years. Of the 608 women, 112 (18.4%) were censored: 33 (29.5%) deaths, 15 (13.4%) had a surgically-induced menopause, four (3.6%) had chemotherapy or radiation-induced menopause, four (3.6%)

transferred out and 56 (50%) missed their scheduled follow-up gynecological visit for more than one year.

Early natural menopause was observed in 36 women, with an incidence rate of 1.09 [95% CI, 0.77-1.49] per 100 person-years. The probability of reaching menopause at age ≤ 45 years was 0.1 (95% CI, 0.06-0.15) (Figure 2). Only three women had premature menopause (at age < 40 years).

The results from univariate and multivariate analysis for early AM are presented in Table 3. The same results of collinearity from AM outcome were observed. “Cigarette smoking during follow-up” and “time-dependent CD4 cell count" covariates were chosen to enter in the in the initial multivariate model.

Early menarche (HR, 2.31; 95% CI, 1.04-5.17), cigarette smoking during follow-up (HR, 2.78; 95% CI, 1.29-5.99), chronic hepatitis C (HR, 4.12,;95% CI, 1.37-12.34), CD4 count less than 50 cells/mm^3 (HR, 5.30; 95% CI, 1.40-20.04) and time under cART exposure less than 10 years (HR, 8.58; 95% CI, 2.01-36.71) were found to be significantly associated to earlier age at AM in the final multivariate model. AIDS defining illnesses remained as confounder variable and was kept in the final multivariate model.

The proportionality of risks was observed in AM and early AM models. Age at menopause was not significantly associated in both models with race/ethnicity, schooling monthly family income, ever exogenous hormone use, alcohol and cocaine use, BMI, type II diabetes, hypothyroidism and cART use.

DISCUSSION

Our study demonstrated that early menarche, hepatitis C co-infection, severe immunosuppression (CD4 count less than 50 cells/mm^3), diagnosis of an AIDS defining

illness and less than 10 years of cART use were predictors of earlier onset of AM. Cigarette smoking during follow-up in the study was also associated with early AM (≤45 years).

The median AM in the preset study falls in the range of values reported among other international studies with HIV-infected women[16-19] and is similar to the median age reported in a Brazilian study.[20] We observed that 27% of postmenopausal women reached natural menopause at an age less than or equal to 45 years, which is defined as early menopause. Similar high rates of early menopause were also observed in the study by Fantry et al[48] (20%) and de Pommerol et al[17] (22%), although these studies included a much smaller sample size. Of note, premature onset of menopause was lower in the present study (2.3%) when compared to other studies conducted in HIV-infected women where high rates of premature menopause was demonstrated.[16,17,25,48] Reproductive factors, such as menarche and parity, may be associated with age at menopause due to the fact that a lower occurrence of menstrual cycles would prevent oocyte depletion and lead to a delay in the cessation of ovarian function. In our study, early menarche (<11 years) was significantly associated with AM and early AM, and this is in agreement with studies done in the general population.[49-51] Studies conducted in populations of HIV-negative [22] or HIV-positive[16-18] women differed from our study as they did not define early menarche, they reported age at menarche. Contradictory reports may be due to different definitions of early menarche or differences in the composition of the study population. Increased parity showed a borderline association with later age of menopause, a finding consistent with prior studies in the general population of women.[22] Schoenbaum et al. found that lower parity was associated with an increased likelihood of onset of menopause in HIV-positive women,[16] although this was not confirmed by other authors.[17,18]

We were not able to observe any association with race/ethnicity or illicit intravenous drug use and menopause status, probably due to the high miscegenation of the Brazilian population and the small number of injecting drug users in our cohort.

We demonstrated that cigarette smoking was related to early AM, but not to AM. This could be partly explained by the large number of former smokers in our cohort Therefore, in the early menopause model the time of smoking cessation until the outcome or censorship is shorter and the association between current cigarette smoking and early onset of menopause could be more clearly observed. Smoking is one of the factors most consistently associated with AM in several studies.[52,53] In the general population studies suggest anticipation of menopause up to two years for women who smoked compared to nonsmokers.[54,55] Other studies suggest that substances present in the cigarette could be associated with irreversible damage to the ovarian follicles and impaired liver estrogen metabolism[56-58] and that women who stopped smoking many years before menopause were more similar to those who never smoked.[9] Therefore, it seems that current smoking near menopause is the main risk factor related to early menopause and not duration (length of time of use of tobacco) or intensity of smoking (pack-years of tobacco intake throughout life).[59,60]

Higher BMI was not a predictor of later AM, and this was consistent with the majority of other studies including those evaluating HIV-infected women.[16,18,54,61] One possible explanation for this is the fact that the information of BMI was analyzed from the study baseline onwards, and that changes in the BMI trajectory throughout life would be more explanatory. A greater weight gain from 20 to 40 years of age has been shown to be associated with later menopause and menopausal age might be mediated by weight changes over time,[62] although this finding has not been consistent among studies.[63]

Hepatitis C co-infection was significantly associated both with an earlier onset of natural menopause and with early menopause, and this is in agreement with data from women in general.[64] Amenorrhea is the most common menstrual disturbance in women with advanced liver disease. Alterations in the hormone metabolism and/or dysfunction of the hypothalamic-pituitary axis[65-67] may be the basis involved in the early onset of menopause in chronic

hepatitis C women but these hypotheses deserve further investigation. Postmenopausal hepatitis C infected women receiving hormone therapy have lower stage fibrosis, similar to premenopausal women.[68,69] The severity of fibrosis worsens in parallel with progressive estrogen deprivation and estradiol/testosterone ratio decrease.[70] Reproductive status was also shown to be an important predictor in the response to pegylated interferon/ribavirin antiviral therapy.[71] Altogether, these data reinforce the hypothesis of an anti-fibrogenic protective role of estrogens[72,73] and the importance of the clinical consequences of an earlier age at menopause in chronic hepatitis C women. The interaction between other chronic hepatitis diseases and early onset of menopause also merits further examination. In the present study we did not explore key hepatitis-related variables related to the severity of liver disease, such as inflammation, fibrosis, steatosis, viral genotype, hepatitis C viral load and treatment history. Other chronic diseases, such as type II diabetes[74] and hypothyroidism[75] have also been associated with earlier menopause, but we did not observe any such association in our study.

We found that severe immunosuppression with very low CD4 cell counts (less than 50 cells/mm^3) and diagnosis of an AIDS defining illness were associated with early onset of menopause. Several studies have shown that HIV related factors such as CD4 cell counts less than 200 cells/mm^3 [16,17,25] and CDC classification B/C[18] or C[25] were associated with an increased risk of earlier menopause.

This is the first study to report the association of less exposure to cART as a predictor of early onset of menopause. Treatment interruption periods during follow-up were not taken into account in this analysis, and therefore this association may be even stronger if we take into account cART adherence. It was still possible to observe the association between early onset of menopause and less exposure to cART even after 10 years of cART use, pointing out the importance of earlier initiation of treatment in women diagnosed with HIV-infection.

Our study had several strengths. Its longitudinal design with prospective measurements of the FMP from a large urban cohort of HIV-infected women reduced the chance of recall bias of the FMP, sometimes observed in retrospective studies as the reliability of the final estimate will be determined by the length of time elapsed since FMP. The inclusion cut off age of 30 years criteria allowed for the evaluation of women who developed the outcome of interest earlier without excluding them from the analyses, which could overestimate median AM. The exclusion of induced menopause as part of the primary outcome reinforces the internal validity of the study.

One of our main limitations is that no hormonal tests were performed to confirm menopausal status. To exclude this possibility, we checked that all women in our sample considered as postmenopausal continued without menses after the end of our study, with a median observation time of 2.5 (IQR: 1.3-5.4) years since FMP until the last follow–up gynecological visit (data not shown), thus confirming their menopausal status.

In conclusion, we found that early menarche, severe immunodeficiency, co-infection with hepatitis C and cigarette smoking were highly associated with early AM. In addition, less exposure to cART was associated with AM and early AM in a large cohort of HIV-infected women in a middle income country where universal access to cART is available for free. These results have significant clinical and public health implications as early onset of menopause has been associated with increased morbidity and mortality. HIV-infected postmenopausal women are an expanding group and a better understanding of aging in these women is of paramount importance for a more appropriate approach and management during this period of life.

REFERENCES

1. Programa Nacional de DST/AIDS. Boletim Epidemiológico de HIV/AIDS - ANO I nº01 - até semana epidemiológica 52ª -dezembro de 2012, Brasília, DF, 2012. Available at:

http://www.aids.gov.br/sites/default/files/anexos/publicacao/2012/52654/boletim_2012_final_pdf_14028.pdf. Accessed October 22, 2013.

2. Global Report: UNAIDS Report on the Global AIDS Epidemic 2012. United Nations Programme on HIV/AIDS (UNAIDS). Available at: http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2012/gr2012/20121120_UNAIDS_Global_Report_2012_en.pdf Accessed October 22, 2013.

3. Santos AFM, Assis M. Vulnerability of the elderly to HIV/AIDS: public politics and health professionals in the context of integral care: a literature review. *Rev Bras Geriatr Geront* 2011;14:147-57.

4. Valadares AL, Pinto-Neto AM, Abdo C, Melo VH. HIV in middle-aged women: associated factors. *Rev Assoc Med Bras* 2010;56(1):112-5.

5. Cejtin HE. Care of the human immunodeficiency virus-infected menopausal woman. *Am J Obstet Gynecol* 2012;207(2):87-93.

6. Research on the menopause in the 1990s. Report of a WHO Scientific Group. *World Health Organ Tech Rep Ser* 1996;866:1-107.

7. Shuster LT, Rhodes DJ, Gostout BS, Grossardt BR, Rocca WA. Premature menopause or early menopause: long-term health consequences. *Maturitas* 2010;65(2):161-6.

8. Thomas F, Renaud F, Benefice E, de Meeus T, Guegan JF. International variability of ages at menarche and menopause: patterns and main determinants. *Human biology* 2001;73(2):271-90.

9. Gold EB, Crawford SL, Avis NE, et al. Factors related to age at natural menopause: longitudinal analyses from SWAN. *Am J Epidemiol* 2013;178(1):70-83.

10. Dratva J, Gomez Real F, Schindler C, et al. Is age at menopause increasing across Europe? Results on age at menopause and determinants from two population-based studies. *Menopause* 2009;16(2):385-94.

11. Yasui T, Hayashi K, Mizunuma H, et al. Factors associated with premature ovarian failure, early menopause and earlier onset of menopause in Japanese women. *Maturitas* 2012;72(3):249-55.

12. Sidibe EH. Menopause in Africa. *Annales d'endocrinologie* 2005;66(2 Pt 1):105-7.

13. Castelo-Branco C, Blumel JE, Chedraui P, et al. Age at menopause in Latin America. *Menopause* 2006;13(4):706-12.

14. Palacios S, Henderson VW, Siseles N, Tan D, Villaseca P. Age of menopause and impact of climacteric symptoms by geographical region. *Climacteric* 2010;13(5):419-28.

15. Pedro AO, Pinto Neto AM, Paiva LH, Osis MJ, Hardy E. Age at natural menopause among Brazilian women: results from a population-based survey. *Cad Saude Publica* 2003;19(1):17-25.

16. Schoenbaum EE, Hartel D, Lo Y, et al. HIV infection, drug use, and onset of natural menopause. *Clin Infect Dis* 2005;41(10):1517-24.

17. de Pommerol M, Hessamfar M, Lawson-Ayayi S, et al. Menopause and HIV infection: age at onset and associated factors, ANRS CO3 Aquitaine cohort. *Int J STD AIDS* 2011;22(2):67-72.

18. Boonyanurak P, Bunupuradah T, Wilawan K, et al. Age at menopause and menopause-related symptoms in human immunodeficiency virus-infected Thai women. *Menopause* 2012;19(7):820-4.

19. Clark RA, Cohn SE, Jarek C, et al. Perimenopausal symptomatology among HIV-infected women at least 40 years of age. *J Acquir Immune Defic Syndr* 2000;23(1):99-100.

20. Ferreira CE, Pinto-Neto AM, Conde DM, Costa-Paiva L, Morais SS, Magalhaes J. Menopause symptoms in women infected with HIV: prevalence and associated factors. *Gynecol Endocrinol* 2007;23(4):198-205.

21. Henderson KD, Bernstein L, Henderson B, Kolonel L, Pike MC. Predictors of the timing of natural menopause in the Multiethnic Cohort Study. *Am J Epidemiol* 2008;167(11):1287-94.

22. Gold EB. The timing of the age at which natural menopause occurs. *Obstet Gynecol Clin North Am* 2011;38(3):425-40.

23. Mishra GD, Cooper R, Tom SE, Kuh D. Early life circumstances and their impact on menarche and menopause. *Womens Health (Lond Engl)* 2009;5(2):175-90.

24. Kanapathipillai R, Hickey M, Giles M. Human immunodeficiency virus and menopause. *Menopause* 2013;20(9):983-90.

25. Cicconi P, Ammassari A, Ladisa N, et al. Prevalence of prolonged amenorrhea in HIV-infected women: results from the Italian DIDI study. *J Acquir Immune Defic Syndr* 2012;61(2):e19-21.

26. Ramezani Tehrani F, Behboudi-Gandevani S, Ghanbarian A, Azizi F. Effect of menopause on cardiovascular disease and its risk factors: a 9-year follow-up study. *Climacteric* 2014;17(2):164-72.

27. Lee JS, Hayashi K, Mishra G, Yasui T, Kubota T, Mizunuma H. Independent association between age at natural menopause and hypercholesterolemia, hypertension, and diabetes mellitus: Japan nurses' health study. *J Atheroscler Thromb* 2013;20(2):161-9.

28. Wellons M, Ouyang P, Schreiner PJ, Herrington DM, Vaidya D. Early menopause predicts future coronary heart disease and stroke: the Multi-Ethnic Study of Atherosclerosis. *Menopause* 2012;19(10):1081-7.

29. Archer DF. Premature menopause increases cardiovascular risk. *Climacteric* 2009;12 Suppl 1:26-31.

30. Fukuda K, Takashima Y, Hashimoto M, Uchino A, Yuzuriha T, Yao H. Early Menopause and the Risk of Silent Brain Infarction in Community-Dwelling Elderly Subjects:

The Sefuri Brain MRI Study. *J Stroke Cerebrovasc Dis*. 2013. doi: 10.1016/j.jstrokecerebrovasdis.2013.07.005. [Epub ahead of print]

31. Rocca WA, Grossardt BR, Miller VM, Shuster LT, Brown RD, Jr. Premature menopause or early menopause and risk of ischemic stroke. *Menopause* 2012;19(3):272-7.

32. Nedergaard A, Henriksen K, Karsdal MA, Christiansen C. Menopause, estrogens and frailty. *Gynecol Endocrinol* 2013;29(5):418-23.

33. Svejme O, Ahlborg HG, Nilsson JA, Karlsson MK. Early menopause and risk of osteoporosis, fracture and mortality: a 34-year prospective observational study in 390 women. *BJOG: an international journal of obstetrics and gynaecology* 2012;119(7):810-6.

34. Cauley JA, Danielson ME, Greendale GA, et al. Bone resorption and fracture across the menopausal transition: the Study of Women's Health Across the Nation. *Menopause* 2012;19(11):1200-7.

35. Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. *J Clin Endocrinol Metab* 2007;92(7):2506-12.

36. Yin MT, Zhang CA, McMahon DJ, et al. Higher rates of bone loss in postmenopausal HIV-infected women: a longitudinal study. *J Clin Endocrinol Metab* 2012;97(2):554-62.

37. Maki PM, Martin-Thormeyer E. HIV, cognition and women. *Neuropsychol Rev* 2009;19(2):204-14.

38. Yin MT, Shi Q, Hoover DR, et al. Fracture incidence in HIV-infected women: results from the Women's Interagency HIV Study. *AIDS* 2010;24(17):2679-86.

39. Grinsztejn B, Bastos FI, Veloso VG, et al. Assessing sexually transmitted infections in a cohort of women living with HIV/AIDS, in Rio de Janeiro, Brazil. *Int J STD AIDS* 2006;17(7):473-8.

40. Friedman RK, Bastos FI, Leite IC, et al. Pregnancy rates and predictors in women with HIV/AIDS in Rio de Janeiro, Southeastern Brazil. *Rev Saude Publica* 2011;45(2):373-81.

41. Cardoso SW, Grinsztejn B, Velasque L, et al. Incidence of modifying or discontinuing first HAART regimen and its determinants in a cohort of HIV-infected patients from Rio de Janeiro, Brazil. *AIDS Res Hum Retroviruses* 2010;26(8):865-74.

42. Moreira RI, Luz PM, Struchiner CJ, et al. Immune status at presentation for HIV clinical care in Rio de Janeiro and Baltimore. *J Acquir Immune Defic Syndr* 2011;57 Suppl 3:S171-8.

43. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. *World Health Organ Tech Rep Ser* 1995;854:1-452.

44. WHO. Global database on body mass index. Available: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html. Accessed October 1, 2013.

45. Centers for Diseases Control and Prevention. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR Recomm Rep* 1992;41(RR-17):1-19.

46. Greenland S. Modeling and variable selection in epidemiologic analysis. *Am J Public Health* 1989;79(3):340-9.

47. Allison PD, ed. Survival analysis using SAS: a practical guide. Cary, North Carolina, USA: SAS Institute, Inc., 1995.

48. Fantry LE, Zhan M, Taylor GH, Sill AM, Flaws JA. Age of menopause and menopausal symptoms in HIV-infected women. *AIDS Patient Care STDS* 2005;19(11):703-11.

49. Parazzini F, Progetto Menopausa Italia Study G. Determinants of age at menopause in women attending menopause clinics in Italy. *Maturitas* 2007;56(3):280-7.

50. Kaczmarek M. The timing of natural menopause in Poland and associated factors. *Maturitas* 2007;57(2):139-53.

51. Gonzales GF, Villena A, De La Cruz D. Age of natural menopause among women in Lima City, Peru. *Int J Gynaecol Obstet* 1997;57(1):69-72.

52. Sun L, Tan L, Yang F, et al. Meta-analysis suggests that smoking is associated with an increased risk of early natural menopause. *Menopause* 2012;19(2):126-32.

53. Parente RC, Faerstein E, Celeste RK, Werneck GL. The relationship between smoking and age at the menopause: A systematic review. *Maturitas* 2008;61(4):287-98.

54. Gold EB, Bromberger J, Crawford S, et al. Factors associated with age at natural menopause in a multiethnic sample of midlife women. *Am J Epidemiol* 2001;153(9):865-74.

55. Aldrighi JM, Alecrin IN, de Oliveira PR, Shinomata HO. Smoking and earlier menopause. *Rev Assoc Med Bras* 2005;51(1):51-3.

56. McLean BK, Rubel A, Nikitovitch-Winer MB. The differential effects of exposure to tobacco smoke on the secretion of luteinizing hormone and prolactin in the proestrous rat. *Endocrinology* 1977;100(6):1566-70.

57. Michnovicz JJ, Hershcopf RJ, Naganuma H, Bradlow HL, Fishman J. Increased 2-hydroxylation of estradiol as a possible mechanism for the anti-estrogenic effect of cigarette smoking. *N Engl J Med* 1986;315(21):1305-9.

58. Tziomalos K, Charsoulis F. Endocrine effects of tobacco smoking. *Clinical endocrinology* 2004;61(6):664-74.

59. Kinney A, Kline J, Levin B. Alcohol, caffeine and smoking in relation to age at menopause. *Maturitas* 2006;54(1):27-38.

60. van Asselt KM, Kok HS, van Der Schouw YT, et al. Current smoking at menopause rather than duration determines the onset of natural menopause. *Epidemiology* 2004;15(5):634-9.

61. Bromberger JT, Matthews KA, Kuller LH, Wing RR, Meilahn EN, Plantinga P. Prospective study of the determinants of age at menopause. *Am J Epidemiol* 1997;145(2):124-33.

62. Morris DH, Jones ME, Schoemaker MJ, McFadden E, Ashworth A, Swerdlow AJ. Body mass index, exercise, and other lifestyle factors in relation to age at natural menopause: analyses from the breakthrough generations study. *Am J Epidemiol* 2012;175(10):998-1005.

63. Hardy R, Mishra GD, Kuh D. Body mass index trajectories and age at menopause in a British birth cohort. *Maturitas* 2008;59(4):304-14.

64. Cieloszyk K, Hartel D, Moskaleva G, Schoenbaum EE. Effects of hepatitis C virus infection on menopause status and symptoms. *Menopause* 2009;16(2):401-6.

65. Jabiry-Zieniewicz Z, Kaminski P, Bobrowska K, et al. Menstrual function in female liver transplant recipients of reproductive age. *Transplantation proceedings* 2009;41(5):1735-9.

66. Valimaki M, Pelkonen R, Salaspuro M, Harkonen M, Hirvonen E, Ylikahri R. Sex hormones in amenorrheic women with alcoholic liver disease. *J Clin Endocrinol Metab* 1984;59(1):133-8.

67. Burra P. Liver abnormalities and endocrine diseases. *Best practice & research Clinical gastroenterology* 2013;27(4):553-63.

68. Codes L, Asselah T, Cazals-Hatem D, et al. Liver fibrosis in women with chronic hepatitis C: evidence for the negative role of the menopause and steatosis and the potential benefit of hormone replacement therapy. *Gut* 2007;56(3):390-5.

69. Di Martino V, Lebray P, Myers RP, et al. Progression of liver fibrosis in women infected with hepatitis C: long-term benefit of estrogen exposure. *Hepatology* 2004;40(6):1426-33.

70. Villa E, Vukotic R, Camma C, et al. Reproductive status is associated with the severity of fibrosis in women with hepatitis C. *PLoS One* 2012;7(9):e44624.

71. Villa E, Karampatou A, Camma C, et al. Early menopause is associated with lack of response to antiviral therapy in women with chronic hepatitis C. *Gastroenterology* 2011;140(3):818-29.

72. Shimizu I, Ito S. Protection of estrogens against the progression of chronic liver disease. *Hepatology research : the official journal of the Japan Society of Hepatology* 2007;37(4):239-47.

73. Yang JD, Abdelmalek MF, Pang H, et al. Gender and menopause impact severity of fibrosis among patients with nonalcoholic steatohepatitis. *Hepatology* 2014;59(4):1406-14.

74. Monterrosa-Castro A, Blumel JE, Portela-Buelvas K, et al. Type II diabetes mellitus and menopause: a multinational study. *Climacteric* 2013;16(6):663-72.

75. Kim TJ, Anasti JN, Flack MR, Kimzey LM, Defensor RA, Nelson LM. Routine endocrine screening for patients with karyotypically normal spontaneous premature ovarian failure. *Obstet Gynecol* 1997;89(5 Pt 1):777-9.

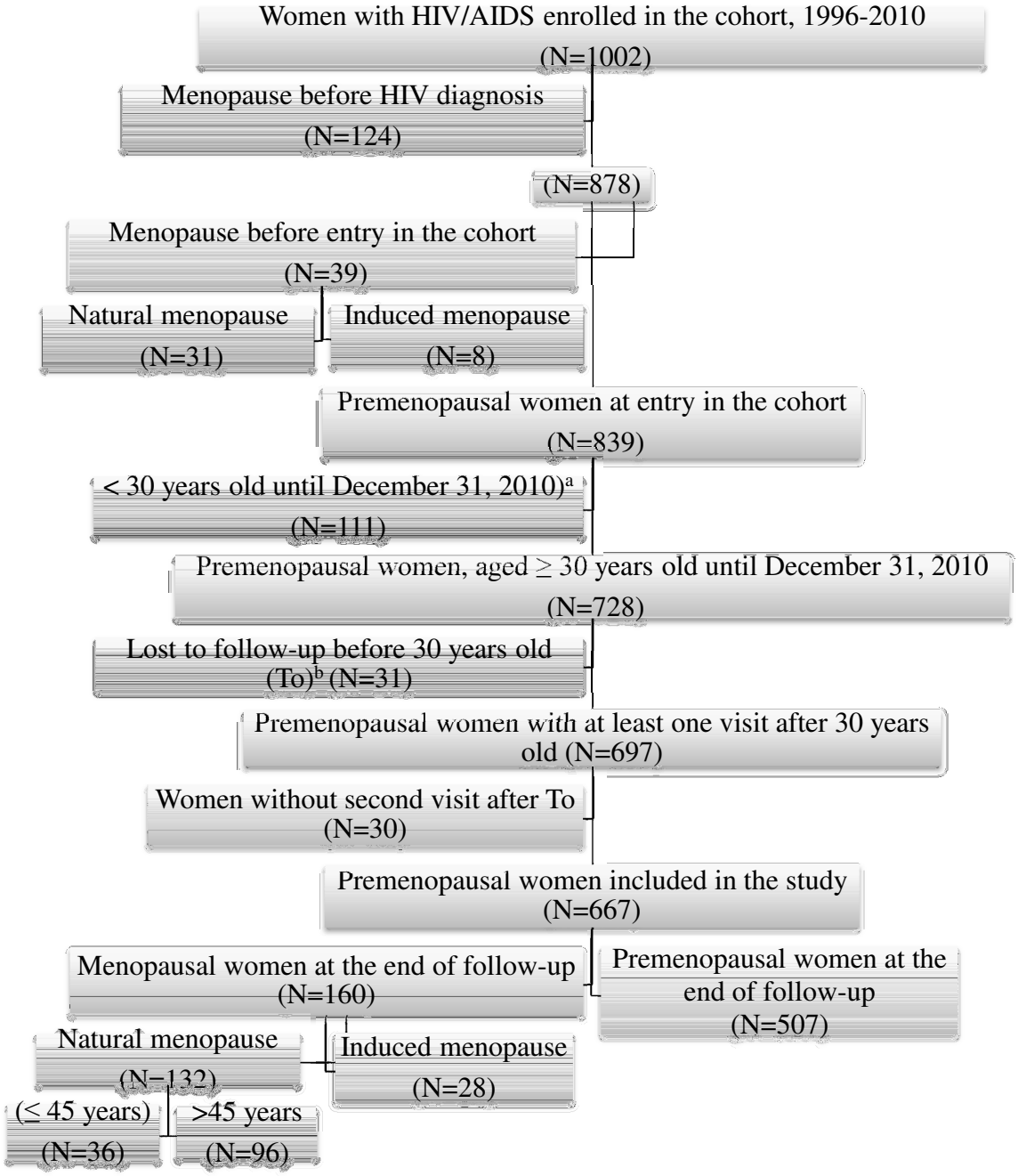

Figure 1: Study profile at Evandro Chagas Clinical Research Institute, Rio de Janeiro, 1996-2011. [a]Women were included if they were 30 years of age or more at cohort entry or if they turned 30 years of age during follow-up until December 31, 2010; [b] To=Initial observation period.

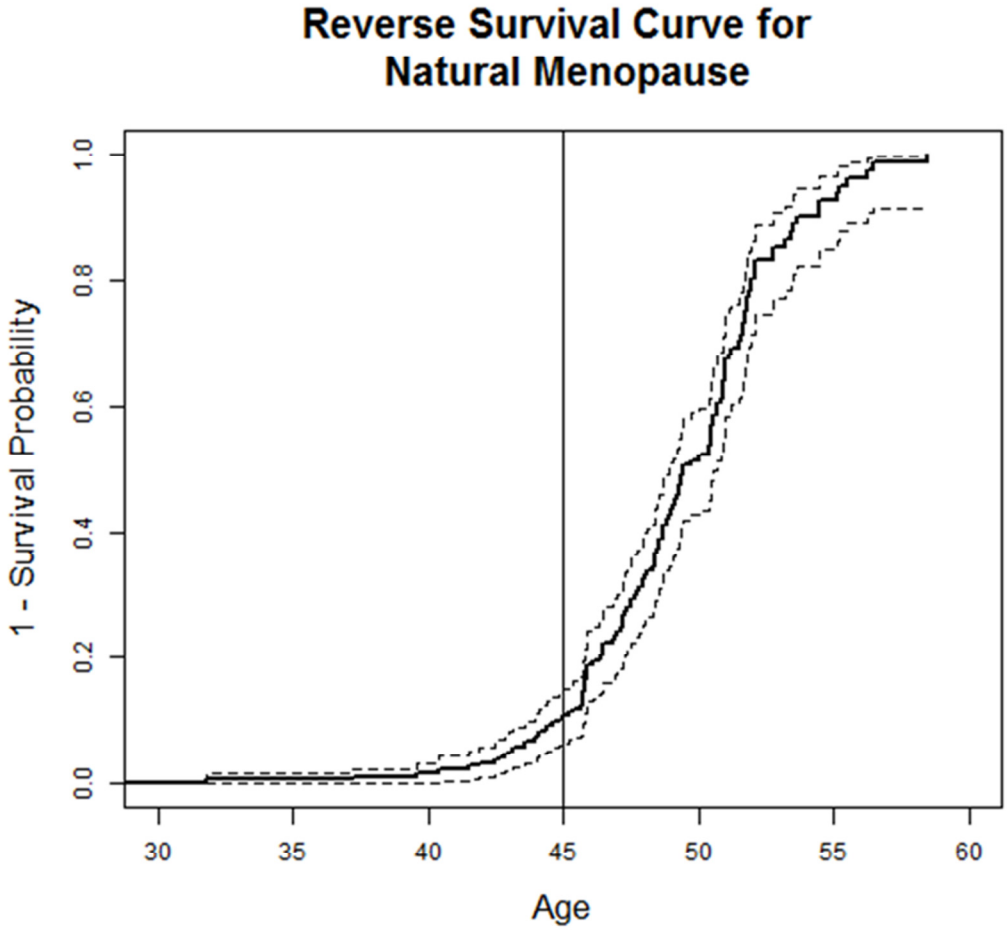

Figure 2: Kaplan-Meier plot of age of natural menopause (solid line) with upper – lower interquartile range (dashed line), Evandro Chagas Clinical Research Institute, Rio de Janeiro, 1996-2011.

TABLE 1. C*haracteristics of 667 participants followed at the Evandro Chagas Clinical Research Institute, Rio de Janeiro, 1996-2011.*

Characteristics	Total (n = 667)	Natural menopausal women (n=132)	Early natural menopausal women (n=36)
Age, years at baseline	34.9 (30.9-40.5)		
Race/ethnicity			
White	264 (39.6)	66 (50.0)	20 (55.6)
Non-white	403 (60.4)	66 (50.0)	16 (44.4)
Schooling, years			
>11	72 (10.8)	11 (8.3)	2 (5.6)
>8-11	220 (33.0)	44 (33.3)	13(36.1)
≤8	375 (56.2)	77 (58.3)	21(58.3)
Monthly family income [a]	560 (300-1,000)		
>5	127 (19.0)	35 (26.5)	9 (25.0)
2-5	215 (32.2)	43 (32.6)	11 (30.6)
0-2	324 (48.6)	54 (40.9)	16 (44.4)
Missing	1 (0.2)	-	-
Age at menarche, years	13 (12-14)		
≥11	600 (90.0)	113 (85.6)	27 (75.0)
<11	66 (9.9)	19 (14.4)	9 (25.0)
Missing	1 (0.1)	-	-
Parity [b]	2 (1-3)		
0	91 (13.6)	12 (9.1)	3 (8.3)
1	170 (25.5)	32 (24.2)	7 (19.4)
2	200 (30.0)	45 (34.1)	20 (55.6)
≥3	206 (30.9)	43 (32.6)	6 (16.7)
Ever exogenous hormone use [b]			
Yes	369 (55.3)	32 (24.2)	11 (30.6)
No	298 (44.7)	100 (75.8)	25 (69.4)
Alcohol use			
No	415 (62.2)	87 (65.9)	18 (50.0)
1-2 drinks	75 (11.2)	9 (6.8)	4 (11.1)
3-4 drinks	64 (9.6)	11 (8.3)	3 (8.3)
≥5 drinks	113 (16.9)	25 (18.9)	11 (30.6)
Cigarette smoking during follow-up [c]			
No	483 (72.41)	83 (62.9)	17 (47.2)
Yes	173 (25.94)	49 (37.1)	19 (52.8)
Missing	11 (1.65)	-	-
Packs-years	9.5 (2.7-23.2)		
Never Smoked	353 (52.9)	51 (38.6)	11 (30.6)
<10	151 (22.6)	30 (22.7)	10 (27.8)

10-19	58 (8.7)	16 (12.1)	3 (8.3)
≥20	90 (13.5)	34 (25.8)	12 (33.3)
Missing	15 (2.2)	1 (0.8)	-
Lifetime illicit drug use			
No	550 (82.5)	111 (84.1)	27 (75.0)
Yes	117 (17.5)	21 (15.9)	9 (25.0)
Lifetime cocaine use [d]			
No	579 (86.8)	118 (89.4)	29 (80.6)
Yes	88 (13.2)	14 (10.6)	7 (19.4)
Body Mass Index (Kg/m^2) [c, e]			
Normal weight	324 (48.6)	74 (56.1)	21(58.3)
Overweight/obese	247 (37.0)	46 (34.8)	10 (27.8)
Underweight	51 (7.6)	9 (6.8)	4 (11.1)
Missing	45 (6.8)	3 (2.3)	1 (2.8)
Type II Diabetes			
No	589 (88.3)	108 (81.8)	29 (80.6)
Yes	78 (11.7)	24 (18.2)	7 (19.4)
Chronic hepatitis C [c]			
No	633 (94.9)	116 (87.9)	31 (86.1)
Yes	34 (5.1)	16 (12.1)	5 (13.9)
Hypothyroidism [c]			
No	654 (98.1)	129 (97.7)	35 (97.2)
Yes	13 (1.9)	3 (2.3)	1 (2.8)
CD4 count nadir ($cells/mm^3$)	182 (74-278)		
≥350	94 (14.1)	12 (9.1)	3 (8.3)
200-349	213 (31.9)	34 (25.8)	7 (19.4)
100-199	155 (23.2)	28 (21.2)	10 (27.9)
50-99	81 (12.1)	23 (17.4)	4 (11.1)
<50	123 (18.4)	35 (26.5)	12 (33.3)
Missing	1 (0.1)	-	-
CD4 count ($cells/mm^3$) [c, e]			
≥350	334 (50.1)	52 (39.4)	12 (33.3)
200-349	153 (22.9)	31 (23.5)	11 (30.6)
100-199	81 (12.1)	24 (18.2)	7 (19.4)
50-99	33 (5.0)	12 (9.1)	3 (8.3)
<50	28 (4.2)	8 (6.0)	2 (5.6)
Missing	38 (5.7)	5 (3.8)	1 (2.8)
AIDS defining illness			
No	373 (55.9)	56 (42.4)	12 (33.3)
Yes	294 (44.1)	76 (57.6)	24 (66.7)
cART exposure			
No	77 (11.5)	14 (10.6)	3 (8.3)
Yes	590 (88.5)	118 (89.4)	33 (91.7)

Time under cART, years	4.9 (2.4-9)		
≥10	137 (20.5)	19 (14.4)	2 (5.6)
<10	530 (79.5)	113 (85.6)	34 (94.4)

Data are presented as n (%) and median (interquartile interval).[a] In Brazilian minimum wages; [b] Ever oral contraceptive and/or other exogenous hormone use; [c] Frequencies presented for baseline but is a time-dependent covariate; [d] intravenous or snorted; [e] Baseline body mass index and CD4 cell counts were defined as the values obtained within six months (before or after) from enrollment; IQR - Interquartile interval; cART- Combination antiretroviral therapy.

TABLE 2. *Unadjusted and adjusted hazard ratios for age at natural menopause from Cox proportional hazards modeling,1996–2011 (n=667)*

Characteristics	Unadjusted analysis			Adjusted analysis		
	Hazard Ratio	95% CI	p-value	Hazard Ratio	95% CI	p-value
Race/ethnicity						
White	1					
Non-white	0.71	0.50-1.01	**0.053**			
Schooling, years						
>11	1					
>8-11	1.21	0.62-2.35	0.578			
≤8	1.34	0.71-2.52	0.373			
Monthly family income [a, b]						
>5	1					
2-5	1.34	0.85-2.10	0.205			
0-2	1.09	0.71-1.68	0.684			
Age at menarche, years [b]						
≥11	1			1		
<11	1.98	1.20-3.24	**0.007**	1.79	1.08-2.98	**0.025**
Parity [b]						
0	1			1		
1	0.57	0.29-1.15	0.117	0.57	0.28-1.19	0.134
2	0.73	0.37-1.44	0.370	0.70	0.35-1.39	0.311
≥3	0.52	0.26-1.02	**0.056**	0.51	0.26-1.03	**0.059**
Ever exogenous hormone use [c]						
Yes	1					
No	1.41	0.94-2.12	**0.102**			
Alcohol use						
No	1					

1-2 drinks	0.73	0.37-1.46	0.377			
3-4 drinks	0.97	0.52-1.83	0.931			
≥5 drinks	0.81	0.52-1.27	0.355			
Cigarette smoking during follow-up [b, d]						
No	1			1		
Yes	1.62	1.12-2.36	**0.011**	1.42	0.96-2.10	0.081
Lifetime cocaine use [e]						
No	1					
Yes	0.77	0.44-1.34	0.348			
Body Mass Index (Kg/m^2) [b, d]						
Normal weight	1			1		
Overweight/obese	0.68	0.48-0.97	**0.034**	0.79	0.55-1.15	0.219
Underweight	0.73	0.26-2.00	0.538	0.61	0.22-1.69	0.336
Type II Diabetes						
No	1					
Yes	0.80	0.50-1.27	0.339			
Chronic hepatitis C [b]						
No	1			1		
Yes	2.02	1.06-3.88	**0.034**	2.77	1.39-5.50	**0.004**
Hypothyroidism [b]						
No	1					
Yes	0.37	0.05-2.80	0.336			
CD4 count (cells/mm^3) [b, d]						
≥350	1			1		
200-349	1.07	0.67-1.72	0.766	0.94	0.58-1.52	0.797
100-199	1.07	0.48-2.36	0.873	1.00	0.44-2.27	0.997
50-99	1.06	0.34-3.37	0.918	1.31	0.39-4.35	0.661

<50	3.47	1.24-9.71	**0.018**	3.41	1.17-9.94	**0.024**
AIDS defining illness						
No	1			1		
Yes	1.51	1.07-2.15	**0.020**	1.68	1.15-2.45	**0.007**
cART exposure						
No	1					
Yes	0.74	0.42-1.30	0.291			
Time under cART, years						
≥10	1			1		
<10	2.57	1.58-4.18	**<0.001**	2.97	1.76-5.01	**<0.001**

[a] In Brazilian minimum wages; [b] Missing data - monthly family income: n=1; menarche: n=1; cigarette smoking: n=11; Body mass index: n=18; CD4 cell count: n=1. [c] Ever oral contraceptive and/or other exogenous hormone use; [d] Time-dependent variable measured during follow-up; [e] intravenous or snorted; cART- Combination antiretroviral therapy; CI - Confidence interval.

TABLE 3. *Unadjusted and adjusted hazard ratios for early (≤45 years) natural menopause from Cox proportional hazards modeling, 1996–2011 (n=608)*

Characteristics	Unadjusted analysis			Adjusted analysis		
	Hazard Ratio	95% CI	p-value	Hazard Ratio	95% CI	p-value
Race/ethnicity						
White	1					
Non-white	0.59	0.30-1.13	**0.113**			
Schooling, years						
>11	1					
>8-11	2.57	0.58-11.4	0.214			
≤8	2.55	0.60-10.87	0.207			
Monthly family income [a, b]						
>5	1					
2-5	1.03	0.43-2.49	0.943			
0-2	1.17	0.52-2.65	0.707			
Age at menarche, years [b]						
≥11	1			1		
<11	3.07	1.44-6.54	**0.004**	2.31	1.04-5.17	**0.041**
Parity [b]						
0	1					
1	0.90	0.23-3.50	0.883			
2	2.06	0.61-7.01	0.247			
≥3	0.93	0.25-3.53	0.920			
Ever exogenous hormone use [c]						
Yes	1					
No	1.80	0.89-3.67	**0.104**			
Alcohol use						

No	1			1		
1-2 drinks	1.33	0.45-3.92	0.611	1.27	0.40-4.04	0.691
3-4 drinks	1.11	0.33-3.76	0.873	0.75	0.19-2.94	0.674
≥5 drinks	2.13	1.01-4.52	**0.048**	1.43	0.60-3.40	0.418
Cigarette smoking [b, d]						
No	1			1		
Yes	4.08	2.11-7.89	**<0.001**	2.78	1.29-5.99	**0.009**
Lifetime cocaine use [e]						
No	1					
Yes	1.91	0.84-4.36	**0.125**			
Body Mass Index (Kg/m^2) [b, d]						
Normal weight	1			1		
Overweight/obese	0.45	0.23-0.91	**0.027**	0.70	0.33-1.50	0.358
Underweight	0.49	0.07-3.63	0.485	0.26	0.033-2.08	0.205
Type II Diabetes						
No	1					
Yes	1.08	0.47-2.47	0.853			
Chronic hepatitis C [b]						
No	1			1		
Yes	5.39	2.09-13.89	**<0.001**	4.12	1.37-12.34	**0.011**
CD4 count ($cells/mm^3$) [b, d]						
≥350	1			1		
200-349	1.14	0.46-2.85	0.773	1.03	0.40-2.67	0.954
100-199	1.99	0.68-5.83	0.212	1.39	0.43-4.56	0.583
50-99	2.60	0.61-11.15	0.199	1.47	0.28-7.88	0.652
<50	8.24	2.77-24.48	**<0.001**	5.30	1.40-20.04	**0.014**
AIDS defining illness						
No	1			1		

Yes	2.38	1.19-4.75	**0.014**	2.02	0.89-4.59	0.092
cART exposure						
No	1					
Yes	0.86	0.26-2.79	0.795			
Time under cART, years						
≥10	1			1		
<10	8.15	1.96-33.94	**0.004**	8.58	2.01-36.71	**0.004**

[a] In Brazilian minimum wages; [b] Missing data - monthly family income: n=1; menarche: n=1; cigarette smoking: n=11; Body mass index: n=18; CD4 cell count: n=1. [c] Ever oral contraceptive and/or other exogenous hormone use; [d] Time-dependent variable measured during follow-up; [e] intravenous or snorted; cART- Combination antiretroviral therapy; CI - Confidence interval.

6.2 ARTIGO 2

6.2.1 Carta de submissão para publicação do artigo 2

PLOS ONE: A manuscript number has been assigned to Menopause... file:///C:/Users/Guilherme Calvet/Dropbox/Tese doutorado HIV...

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Dear Dr Calvet,

On Sep 29 2013 04:24PM, we received your Research Article entitled "Menopause and first-line antiretroviral therapy effectiveness in HIV-infected women in Brazil" by Guilherme Amaral Calvet, MD, MSc; Luciane Velasque; Paula Mendes Luz; Sandra Wagner Cardoso; Monica Derrico; Ronaldo Ismério Moreira; Angela Vasconcelos Andrade; Andrea Cytryn; Elaine Pires; Valdiléa Gonçalves Veloso; Beatriz Grinsztejn; Ruth Khalili Friedman.

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6.2.2 Artigo 2 aceito para publicação na revista PLOS ONE em 20/01/2014.

TITLE: Absence of Effect of Menopause Status at Initiation of First-Line Antiretroviral Therapy on Immunologic or Virologic Responses: A Cohort Study from Rio de Janeiro, Brazil

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PRESENTED at the XIX World AIDS Conference; Washington, DC; July 22 - 27, 2012 [abstract THPE040]

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ABSTRACT

Objective: To compare the effectiveness of first-line combination antiretroviral therapy (cART) between premenopausal and postmenopausal women.

Methods: ART-naïve women initiating cART between January 2000/June 2010 at the Instituto de Pesquisa Clínica Evandro Chagas Cohort were studied. Women were defined as postmenopausal after 12 consecutive months of amenorrhea. CD4 cell counts and HIV-1 RNA viral load (VL) measurements were compared between pre- and postmenopausal at 6, 12 and 24 months after cART initiation. Women who modified/discontinued a drug class or died due to an AIDS defining illness were classified as ART-failures. Variables were compared using Wilcoxon test, $\chi 2$ or Fisher's exact test. The odds of cART effectiveness (VL<400 copies/mL and/or no need to change cART) were compared using logistic regression. Linear model was used to access relationship between CD4 change and menopause.

Results: Among 383 women, 328 (85%) were premenopausal and 55 (15%) postmenopausal. Median pre cART CD4 counts were 231 and 208 cells/mm^3 ($p = 0.14$) in pre- and postmenopausal women, respectively. No difference in the median pre cART VL was found (both 4.8 copies/mL). Median CD4 changes were similar at 6 and 12 months. At 24 months after cART initiation, CD4 changes among postmenopausal women were significantly lower among premenopausal women ($p = 0.01$). When the analysis was restricted to women with VL<400 copies/mL, no statistical difference was observed. Overall, 63.7% achieved cART effectiveness at 24 months without differences between groups at 6, 12 and 24 months.

Conclusion: Menopause status at the time of first-line cART initiation does not impact CD4 cell changes at 24 months among women with a virologic response. No relationship between menopause status and virologic response was observed.

Introduction

Women account for 50% of people living with HIV, the majority of them living in low and middle-income countries [1]. Worldwide, life expectancy has been increasing over the last several decades, even in developing countries, leading to a greater number of individuals older than 60 years. Brazil has one of the fastest aging populations in the world. In a half of a century (1960–2010), life expectancy of the Brazilian population increased by 25.4 years, having changed from 48.0 to 73.4 years [2].

The expansion of combination antiretroviral therapy (cART) coverage was crucial to reduce HIV-related morbidity and mortality rates turning HIV infection into a chronic condition. Antiretroviral therapy (ART) global coverage has significantly grown in the latest years, with 11.7 million life-years added to the world between 1996 and 2008 [3]. Consequently, the HIV/AIDS population is becoming older.

The number of older women who will become HIV-infected or who will live with HIV is expected to increase as overall life expectancy increases, and many of them will undergo menopause during the course of the HIV disease [4]. Prior to receiving antiretroviral therapy, younger HIV-infected women have higher CD4 cell counts and lower HIV RNA levels [5]–[7] when compared with HIV-infected men.

Recent studies have shown that aging has very specific effects on T cell function [8]. Natural sex steroids can mediate changes in the immune system and estrogens can regulate humoral and cellular immune responses with a decrease in CD4+ T and B lymphocytes subpopulations in postmenopausal women [9]–[11].

Several studies have evaluated the influence of gender and age on cART outcomes [12]–[15]. cART effectiveness in women may be different according to menopausal status, and previous studies have shown conflicting results [16], [17].

Data on the cART outcomes in HIV-infected postmenopausal women remain scarce, especially in low- and middle-income settings, including Brazil, where universal access to cART free of cost has been provided by the Ministry of Heath since 1997. The purpose of this study was to compare the effectiveness of first-line cART among HIV-infected pre- and postmenopausal women in a cohort of HIV-infected women in Rio de Janeiro, Brazil.

Methods

Ethical Statement

The study protocol was reviewed and approved by the ethics committee of Evandro Chagas Clinical Research Institute, Oswaldo Cruz Foundation (CAE 0032.0.009.000-10). Written informed consent was obtained from all women.

Description of the Cohort and Study Population

This study was conducted at the Instituto de Pesquisa Clínica Evandro Chagas (IPEC) AIDS Service at Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro, Brazil, where care has been provided to HIV/AIDS patients since 1986. An observational, longitudinal, clinical database is maintained on patients receiving primary and specialized HIV care at the clinic. Details of the HIV/AIDS cohort can be found elsewhere [18], [19]. To study the natural history of HIV infection in women, a prospective open cohort was established at IPEC in 1996. Study visits occur every 6 months; sociodemographic, behavior, reproductive, gynecologic and laboratory data are collected using structured questionnaires [20], [21].

For this study, data from 386 antiretroviral-naïve women who initiated cART between January 1, 2000 and June 30, 2010 were considered. Women without gynecological data defining menopause status were excluded (N = 3). Thus, we analyzed data from 383 cART-naïve patients who initiated cART within the study period. Follow up information includes data up to September 30, 2011.

Study Definitions

Menopausal status was prospectively captured during bi-anual interviews. Women were defined as postmenopausal after twelve consecutive months of amenorrhea, for which there was no other obvious pathological or physiological cause [22]. Women with a history of hysterectomy were only considered postmenopausal if they had undergone bilateral oophorectomy regardless of age. All other women were classified as premenopausal.

Race was based on provider report and categorized as white and nonwhite.

cART was defined as two nucleosides transcriptase reverse inhibitors (NTRI) in combination with one non nucleoside transcriptase reverse inhibitor (NNRTI) or one protease inhibitor (PI); antiretroviral regimens were classified as NNRTI or PI-based. The calendar year of cART initiation was stratified into two groups: 2000–04 and 2005–09.

Drug class modifications and discontinuation were defined as any NNRTI or PI drug modification or interruption (including deaths due to AIDS defining illnesses). Neither NTRI substitutions nor regimen dosage adjustments were considered regimen modifications.

The effectiveness of first-line cART at 6, 12 and 24 months was defined as an HIV-1 RNA viral load (VL) measurement of less than 400 copies/mL at these time points without drug class modification. Window periods were defined for each time point as 5–9 months, 9–15 months, and 21–27 months, respectively. Within each window, the viral load and CD4 cell counts (cells/mm^3) measurement recorded closest to the time points were evaluated.

Baseline CD4+ lymphocyte counts was defined as the value obtained within 90 days of cART initiation (before or after) and baseline HIV-1 RNA as any available value obtained before or up to 7 days after cART initiation. CD4+ cell counts and HIV-1 RNA results were obtained from the medical records. CD4+ cell counts were categorized into <200, 200–500 and >500 cells/mm^3. Plasma HIV VL was categorized into 401–10,000, 10,001–100.000 and >100,000 copies/mL.

CD4+ T-cell counts samples were evaluated on a BD FACSCalibur cytometer (Becton Dickinson, USA). The assays used for measurement of HIV-1 viral load were nucleic acid sequence–based amplification (NASBA, Organon Teknika, Boxtel, The Netherlands), Roche Amplicor reverse transcriptase polymerase chain reaction (RT-PCR) assay (Roche Molecular Diagnostics, USA) and branched DNA assay, Versant HIV-1 RNA 3.0 (Siemens, Tarrytown, USA). The assays used for measurement of HIV-1 viral load varied according to the year and whether or not the woman was enrolled in a clinical trial. For the purpose of statistical analysis, the cut-off value, defined as undetectable VL, was set as a VL ≤400 copies/mL, regardless of the method used.

AIDS defining illnesses (ADI) was defined as the presence of any 1993 Centers for Disease Control and Prevention (CDC)-defined ADI [23] at 90 days prior up to 30 days after cART initiation baseline.

Participation in a clinical trial was defined when a patient started their first-line regimen in a cART-naïve clinical trial.

Statistical Analysis

Quantitative variables between pre- and postmenopausal women were compared using Wilcoxon test. Categorical variables were compared using chi-squared test or Fisher's exact test. Median changes in CD4 cell counts and HIV-1 RNA VL measurements were compared between pre- and postmenopausal women at 6, 12 and 24 months after cART initiation.

We calculated the odds ratio for effective cART and the 95% confidence interval (95%CI) of first-line cART at 6, 12 and 24 months after treatment initiation, adjusting for baseline log 10 VL, baseline CD4, cART regimen and ADI, using logistic regression model.

The impact of missing VL measurement on the effectiveness of first-line cART was evaluated in a sensitivity analyses. We compared the estimates and confidence intervals of three different models. 1) Model A: when individuals with missing VL measurements were

excluded; 2) Model B: when the missing VL measurements were coded as 1 (missing values assumed to be failure yielding a worse-case scenario) and; 3) Model C: when the missing VL measurements were coded 0 (missing values assumed to be effective yielding a best-case scenario).

Linear regression was used to access the relationship between CD4 change and menopause at 6, 12 and 24 months. In the other analyses, immunological reconstitution was defined as a >25% increase in CD4 cell count from baseline and logistic regression was used to access the menopause effect at 6, 12 and 24 months. All models were adjusted for baseline log 10 VL, baseline CD4, cART regimen and ADI.

For all statistical analyses we used the statistical software R, version 2.14.2 (www.r-project.org).

Results

Among 383 women, 328 (85%) were premenopausal and 55 (15%) postmenopausal. Demographic and clinical characteristics are shown in table 1. There were no significant differences in characteristics between the two subgroups, except for age and history of hysterectomy. The median age was 34 years [interquartile interval (IQR): 28–40] for premenopausal and 52 years (IQR: 48–55) for postmenopausal women (P<.001). A hysterectomy history was more frequent among postmenopausal women (18.2% vs. 1.2% in premenopausal women, p<0.001).

Median pre-cART CD4 counts were 231 (IQR:132–334) and 208 (IQR:85–287) cells/mm3 (p = 0.14) in pre- and postmenopausal women, respectively. No difference in median pre cART VL was observed (both 4.8 copies/mL). Almost two- thirds of the study population initiated cART between 2005 and 2009. NNRTI-based cART was the most frequently prescribed (72.3% and 67.3% in pre- and post-menopausal women, respectively). Approximately 40% of all women initiated cART within a clinical trial.

The most frequent first cART regimens stratified by menopausal status are depicted in Table 2. A combination of zidovudine (ZDV)+lamivudine (3TC)+efavirenz (EFV) was used by almost two-fifths of the study population (148, 38.6%).

CD4 count median changes were similar in pre- and postmenopausal women at 6 (101 vs. 106 cells/mm^3; p = 0.73) and 12 months (171 vs. 147 cells/mm^3; p = 0.42). At 24 months after cART initiation, CD4 median changes among postmenopausal women were significantly lower than among premenopausal women (184 vs. 273 cells/mm^3; respectively; p = 0.02) (table 3).

When analysis was restricted to women with VL<400 copies/mL in both groups, no statistical differences were observed between pre- and postmenopausal, although CD4 median changes were lower among postmenopausal women at all-time points (6, 12 and 24 months) (table 3).

No differences were found between the proportions of pre- and postmenopausal women who achieved VL<400 copies/mL at 6 (71.3% vs. 72.7%, respectively; p = 0.99), 12 (71.3% vs. 73.3%, respectively; p = 0.94), and 24 months (64.3% vs. 60.5%, respectively; p = 0.60) (table 3).

There were no differences in the odds ratio of achieving an HIV-1 RNA level <400 copies/mL for premenopausal compared with postmenopausal women after adjusting for baseline log10 HIV-1 RNA levels, baseline CD4, cART regimen and ADI at 6 months (OR = 1.00; 95% CI: 0.86–1.17), 12 months (OR = 1.02; 95% CI: 0.87–1.19), and 24 months (OR = 1.02; 95% CI: 0.85–1.22) (table 4). Sensitivity analyses performed to assess the impact of missing information in the evaluation of cART effectiveness did not show potential selection bias. In this analysis (data not shown), the estimates of all the variables retained in the final model A did not differ significantly (overlap 95% CI) from the ones estimated in models B and C.

At 24 months after cART initiation (and after adjusting for the same covariates), postmenopausal women had significantly lower CD4 cell changes (−97.8 cells/mm^3; SE: 39.9 cells/mm^3) than premenopausal women (p = 0.01). This difference was not observed when analysis was restricted to women with VL<400 copies (−61cells/mm^3; SE: 44.4 cells/mm^3) (p = 0.26) (table 4).

When immunological reconstitution was evaluated as a >25% increase in CD4 cell count from baseline, no statistical differences were observed in unadjusted or adjusted analyses comparing pre- and postmenopausal women at 6, 12 and 24 months after cART initiation in the overall group or when the analysis was restricted to virologically suppressed women (tables 3and 4).

Discussion

Our results demonstrated that menopause status at the time of initiation of first-line combination antiretroviral therapy does not impact CD4 cell count changes at 24 months among women with virologic response, regardless of the ART class prescribed, year of ART initiation or having started ART in a clinical trial as compared to regular care. Nevertheless, CD4 median changes remained lower among postmenopausal women.

We found no evidence of any interaction between menopause and virologic responses. To our knowledge, our analysis is the first to report virologic and immunologic outcomes following cART initiation in women from a middle-income country stratified by menopause status.

Most cohort studies found that overall, older people can achieve treatment success rates similar to younger individuals [24], [25]. Some investigators indirectly evaluated the impact of reproductive hormones levels on CD4 lymphocyte counts [17], [26]. In a study from the Swiss cohort, postmenopausal women had lower CD4 lymphocyte counts 3 years after seroconversion as compared to premenopausal women, although the differences were not statistically significant [17]. A meta-analysis involving 4414 antiretroviral-naïve women

enrolled in 32 randomized trials between 2000 and 2010 concluded that women aged 50 or older had significantly greater chances of viral suppression in response to a first-line cART regimen than women 35 or younger at weeks 24 and 48. No significant age differences on immunologic responses at weeks 24 or 48 were observed, although younger women taking a NNRTI based cART regimen had significantly better CD4-cell responses than the older group through 48 weeks of treatment [26]. Treatment compliance may explain these outcomes because older patients have been found to be more adherent to HIV medications than younger patients [27]–[29].

Several studies have shown a decreased CD4 cell response among older patients, regardless of sex, and some of them have observed increased HIV disease progression and a negative impact on survival [14], [15], [30]–[34]. Among 24107 HIV-infected adults enrolled in the International epidemiological Database to Evaluate AIDS (IeDEA) Collaboration in the West African region, a significantly higher mean CD4 gain was observed among younger patients when compared to elderly patients after 12 months of cART initiation [15]. A study from the French Hospital Database including 3015 antiretroviral-naïve patients found that patients over 50 years old had a significantly slower CD4 cell reconstitution and a significantly higher risk of clinical progression than younger patients, despite a better virologic response [30]. Althoff et al in a pooled analysis of 19 prospective cohort studies in the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) showed that the immunologic response decreased with increasing age after 24 months of initial cART regimen, regardless of ART class and that older individuals were less likely to have a CD4 increase greater than 100 cells [31].

Abrogoua et al, in a study conducted in a resource-limited setting, found that age and baseline clinical status had no significant influence on immunological outcomes at 24 months, although an optimal CD4 cell response was significantly influenced by adherence [29]. We

were unable to evaluate the impact of adherence on virological and immunological outcomes in our study population as adherence data were not available. Paterson et al, in an analysis comparing the effect of sex by age strata, did not find differences at 6 months from the initiation of a first HAART regimen among previously ART naïve patients in either immunological reconstitution or virological response. The potency of a first HAART regimen in controlling HIV-1 replication and subsequent immunological reconstitution have probably been able to mask any more subtle effect of biological sex differences on treatment responses [35]. In a subsequent study evaluating long-term immunologic and virologic responses to initial ART in pre- and post-menopausal women participating in two treatment trials similar virologic and immunologic responses to ART in treatment naïve pre- and post-menopausal women initiating ART in a clinical trial setting were observed [16].

Our results are reassuring that women can be treated similarly irrespective of age. Our analysis, however, did not include evaluations of antiretroviral-related toxicities or other adverse events which have been reported to be increased among older individuals and women including in our cohort [18], [27], [36], [37]. Despite this, 73.3% of the post-menopausal women and 71.3% of premenopausal women with HIV-1 RNA measurements were virologically suppressed at 24 months.

Strengths of this study include a large urban cohort of women receiving HIV clinical care coupled with specialized gynecological care with prospective standardized bi-annual data collection on reproductive health, which reduces the risk of recall bias in regards to age at menopause.

Limitations of this study include the retrospective nature of the HIV clinical and laboratory data collection, and a certain level of missing data on CD4 and viral load, although sensitivity

analysis did not show potential selection bias. Moreover, the lack of adherence data precluded a systematic adherence evaluation.

In summary, in our study population, menopause status at cART initiation did not impact long-term immunological outcomes in women with favorable virologic responses. We found no evidence of an interaction between menopause status and virologic responses. These results are reassuring, given the increasing number of older HIV-infected women initiating cART and the impact of immune reconstitution on long-term survival.

Author Contributions

Conceived and designed the experiments: GAC LV PML SWC MD RIM VGV BG RKF. Performed the experiments: GAC SWC ACVdA AC EP RKF. Analyzed the data: LV PML MD RIM. Wrote the paper: GAC LV SWC VGV BG RKF.

References

1.UNAIDS World AIDS Day report (2011) Available:http://www.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2011/jc2216_worldaidsday_report_2011_en.pdf.Accessed 28 August 2013.

2.Instituto Brasileiro de Geografia e Estatística – IBGE (Brazil) (2002) Perfil dos Idosos Responsáveis pelos Domicílios no Brasil 2000. Rio de Janeiro. Available:http://www.ibge.gov.br/home/estatistica/populacao/perfilidoso/perfidosos2000.pdf. Accessed 28 September 2013.

3.Lazarus JV, Nielsen KK (2010) HIV and people over 50 years old in Europe. HIV Med 11: 479–481.

4.Cejtin HE (2012) Care of the human immunodeficiency virus-infected menopausal woman. Am J Obstet Gynecol 207: 87–93.

5.Napravnik S, Poole C, Thomas JC, Eron JJ Jr (2002) Gender difference in HIV RNA levels: a meta-analysis of published studies. J Acquir Immune Defic Syndr 31: 11–19.

6.Gandhi M, Bacchetti P, Miotti P, Quinn TC, Veronese F, et al. (2002) Does patient sex affect human immunodeficiency virus levels? Clin Infect Dis 35: 313–322.

7.Grinsztejn B, Smeaton L, Barnett R, Klingman K, Hakim J, et al. (2011) Sex-associated differences in pre-antiretroviral therapy plasma HIV-1 RNA in diverse areas of the world vary by CD4(+) T-cell count. Antivir Ther 16: 1057–1062.

8.Haynes L, Maue AC (2009) Effects of aging on T cell function. Curr Opin Immunol 21: 414–417.

9.Yao G, Hou Y (2004) Recent advances for research on effect of estrogen on immune system. Wei Sheng Yan Jiu 33: 759–761.

10.Gameiro CM, Romao F, Castelo-Branco C (2010) Menopause and aging: changes in the immune system–a review. Maturitas 67: 316–320.

11.Oertelt-Prigione S (2012) The influence of sex and gender on the immune response. Autoimmun Rev 11: A479–485.

12.Raboud J, Blitz S, Walmsley S, Thompson C, Rourke SB, et al. (2010) Effect of gender and calendar year on time to and duration of virologic suppression among antiretroviral-naive HIV-infected individuals initiating combination antiretroviral therapy. HIV Clin Trials 11: 340–350

13.Barber TJ, Geretti AM, Anderson J, Schwenk A, Phillips AN, et al. (2011) Outcomes in the first year after initiation of first-line HAART among heterosexual men and women in the UK CHIC Study. Antivir Ther 16: 805–814.

14.Greenbaum AH, Wilson LE, Keruly JC, Moore RD, Gebo KA (2008) Effect of age and HAART regimen on clinical response in an urban cohort of HIV-infected individuals. AIDS 22: 2331–2339.

15.Balestre E, Eholie SP, Lokossue A, Sow PS, Charurat M, et al. (2012) Effect of age on immunological response in the first year of antiretroviral therapy in HIV-1-infected adults in West Africa. AIDS 26: 951–957.

16.Patterson KB, Cohn SE, Uyanik J, Hughes M, Smurzynski M, et al. (2009) Treatment responses in antiretroviral treatment-naive premenopausal and postmenopausal HIV-1-infected women: an analysis from AIDS Clinical Trials Group Studies. Clin Infect Dis 49: 473–476.

17.van Benthem BH, Vernazza P, Coutinho RA, Prins M, European Study on the Natural History of HIV Infection in Women, et al. (2002) The impact of pregnancy and menopause on CD4 lymphocyte counts in HIV-infected women. AIDS 16: 919–924.

18.Cardoso SW, Grinsztejn B, Velasque L, Veloso VG, Luz PM, et al. (2010) Incidence of modifying or discontinuing first HAART regimen and its determinants in a cohort of HIV-infected patients from Rio de Janeiro, Brazil. AIDS Res Hum Retroviruses 26: 865–874.

19.Moreira RI, Luz PM, Struchiner CJ, Morgado M, Veloso VG, et al. (2011) Immune status at presentation for HIV clinical care in Rio de Janeiro and Baltimore. J Acquir Immune Defic Syndr 57 Suppl 3S171–178.

20.Grinsztejn B, Bastos FI, Veloso VG, Friedman RK, Pilotto JH, et al. (2006) Assessing sexually transmitted infections in a cohort of women living with HIV/AIDS, in Rio de Janeiro, Brazil. Int J STD AIDS 17: 473–478.

21.Friedman RK, Bastos FI, Leite IC, Veloso VG, Moreira RI, et al. (2011) Pregnancy rates and predictors in women with HIV/AIDS in Rio de Janeiro, Southeastern Brazil. Rev Saude Publica 45: 373–381.

22.Research on the menopause in the 1990s. Report of a WHO Scientific Group. World Health Organ Tech Rep Ser 866: 1–107.

23.Centers for Diseases Control and Prevention (1992) 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. MMWR Recomm Rep 41: 1–19.

24.Tumbarello M, Rabagliati R, de Gaetano Donati K, Bertagnolio S, Montuori E, et al. (2004) Older age does not influence CD4 cell recovery in HIV-1 infected patients receiving highly active antiretroviral therapy. BMC Infect Dis 4: 46.

25.Wellons MF, Sanders L, Edwards LJ, Bartlett JA, Heald AE, et al. (2002) HIV infection: treatment outcomes in older and younger adults. J Am Geriatr Soc 50: 603–607.

26.Yan J, Soon G, Zhou S, Min M, Chan-Tack K, et al. (2013) Differences by age for women in the response to initial HAARTs: Meta-analysis from clinical studies submitted to the FDA (2000–2010). Third International Workshop on HIV and Women. Proceedings. Toronto, Canada, 2013. Reviews in Antiviral Therapy & Infectious Diseases 1: O–11.

27.Silverberg MJ, Leyden W, Horberg MA, DeLorenze GN, Klein D, et al. (2007) Older age and the response to and tolerability of antiretroviral therapy. Arch Intern Med 167: 684–691.

28.Newman J, Iriondo-Perez J, Hemingway-Foday J, Freeman A, Akam W, et al. (2012) Older Adults Accessing HIV Care and Treatment and Adherence in the IeDEA Central Africa Cohort. AIDS Res Treat 2012: 725713.

29.Abrogoua DP, Kablan BJ, Kamenan BA, Aulagner G, N'Guessan K, et al. (2012) Assessment of the impact of adherence and other predictors during HAART on various CD4 cell responses in resource-limited settings. Patient Prefer Adherence 6: 227–237.

30.Grabar S, Kousignian I, Sobel A, Le Bras P, Gasnault J, et al. (2004) Immunologic and clinical responses to highly active antiretroviral therapy over 50 years of age. Results from the French Hospital Database on HIV. AIDS 18: 2029–2038.

31.Althoff KN, Justice AC, Gange SJ, Deeks SG, Saag MS, et al. (2010) Virologic and immunologic response to HAART, by age and regimen class. AIDS 24: 2469–2479.

32.Maman D, Pujades-Rodriguez M, Nicholas S, McGuire M, Szumilin E, et al. (2012) Response to antiretroviral therapy: improved survival associated with CD4 above 500 cells/mul. AIDS 26: 1393–1398.

33.Blanco JR, Jarrin I, Vallejo M, Berenguer J, Solera C, et al. (2012) Definition of advanced age in HIV infection: looking for an age cut-off. AIDS Res Hum Retroviruses 28: 1000–1006.

34.Opportunistic Infections Project Team of the Collaboration of Observational HIV Epidemiological Research in Europe in EuroCoord, Young J, Psichogiou M, Meyer L, Ayayi S, et al (2012) CD4 cell count and the risk of AIDS or death in HIV-Infected adults on combination antiretroviral therapy with a suppressed viral load: a longitudinal cohort study from COHERE. PLoS Med 9: e1001194.

35.Patterson K, Napravnik S, Eron J, Keruly J, Moore R (2007) Effects of age and sex on immunological and virological responses to initial highly active antiretroviral therapy. HIV Med 8: 406–410.

36.Hermes A, Squires K, Fredrick L, Martinez M, Pasley M, et al. (2012) Meta-analysis of the safety, tolerability, and efficacy of lopinavir/ritonavir-containing antiretroviral therapy in HIV-1-infected women. HIV Clin Trials 13: 308–323.

37.Torres TS, Cardoso SW, Velasque LS, Veloso VG, Grinsztejn B (2014) Incidence rate of modifying or discontinuing first combined antiretroviral therapy regimen due to toxicity during the first year of treatment stratified by age. Braz J Infect Dis 18: 34–41.

Table 1. Demographic and clinical characteristics for premenopausal and postmenopausal women at the start of antiretroviral therapy (baseline).

Characteristic		Premenopause (N=328)	Postmenopause (N=55)	Total (N=383)	p-value
Age, median years (IQR)		34 (28-40)	52 (48-55)	36 (30-43)	<0.001
Race/ethnicity, N (%)	White	136 (41.5)	28 (50.9)	164 (42.8)	0.239
	Non-white	192 (58.5)	27 (49.1)	219 (57.2)	
Hysterectomy, N (%)		4 (1.2)	10 (18.2)	14 (3.6)	<0.001
Baseline CD4 cell count, median (IQR, cells/mm^3)		231(132-334)	208 (85-287)	227(127-329)	0.14
Baseline CD4 cell count (cells/mm^3), N (%)	<200	112 (40.3)	24 (49.0)	136 (41.6)	0.495
	200-500	149 (53.6)	23 (46.9)	172 (52.6)	
	>500	17(6.1)	2 (4.1)	19 (5.8)	
Baseline HIV viral load, median (IQR, log 10 copies/mL)		4.8 (4.1-5.4)	4.8 (4.4-5.3)	4.8 (4.2-5.4)	0.18
Baseline HIV viral load (copies/mL), N (%)	401 - 10,000	51 (20.4)	3 (6.5)	54 (18.2)	0.071
	10,001 - 100,000	98 (39.2)	23 (50.0)	121 (40.9)	
	> 100,000	101 (40.4)	20 (43.5)	121 (40.9)	
AIDS defining illness, N (%)	Yes	68 (20.7)	13 (23.6)	81 (21.2)	0.597
cART regimen, N (%)	PI	91 (27.7)	18 (32.7)	109 (28.5)	0.518
	NNRTI	237 (72.3)	37 (67.3)	274 (71.5)	
Year of starting cART, N (%)	2000-2004	118 (36.0)	21 (38.2)	139 (36.3)	0.764
	2005-2009	210 (64.0)	34 (61.8)	244 (63.7)	
In clinical trial, N (%)	Yes	125 (38.1)	20 (36.4)	145 (37.9)	0.881

IQR, interquartile interval;

cART: Combination Antiretroviral Therapy; PI: Protease inhibitor;

NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitors

Table 2. Most frequent first cART regimens stratified by menopausal status at the initiation of antiretroviral therapy (baseline).

First cART regimens	Premenopause N=328 (%)	Postmenopause N=55 (%)	Total N=383 (%)
ZDV + 3TC + EFV	123 (37.5)	25 (45.4)	148 (38.6)
FTC + TDF + EFV	42 (12.8)	5 (9.1)	47 (12.3)
TDF + 3TC + EFV	27 (8.2)	4 (7.3)	31(8.1)
d4T + 3TC + EFV	12 (3.7)	3 (5.5)	15 (3.9)
ZDV + 3TC + ATV	14 (4.3)	1 (1.8)	15 (3.9)
FTC + ddI + ATV	10 (3.1)	3 (5.5)	13 (3.4)
ZDV + 3TC + NFV	11 (3.4)	2 (3.6)	13 (3.4)
ZDV + 3TC + LOP/r	9 (2.7)	3 (5.5)	12 (3.1)
TDF + 3TC + ATV/r	8 (2.4)	1 (1.8)	9 (2.3)
d4T + 3TC + NFV	4 (1.2)	3 (5.5)	7 (1.8)
ZDV + 3TC + ATV/r	6 (1.8)	1 (1.8)	7 (1.8)
ZDV + 3TC + SQV/r	5 (1.5)	1 (1.8)	6 (1.6)
ZDV + 3TC + IDV/r	2 (0.6)	1 (1.8)	3 (0.8)
TDF + 3TC + LOP/r	1 (0.3)	2 (3.6)	3 (0.8)
Other NNRTI-based cART	33 (10.1)	-	33 (8.6)
Other PI-based cART	21 (6.4)	-	21 (5.5)

3TC, lamivudine; ATV/r, atazanavir/ritonavir; d4T, stavudine; ddI, didanosine; EFV, efavirenz;

FTC, emtricitabine; IDV/r, indinavir/ritonavir; LOP/r, lopinavir/ritonavir; SQV/r, saquinavir/ritonavir;

NFV, nelfinavir; TDF, tenofovir; ZDV, zidovudine;

cART: combination antiretroviral therapy; PI: Protease inhibitor;

NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitors.

Table 3. cART effectiveness at 6, 12, and 24 months and the median change in CD4 cell count for premenopausal and postmenopausal antiretroviral-naïve women.

Characteristic	Time points	N	Premenopause	Postmenopause	total	p-value
All patients						
cART effectiveness, N (%)	6 months	309	189 (71.3)	32 (72.7)	221 (71.4)	0.99
	12 months	310	189 (71.3)	33 (73.3)	223 (71.2)	0.94
	24 months	293	164 (64.3)	24 (60.5)	188 (63.7)	0.60
Change CD4, median (IQR, cells/mm^3)	6 months	260	101 (31-193)	106 (69-201)	101 (31-194)	0.73
	12 months	273	171 (76-290)	147 (75-278)	163 (72-285)	0.42
	24 months	251	273 (156-395)	184 (116-282)	262 (149-384)	**0.02**
>25% CD4 cell count increase [N (%)]	6 months	260	145 (65.3)	27 (71.1)	172 (66.2)	0.61
	12 months	273	175 (75.4)	31 (75.6)	206 (75.5)	0.86
	24 months	251	187 (86.6)	28 (80.0)	215 (85.7)	0.44
In patients with viral load ≤400 copies/mL						
Change CD4, median (IQR, cells/mm^3)	6 months	200	119 (56-204)	99 (60-199)	113 (56-202)	0.79
	12 months	207	182 (106-300)	152 (52-268)	179 (97-299)	0.20
	24 months	204	298 (188-439)	244 (154-396)	291 (178-432)	0.27
>25% CD4 cell count increase [N (%)]	6 months	200	122 (70.9)	19 (67.9)	141 (70.5)	0.92
	12 months	207	143 (80.8)	21 (70.0)	164 (79.2)	0.27
	24 months	204	161 (90.4)	23 (88.5)	184 (90.2)	0.73

IQR, interquartile interval;

cART: Combination Antiretroviral Therapy;

Table 4. cART effectiveness and CD4 change models at 6, 12, and 24 months for premenopausal and postmenopausal antiretroviral-naïve women.

	6 months	**12 months**	**24 months**
All patients	N=230	N=228	N=210
cART effectiveness [OR (95%CI)]	1.00 (0.86-1.17)	1.02 (0.87-1.19)	1.02 (0.85-1.22)
	N=213	N=222	N=207
CD4 Change coefficients (Standard error)	-7.6(29.0)	-27.4(31.2)	**-97.8(39.9)**
	N=213	N=222	N=207
>25% CD4 cell count increase [OR(95%CI)]	1.06 (0.43-2.59)	0.69 (0.29-1.69)	0.46 (0.17-1.26)
In patients with viral load ≤400 copies/mL	N=164	N=167	N=166
CD4 Change coefficients (Standard error)	-14.3(34.8)	-35.4(35.9)	-61.1(44.4)
	N=164	N=167	N=166
>25% CD4 cell count increase [OR(95%CI)]	0.69 (0.24-1.95)	0.38 (0.14-1.07)	0.46 (0.11-1.92)

cART: Combination Antiretroviral Therapy; OR: Odds Ratio

Models adjusted for baseline log 10 HIV-1 RNA levels, baseline CD4, HAART regimen and AIDS defining illness

$p<0.05$ in bold

7 CONCLUSÕES

A idade da ocorrência natural da menopausa na coorte de mulheres HIV-positivo foi de 48 anos, inferior à média observada em um estudo brasileiro de base populacional (51,2 anos) em mulheres HIV-negativo.

Uma alta prevalência (27%) de menopausa precoce (≤ 45 anos) foi observada na coorte.

Vários fatores foram associados com antecipação da idade natural da menopausa: Menarca precoce (<11 anos), imunodeficiência avançada relacionada ao HIV (contagem de células T CD4+ < 50 células/mm^3), exposição inferior a 10 anos ao esquema antirretroviral, coinfecção hepatite C/HIV e tabagismo.

Mulheres pós-menopausadas, previamente virgens de terapia antirretroviral, com resposta virológica após início da terapia antirretroviral combinada de primeira linha, respondem de forma semelhante a mulheres pré-menopausadas em relação à resposta imunológica em até 24 meses após início do tratamento.

Não foi observada relação entre o status da menopausa e resposta virológica à terapia antirretroviral combinada de primeira linha em até 24 meses após início do tratamento.

8 RECOMENDAÇÕES

-Necessidade de mais estudos para entender o impacto da infecção pelo HIV no processo de envelhecimento feminino, particularmente em desfechos como osteoporose e doenças cardiovasculares, mais comuns em mulheres de faixa etária mais elevada, em função do crescente número de mulheres pós-menopausadas em tratamento antirretroviral.

-Avaliar o impacto da menopausa precoce em relação às possíveis complicações comuns a esta faixa etária.

-Estudar o uso da reposição hormonal em mulheres HIV-positivo, especialmente nas mulheres com menopausa precoce, avaliando benefícios à saúde, redução de sintomatologia, aumento da qualidade de vida, análise das interações medicamentosas e segurança nessa população.

-Estabelecer um fluxograma para definir adequadamente a ocorrência de menopausa em mulheres infectadas pelo HIV, considerando a possibilidade do uso de marcadores hormonais para estabelecer seu status menopáusico, e construir um protocolo para atendimento dessas mulheres nos períodos pré, peri e pós-menopáusico, voltado para redução de sintomatologia, prevenção de câncer ginecológico e outros agravos associados ao processo de envelhecimento feminino, à infecção pelo HIV e ao uso da terapia antirretroviral.

-Incluir dosagem de FSH na rotina laboratorial do IPEC para avaliação hormonal de pacientes na perimenopausa.

-Estudos futuros são necessários para avaliar estratégias de otimização da terapia antirretroviral nesta população, em função do número crescente de mulheres pós-menopausadas em uso de terapia antirretroviral e do impacto da reconstituição imune na sobrevida.

9 REFERÊNCIAS

Aldrighi JM, Alecrin IN, de Oliveira PR, Shinomata HO. Smoking and earlier menopause. Rev Assoc Med Bras 2005;51(1):51-3.

Althoff KN, Justice AC, Gange SJ, Deeks SG, Saag MS, Silverberg MJ, et al. Virologic and immunologic response to HAART, by age and regimen class. AIDS 2010;24(16):2469-79.

Alvarez C, Salazar R, Galindez J, Rangel F, Castaaeda ML, Lopardo G, et al. Metabolic syndrome in HIV-infected patients receiving antiretroviral therapy in Latin America. Braz J Infect Dis 2010;14(3):256-63.

Archer DF. Premature menopause increases cardiovascular risk. Climacteric 2009;12 Suppl 1:26-31.

Balestre E, Eholie SP, Lokossue A, Sow PS, Charurat M, Minga A, et al. Effect of age on immunological response in the first year of antiretroviral therapy in HIV-1-infected adults in West Africa. AIDS 2012;26(8):951-7.

Ballon G, Ometto L, Righetti E, Cattelan AM, Masiero S, Zanchetta M, et al. Human immunodeficiency virus type 1 modulates telomerase activity in peripheral blood lymphocytes. J Infect Dis 2001;183(3):417-24.

Barber TJ, Geretti AM, Anderson J, Schwenk A, Phillips AN, Bansi L, et al. Outcomes in the first year after initiation of first-line HAART among heterosexual men and women in the UK CHIC Study. Antivir Ther 201116(6):805-14.

Boonyanurak P, Bunupuradah T, Wilawan K, Lueanyod A, Thongpaeng P, Chatvong D, et al. Age at menopause and menopause-related symptoms in human immunodeficiency virus-infected Thai women. Menopause 2012;19(7):820-4.

Cardoso SW, Torres TS, Santini-Oliveira M, Marins LM, Veloso VG, Grinsztejn B. Aging with HIV: a practical review. Braz J Infect Dis 2013;17(4):464-79.
Castelo-Branco C, Blumel JE, Chedraui P, Calle A, Bocanera R, Depiano E, et al.
Age at menopause in Latin America. Menopause 2006;13(4):706-12.

Cauley JA, Danielson ME, Greendale GA, Finkelstein JS, Chang YF, Lo JC, et al. Bone resorption and fracture across the menopausal transition: the Study of Women's Health Across the Nation. Menopause 2012;19(11):1200-7.

Cejtin HE. Care of the human immunodeficiency virus-infected menopausal woman. Am J Obstet Gynecol 2012;207(2):87-93.

Cejtin HE, Kalinowski A, Bacchetti P, Taylor RN, Watts DH, Kim S, et al. Effects of human immunodeficiency virus on protracted amenorrhea and ovarian dysfunction. Obstet Gynecol 2006;108(6):1423-31.

Cejtin H E, Kim S, Taylor RN, Watts DH, Minkoff HL, Massad LS, Preston-Martin S, Anastos K. Menopause in women in the Women's Interagency HIV Study (WIHS). Poster Exhibition: The XV International AIDS Conference: Abstract no. WePeD6504". Disponível em: http://www.iasociety.org/Default.aspx?pageId=11&abstractId=2169564. Acesso 25 Out 2013.

Centers for Disease C, Prevention. Cigarette smoking among adults--United States, 1992, and changes in the definition of current cigarette smoking. MMWR Morbidity and mortality weekly report 1994;43(19):342-6.

Cicconi P, Ammassari A, Ladisa N, Di Sora F, Bini T, Pierro P, et al. Prevalence of prolonged amenorrhea in HIV-infected women: results from the Italian DIDI study. J Acquir Immune Defic Syndr 2012;61(2):e19-21.

Clark RA, Cohn SE, Jarek C, Craven KS, Lyons C, Jacobson M, et al. Perimenopausal symptomatology among HIV-infected women at least 40 years of age. J Acquir Immune Defic Syndr 2000;23(1):99-100.

Collaborative Group on Hormonal Factors in Breast Cancer. Menarche, menopause, and breast cancer risk: individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. The lancet oncology 2012;13(11):1141-51.

Cramer DW. The epidemiology of endometrial and ovarian cancer. Hematology/oncology clinics of North America 2012;26(1):1-12.

de Pommerol M, Hessamfar M, Lawson-Ayayi S, Neau D, Geffard S, Farbos S, et al. Menopause and HIV infection: age at onset and associated factors, ANRS CO3 Aquitaine cohort. Int J STD AIDS 2011;22(2):67-72.

Douek DC, McFarland RD, Keiser PH, Gage EA, Massey JM, Haynes BF, et al. Changes in thymic function with age and during the treatment of HIV infection. Nature 1998;396(6712):690-5.

Dratva J, Gomez Real F, Schindler C, Ackermann-Liebrich U, Gerbase MW, Probst-Hensch NM, et al. Is age at menopause increasing across Europe? Results on age at menopause and determinants from two population-based studies. Menopause 2009;16(2):385-94.

Edwards BJ, Li J. Endocrinology of menopause. Periodontology 2000 2013;61(1):177-94.

Ellerbrock TV, Wright TC, Bush TJ, Dole P, Brudney K, Chiasson MA. Characteristics of menstruation in women infected with human immunodeficiency virus. Obstet Gynecol 1996;87(6):1030-4.

Fantry LE, Zhan M, Taylor GH, Sill AM, Flaws JA. Age of menopause and menopausal symptoms in HIV-infected women. AIDS Patient Care STDS 2005;19(11):703-11.

Ferrando-Martinez S, Ruiz-Mateos E, Hernandez A, Gutierrez E, Rodriguez-Mendez Mdel M, Ordonez A, et al. Age-related deregulation of naive T cell homeostasis in elderly humans. Age (Dordr) 2011;33(2):197-207.

Ferreira CE, Pinto-Neto AM, Conde DM, Costa-Paiva L, Morais SS, Magalhaes J. Menopause symptoms in women infected with HIV: prevalence and associated factors. Gynecol Endocrinol 2007;23(4):198-205.

Fukuda K, Takashima Y, Hashimoto M, Uchino A, Yuzuriha T, Yao H. Early Menopause and the Risk of Silent Brain Infarction in Community-Dwelling Elderly Subjects: The Sefuri Brain MRI Study. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association 2013.[epub ahead of print].

Gameiro CM, Romao F, Castelo-Branco C. Menopause and aging: changes in the immune system--a review. Maturitas 2010;67(4):316-20.

Gandhi M, Bacchetti P, Miotti P, Quinn TC, Veronese F, Greenblatt RM. Does patient sex affect human immunodeficiency virus levels? Clin Infect Dis 2002;35(3):313-22.

Giannarelli C, Klein RS, Badimon JJ. Cardiovascular implications of HIV-induced dyslipidemia. Atherosclerosis 2011;219(2):384-9.

Gold EB. The timing of the age at which natural menopause occurs. Obstet Gynecol Clin North Am 2011;38(3):425-40.

Gold EB, Crawford SL, Avis NE, Crandall CJ, Matthews KA, Waetjen LE, et al. Factors related to age at natural menopause: longitudinal analyses from SWAN. Am J Epidemiol 2013;178(1):70-83.

Grabar S, Kousignian I, Sobel A, Le Bras P, Gasnault J, Enel P, et al. Immunologic and clinical responses to highly active antiretroviral therapy over 50 years of age. Results from the French Hospital Database on HIV. AIDS 2004;18(15):2029-38.

Greenbaum AH, Wilson LE, Keruly JC, Moore RD, Gebo KA. Effect of age and HAART regimen on clinical response in an urban cohort of HIV-infected individuals. AIDS 2008;22(17):2331-9.

Grinsztejn B, Smeaton L, Barnett R, Klingman K, Hakim J, Flanigan T, et al. Sex-associated differences in pre-antiretroviral therapy plasma HIV-1 RNA in diverse areas of the world vary by CD4(+) T-cell count. Antivir Ther 2011;16(7):1057-62.

Hacker MA, Petersen ML, Enriquez M, Bastos FI. Highly active antiretroviral therapy in Brazil: the challenge of universal access in a context of social inequality. Rev Panam Salud Publica 2004;16(2):78-83.

Harlow SD, Cohen M, Ohmit SE, Schuman P, Cu-Uvin S, Lin X, et al. Substance use and psychotherapeutic medications: a likely contributor to menstrual disorders in women who are seropositive for human immunodeficiency virus. Am J Obstet Gynecol 2003;188(4):881-6.

Harlow SD, Gass M, Hall JE, Lobo R, Maki P, Rebar RW, et al. Executive summary of the Stages of Reproductive Aging Workshop + 10: addressing the unfinished agenda of staging reproductive aging. Menopause 2012;19(4):387-95.

Haynes L, Eaton SM, Burns EM, Randall TD, Swain SL. CD4 T cell memory derived from young naive cells functions well into old age, but memory generated from aged naive cells functions poorly. Proc Natl Acad Sci U S A 2003;100(25):15053-8.

Haynes L, Maue AC. Effects of aging on T cell function. Curr Opin Immunol 2009;21(4):414-7.

Hearps AC, Angelovich TA, Jaworowski A, Mills J, Landay AL, Crowe SM. HIV infection and aging of the innate immune system. Sex Health 2011;8(4):453-64.

Henderson KD, Bernstein L, Henderson B, Kolonel L, Pike MC. Predictors of the timing of natural menopause in the Multiethnic Cohort Study. Am J Epidemiol 2008;167(11):1287-94.

Jin M, Yu Y, Huang H. An update on primary ovarian insufficiency. Sci China Life Sci 2012;55(8):677-86.

Kanapathipillai R, Hickey M, Giles M. Human immunodeficiency virus and menopause. Menopause 2013;20(9):983-90.

Kolte L. Thymic function in HIV-infection. Dan Med J 2013;60(4):B4622.

Kwakwa H, Spencer D, Evans C, Garner W, Walker I, Temme L. Gender differences in virologic outcomes in a meta-analysis of randomized controlled clinical trials in HIV-1-infected patients on antiretroviral therapy. XIX International AIDS Conference; 2012 Jul 22-27; Washington DC. Abstract THPE041.

Lee JS, Hayashi K, Mishra G, Yasui T, Kubota T, Mizunuma H. Independent association between age at natural menopause and hypercholesterolemia, hypertension, and diabetes mellitus: Japan nurses' health study. J Atheroscler Thromb 2013;20(2):161-9.

Li S, Rosenberg L, Wise LA, Boggs DA, LaValley M, Palmer JR. Age at natural menopause in relation to all-cause and cause-specific mortality in a follow-up study of US black women. Maturitas 2013;75(3):246-52.

Li T, Wu N, Dai Y, Qiu Z, Han Y, Xie J, et al. Reduced thymic output is a major mechanism of immune reconstitution failure in HIV-infected patients after long-term antiretroviral therapy. Clin Infect Dis 2011;53(9):944-51.

Libois A, Clumeck N, Kabeya K, Gerard M, De Wit S, Poll B, et al. Risk factors of osteopenia in HIV-infected women: no role of antiretroviral therapy. Maturitas 2010;65(1):51-4.

Maki PM, Martin-Thormeyer E. HIV, cognition and women. Neuropsychol Rev 2009;19(2):204-14.

Malaguarnera L, Ferlito L, Imbesi RM, Gulizia GS, Di Mauro S, Maugeri D, et al. Immunosenescence: a review. Arch Gerontol Geriatr 2001;32(1):1-14.

Manfredi R, Chiodo F. A case-control study of virological and immunological effects of highly active antiretroviral therapy in HIV-infected patients with advanced age. AIDS 2000;14(10):1475-7.

Maue AC, Haynes L. CD4+ T cells and immunosenescence--a mini-review. Gerontology 2009;55(5):491-5.

Mishra GD, Cooper R, Tom SE, Kuh D. Early life circumstances and their impact on menarche and menopause. Womens Health (Lond Engl) 2009;5(2):175-90.

Mondul AM, Rodriguez C, Jacobs EJ, Calle EE. Age at natural menopause and cause-specific mortality. Am J Epidemiol 2005;162(11):1089-97.

Moro-Garcia MA, Alonso-Arias R, Lopez-Larrea C. Molecular mechanisms involved in the aging of the T-cell immune response. Curr Genomics 2012;13(8):589-602.

Mutevedzi PC, Lessells RJ, Rodger AJ, Newell ML. Association of age with mortality and virological and immunological response to antiretroviral therapy in rural South African adults. PLoS One 2011;6(7):e21795.

Nagy GS. Diabetes mellitus in HIV-infected women. AIDS Clin Care 2003;15(5):44-5.

Napravnik S, Poole C, Thomas JC, Eron JJ, Jr. Gender difference in HIV RNA levels: a meta-analysis of published studies. J Acquir Immune Defic Syndr 2002;31(1):11-9.

Nedergaard A, Henriksen K, Karsdal MA, Christiansen C. Menopause, estrogens and frailty. Gynecol Endocrinol 2013;29(5):418-23.

Nguyen N, Holodniy M. HIV infection in the elderly. Clin Interv Aging 2008;3(3):453-72.

Oertelt-Prigione S. The influence of sex and gender on the immune response. Autoimmun Rev 2012;11(6-7):A479-85.

Otero UB, Chor D, Carvalho MS, Faerstein E, Lopes Cde S, Werneck GL. Lack of association between age at menarche and age at menopause: Pro-Saude Study, Rio de Janeiro, Brazil. Maturitas 2010;67(3):245-50.

Palacios S, Henderson VW, Siseles N, Tan D, Villaseca P. Age of menopause and impact of climacteric symptoms by geographical region. Climacteric 2010;13(5):419-28.

Patterson K, Napravnik S, Eron J, Keruly J, Moore R. Effects of age and sex on immunological and virological responses to initial highly active antiretroviral therapy. HIV Med 2007;8(6):406-10.

Patterson KB, Cohn SE, Uyanik J, Hughes M, Smurzynski M, Eron JJ. Treatment responses in antiretroviral treatment-naive premenopausal and postmenopausal HIV-1-infected women: an analysis from AIDS Clinical Trials Group Studies. Clin Infect Dis 2009;49(3):473-6.

Pedro AO, Pinto Neto AM, Paiva LH, Osis MJ, Hardy E. [Age at natural menopause among Brazilian women: results from a population-based survey]. Cad Saude Publica 2003;19(1):17-25.

Perez-Molina JA, Mora Rillo M, Suarez-Lozano I, Casado-Osorio JL, Teira Cobo R, Rivas Gonzalez P, et al. Response to combined antiretroviral therapy according to gender and origin in a cohort of naive HIV-infected patients: GESIDA-5808 study. HIV Clin Trials 2012;13(3):131-41.

Pirrone V, Libon DJ, Sell C, Lerner CA, Nonnemacher MR, Wigdahl B. Impact of age on markers of HIV-1 disease. Future Virol 2013;8(1):81-101.

Programa Nacional de DST/AIDS. Boletim Epidemiológico de HIV/AIDS - ANO I nº01 - até semana epidemiológica 52ª -dezembro de 2012, Brasília, DF, 2012. Disponível em: http://www.aids.gov.br/sites/default/files/anexos/publicacao/2012/52654/boletim_2012_final_pdf_14028.pdf. Acesso 22 Out 2013.

Raboud J, Blitz S, Walmsley S, Thompson C, Rourke SB, Loutfy MR, et al. Effect of gender and calendar year on time to and duration of virologic suppression among antiretroviral-naive

HIV-infected individuals initiating combination antiretroviral therapy. HIV Clin Trials 2010;11(6):340-50.

Ramezani Tehrani F, Behboudi-Gandevani S, Ghanbarian A, Azizi F. Effect of menopause on cardiovascular disease and its risk factors: a 9-year follow-up study. Climacteric 2013. [epub ahead of print].

Research on the menopause in the 1990s. Report of a WHO Scientific Group. World Health Organ Tech Rep Ser 1996;866:1-107.

Rocca WA, Grossardt BR, Miller VM, Shuster LT, Brown RD, Jr. Premature menopause or early menopause and risk of ischemic stroke. Menopause 2012;19(3):272-7.

Schoenbaum EE, Hartel D, Lo Y, Howard AA, Floris-Moore M, Arnsten JH, et al. HIV infection, drug use, and onset of natural menopause. Clin Infect Dis 2005;41(10):1517-24.

Seifer DB, Golub ET, Lambert-Messerlian G, Springer G, Holman S, Moxley M, et al. Biologic markers of ovarian reserve and reproductive aging: application in a cohort study of HIV infection in women. Fertil Steril 2007;88(6):1645-52.

Shah PN, Smith JR, Wells C, Barton SE, Kitchen VS, Steer PJ. Menstrual symptoms in women infected by the human immunodeficiency virus. Obstet Gynecol 1994;83(3):397-400.

Shelling AN. Premature ovarian failure. Reproduction 2010;140(5):633-41.

Shuster LT, Rhodes DJ, Gostout BS, Grossardt BR, Rocca WA. Premature menopause or early menopause: long-term health consequences. Maturitas 2010;65(2):161-6.

Sidibe EH. Menopause in Africa. Ann Endocrinol (Paris) 2005;66(2 Pt 1):105-7.

Soon GG, Min M, Struble KA, Chan-Tack KM, Hammerstrom T, Qi K, et al. Meta-analysis of gender differences in efficacy outcomes for HIV-positive subjects in randomized controlled clinical trials of antiretroviral therapy (2000-2008). AIDS Patient Care STDS 2012;26(8):444-53.

Svejme O, Ahlborg HG, Nilsson JA, Karlsson MK. Early menopause and risk of osteoporosis, fracture and mortality: a 34-year prospective observational study in 390 women. BJOG 2012;119(7):810-6.

Thomas F, Renaud F, Benefice E, de Meeus T, Guegan JF. International variability of ages at menarche and menopause: patterns and main determinants. Hum Biol 2001;73(2):271-90.

Thorsteinsson K, Ladelund S, Jensen-Fangel S, Johansen IS, Katzenstein TL, Pedersen G, et al. Impact of gender on response to highly active antiretroviral therapy in HIV-1 infected patients: a nationwide population-based cohort study. BMC Infect Dis 2012;12:293.

Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. J Clin Endocrinol Metab 2007;92(7):2506-12.

Tumbarello M, Rabagliati R, de Gaetano Donati K, Bertagnolio S, Montuori E, Tamburrini E, et al. Older age does not influence CD4 cell recovery in HIV-1 infected patients receiving highly active antiretroviral therapy. BMC Infect Dis 2004;4:46.

UNAIDS Report on the Global AIDS Epidemic 2012. United Nations Programme on HIV/AIDS (UNAIDS). Disponível em: http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2012/gr2012/20121120_UNAIDS_Global_Report_2012_en.pdf Acesso em 22 Out 2013.

Utian WH, Woods NF. Impact of hormone therapy on quality of life after menopause. Menopause 2013;20(10):1098-105.

Valadares AL, Pinto-Neto AM, Abdo C, Melo VH. [HIV in middle-aged women: associated factors]. Rev Assoc Med Bras 2010;56(1):112-5.

van Benthem BH, Vernazza P, Coutinho RA, Prins M, European Study on the Natural History of HIV Infection in Women, the Swiss HIV Cohort Study. The impact of pregnancy and menopause on CD4 lymphocyte counts in HIV-infected women. AIDS 2002;16(6):919-24.

Viard JP, Mocroft A, Chiesi A, Kirk O, Roge B, Panos G, et al. Influence of age on CD4 cell recovery in human immunodeficiency virus-infected patients receiving highly active antiretroviral therapy: evidence from the EuroSIDA study. J Infect Dis 2001;183(8):1290-4.

Wellons M, Ouyang P, Schreiner PJ, Herrington DM, Vaidya D. Early menopause predicts future coronary heart disease and stroke: the Multi-Ethnic Study of Atherosclerosis. Menopause 2012;19(10):1081-7.

Wellons MF, Sanders L, Edwards LJ, Bartlett JA, Heald AE, Schmader KE. HIV infection: treatment outcomes in older and younger adults. J Am Geriatr Soc 2002;50(4):603-7.

Yan J, Soon G, Zhou S, Min M, Chan-Tack K, Struble K, et al. (2013) Differences by age for women in the response to initial HAARTs: Meta-analysis from clinical studies submitted to the FDA (2000-2010). Third International Workshop on HIV and Women. Proceedings. Toronto, Canada, 2013. Reviews in Antiviral Therapy & Infectious Diseases 1: O-11.

Yao G, Hou Y. Recent advances for research on effect of estrogen on immune system. Wei Sheng Yan Jiu 2004;33(6):759-61.

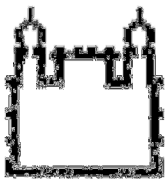
Yasui T, Hayashi K, Mizunuma H, Kubota T, Aso T, Matsumura Y, et al. Factors associated with premature ovarian failure, early menopause and earlier onset of menopause in Japanese women. Maturitas 2012 ;72(3):249-55.

Yin MT, Shi Q, Hoover DR, Anastos K, Sharma A, Young M, et al. Fracture incidence in HIV-infected women: results from the Women's Interagency HIV Study. AIDS 2010;24(17):2679-86.

Yin MT, Zhang CA, McMahon DJ, Ferris DC, Irani D, Colon I, et al. Higher rates of bone loss in postmenopausal HIV-infected women: a longitudinal study. J Clin Endocrinol Metab 2012;97(2):554-62.

ANEXOS

ANEXO A–Cartas de Aprovação do Comitê de Ética em Pesquisa do IPEC



Ministério da Saúde

Fundação Oswaldo Cruz — FIOCRUZ

CENTRO DE PESQUISA HOSPITAL EVANDRO CHAGAS

COMITÊ DE ÉTICA EM PESQUISA - CEP/CPqHEC

PARECER

Rio de Janeiro, 10 de dezembro de 2001

Deliberação: APROVADO
Título do Projeto: "História Natural da Infecção pelo Papilomavírus Humano em uma Coorte de Mulheres Infectadas pelo HIV no Rio de Janeiro".
Protocolo nº CEP: 020/2001
Pesquisador Responsável: Beatriz Grinsztejn
Instituição: CPqHEC / FIOCRUZ

Projeto visa descrever a história natural da infecção pelo HPV em uma coorte de mulheres infectadas pelo HIV no Rio de Janeiro, considerando-se: a) a prevalência e a incidência da infecção pelo HPV nesta coorte; b) o tempo de persistência da infecção pelo HPV nesta coorte; c) a prevalência e incidência de lesões intra-epiteliais cervicais nesta coorte, e a proporção de remissões espontâneas, recorrências, persistência e progressão destas lesões nesta coorte.

A metodologia está adequada para o tipo de estudo a ser realizado e o termo de consentimento bem elaborado em linguagem accessível.

Após análise deste CEP quanto à viabilidade e aos aspectos éticos do estudo e de acordo com a Resolução 196/96 do CNS-MS, concluiu-se pela **aprovação** do protocolo de pesquisa apresentado.

Atenciosamente,

Dra. Léa Camillo - Coura
Coordenadora do Comitê de
Ética em Pesquisa
CPqHEC / FIOCRUZ

Ministério da Saúde

FIOCRUZ
Fundação Oswaldo Cruz
Instituto de Pesquisa Clínica Evandro Chagas

I P E C

Comitê de Ética em Pesquisa

PARECER CONSUBSTANCIADO – 043/2010

Protocolo 0032.0.009.000-10

1. Identificação:
Título do Projeto: "Estudo Longitudinal da História Natural da Infecção pelo HIV em pacientes acompanhados no IPEC-FIOCRUZ".
Pesquisador Responsável: Beatriz Grinsztejn.
Instituição Responsável: Instituto de Pesquisa Clínica Evandro Chagas/FIOCRUZ.
Data de Apresentação ao CEP: 30/07/2010.

2. Sumário:
Trata-se de um estudo de coorte, retrospectivo, entre os pacientes acompanhados no IPEC a partir de janeiro de 1986. Tem como objetivos: a) Avaliar a resposta a terapia antirretroviral e subsequente e seus preditores no contexto de uma coorte urbana de apcientes infectados pelo HIV/AIDS em acompanhamento num serviço de referência, o Instituto de Pesquisa Clínica Evandro-IPEC/Fiocruz, que iniciaram o uso de terapia antirretroviral potente (HAART); b) Caracterizar o perfil dos eventos não associados ao HIV/AIDS, tais como eventos cardiovasculares, renais, hepáticos, neoplasias, ósseos, metabólicos entre outros, estimar sua incidência e fatores de risco nos períodos pré e pós disponibilização da terapia antirretroviral potente no Brasil; c) Descrever as causas de morte nessa coorte, analisando o risco competitivo das diferentes causas de morte envolvidas; d) Estudar o diagnóstico tardio da infecção pelo HIV: seus preditores e impacto do diagnóstico tardio na sobrevida; e) Estimar a frequencia de eventos graves e que motivaram a troca de medicamentos/esquemas terapêuticos relacionados à toxicidade dos ARV, os fatores associados à sua ocorrência nesta coorte de pacientes com HIV/AIDS acompanhados no IPEC/Fiocruz; f) Estimar a prevalência de toxicidadfe de curto e longo prazo nessa coorte; g) Estimar as taxas de interrupção e modificação dos esquemas HAART inicial e subsequentemantes e seus fatores associados; h) Estabelecer o tempo até a modificação ou interrupção do primeiro esquema e dos esuqemas HAART subsequentes destes pacientes; i) Estudar o processo de senescência nos indivíduos infectados pelo HIV e suas repercussões no perfil de morbimortalidade, no tratamento antirretroviral e consumo de serviço de saúde; j) Estudar a efetividade dos esquemas HAART na coorte de pacientes com idade igual ou superior a 50 anos acompanhados no IPEC/Fiocruz; k) Estudar a tolerabilidadade e segurança dos esquemas HAART na coorte de pacientes com idade igual ou superior a 50 anos acompanhados no IPEC/Fiocruz; l) Acessar o perfil de morbimortalidade na coorte de pacientes com igual ou superior a 50 anos acompanhados no IPEC/Fiocruz; m) Estudar o consumo de serviços hospitalares e ambulatoriais dessa população de pacinets. Os dados serão obtidos através da base de dados da coorte de pacientes com HIV/AIDS do IPEC/Fiocruz e sempre que necessário através da revisão dos prontuários. Para o cálculo da frequência absoluta e relativa das doenças indicativas presentes na definição do caso de AIDS, as análises serão realizadas para o período como um todo (1986 até 2009 podendo se estender posteriormente em novas revisões), e para cada um dos períodos específicos: 1986-1990; 1991-1995 e 1996-2005, 2006-2009, que correspondem às eras de ausência de terapia antirretroviral; instituição da monoterapia e terapia dupla; instituição da terapia potente

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(HAART) e períodos subseqüentes englobando importantes modificações nas rotinas de tratamento.

3. Observações Gerais: (Atendendo à Resolução CNS 196/96).
Projeto com delineamento adequado. Para os pacientes em acompanhamento ativo na coorte ou iniciando acompanhamento será aplicado Termo de Consentimento Livre e Esclarecido, elaborado em linguagem acessível ao sujeito de pesquisa. Para os pacientes cujo acompanhamento resultou em perda de seguimento ou óbito, para os quais não será possível a utilização de TCLE, foi confeccionado um Termo de Confidencialidade para que seja assegurado que as informações obtidas serão de caráter confidencial e serão utilizadas apenas para fins científicos. Os atendimentos ambulatoriais e os exames laboratoriais de rotina e para diagnóstico serão realizados de acordo com o preconizado para cortejo de portadores de HIV no IPEC, sem custo adicional.

4. Diligências:
Não houve.

5. Parecer: APROVADO.

Data da Reunião: 13 de setembro de 2010.

Assinatura do Coordenador:

Dr.ª Léa Camillo-Coura
Coordenadora do Comitê
de Ética em Pesquisa

ANEXO B– Análise para verificar o pressuposto de proporcionalidade do modelo de Cox do artigo *Factors associated with earlier age at natural menopause in HIV-infected women in Brazil*.

Outputs do pacote estatístico "R" das análises de correlação do resíduo de Schoenfeld.

1) Modelo 1: Idade natural da menopausa.

Variáveis	rho	chisq	p
menarcacod11<11	-0.04589	0.27650	0.5990
paratdcat>=3	-0.07568	0.78414	0.3759
paratdcat1	-0.06173	0.53416	0.4649
paratdcat2	-0.08430	0.98603	0.3207
fumatdSim	-0.08234	1.06138	0.3029
imctdOverweight/Obesity	0.07386	0.77397	0.3790
imctdUnderweight	0.09294	1.17061	0.2793
HCVSEDtdSim	-0.10510	1.57261	0.2098
cd4td200-349	-0.04824	0.31752	0.5731
cd4td100-199	-0.08938	1.22317	0.2687
cd4td50-99	-0.00867	0.00988	0.9208
cd4td<50	-0.14305	2.82640	0.0927
aidsxnaoaidsAIDS	-0.14335	2.65050	0.1035
tempoHAARTcat0|-10	-0.14888	3.18826	0.0742
GLOBAL	NA	17.42379	0.2343

2) Modelo 2: Idade natural precoce da menopausa (≤45 anos).

Variáveis	rho	chisq	p
menarcacod11<11	-0.04769	0.08358	0.7725
media25 ou mais copos	0.03241	0.03835	0.8447
media23 a 4 copos	-0.09402	0.28402	0.5941
media21 a 2 copos	0.23218	2.29504	0.1298
fumatdSim	0.25312	2.76467	0.0964
imctdOverweight/Obesity	0.07412	0.21295	0.6445
imctdUnderweight	-0.16263	1.17628	0.2781
HCVSEDtdSim	-0.00941	0.00324	0.9546
cd4td200-349	-0.07150	0.18395	0.6680
cd4td100-199	0.02773	0.03424	0.8532
cd4td50-99	-0.11232	0.56266	0.4532
cd4td<50	-0.09292	0.33566	0.5623
aidsxnaoaidsAIDS	0.12259	0.52017	0.4708
tempoHAARTcat0|-10	-0.21792	1.65430	0.1984
GLOBAL	NA	11.70760	0.6298

Conclusão: para nível de significância de 0,05 não rejeitamos a hipótese de que os *hazards* são proporcionais tanto para nenhuma covariável quanto para a avaliação global do ajuste do modelo. Ou seja, para nenhuma variável ajustada no modelo houve violação do pressuposto de proporcionalidade.

ANEXO C– Análise de sensibilidade para avaliar o impacto da ausência de informação da variável carga viral para o HIV na resposta virológica ao primeiro esquema antirretroviral do artigo *Absence of Effect of Menopause Status at Initiation of First-Line Antiretroviral Therapy on Immunologic or Virologic Responses: A Cohort Study from Rio de Janeiro, Brazil*

	Valores observados				***Missing*=não sucesso**				***Missing*=sucesso**			
Variáveis	**OR**	**LI**	**LS**	**p**	**OR**	**LI**	**LS**	**p**	**OR**	**LI**	**LS**	**p**
6 meses												
factor(meno)1	**1,01**	**0,86**	**1,18**	**0,93**	**1,02**	**0,87**	**1,20**	**0,79**	**1,00**	**0,88**	**1,14**	**0,97**
logcv	0,94	0,87	1,01	0,08	1,00	0,94	1,08	0,92	0,93	0,88	0,99	0,02
ITRNN	1,06	0,92	1,21	0,44	1,10	0,96	1,26	0,18	1,03	0,92	1,15	0,66
cd4_baseline	1,00	1,00	1,00	0,92	1,00	1,00	1,00	0,19	1,00	1,00	1,00	0,72
factor(do_concomitante)1	1,11	0,94	1,31	0,24	0,97	0,82	1,14	0,73	1,13	0,98	1,29	0,09
12 meses												
factor(meno)11	**1,02**	**0,88**	**1,19**	**0,76**	**1,03**	**0,88**	**1,20**	**0,74**	**1,01**	**0,89**	**1,16**	**0,83**
logcv1	0,96	0,90	1,02	0,20	0,94	0,88	1,01	0,09	0,97	0,91	1,03	0,29
ITRNN	0,97	0,85	1,11	0,67	0,91	0,80	1,05	0,19	1,00	0,89	1,12	1,00
cd4_baseline1	1,00	1,00	1,00	0,91	1,00	1,00	1,00	0,14	1,00	1,00	1,00	0,65
factor(do_concomitante)11	1,07	0,91	1,27	0,41	1,00	0,85	1,18	0,98	1,08	0,94	1,24	0,27
24 meses												
factor(meno)12	**1,01**	**0,85**	**1,20**	**0,87**	**1,00**	**0,85**	**1,17**	**0,99**	**1,02**	**0,88**	**1,18**	**0,81**
logcv2	0,96	0,89	1,03	0,27	0,98	0,91	1,05	0,51	0,96	0,90	1,02	0,20
ITRNN	1,09	0,94	1,26	0,26	1,08	0,94	1,24	0,29	1,07	0,94	1,21	0,29
cd4_baseline2	1,00	1,00	1,00	0,98	1,00	1,00	1,00	0,54	1,00	1,00	1,00	0,73
factor(do_concomitante)12	1,05	0,87	1,26	0,63	1,02	0,86	1,21	0,80	1,05	0,90	1,22	0,52

OR: Razão de chance; LI: Limite inferior; LS: Limite superior; logcv: log 10 carga viral do HIV basal; ITRNN: Inibidores da Transcriptase Reversa Não Análogos de Nucleosídeos; do_concomitante: Doença definidora de aids; Variável de interesse: factor (meno); Variáveis de ajuste: logcv, ITRNN, cd4_baseline e factor(do_concomitante).

Conclusão: As análises de sensibilidade realizadas para avaliar o impacto da ausência de informação na avaliação da eficácia do esquema antirretroviral não mostraram potencial viés de seleção.