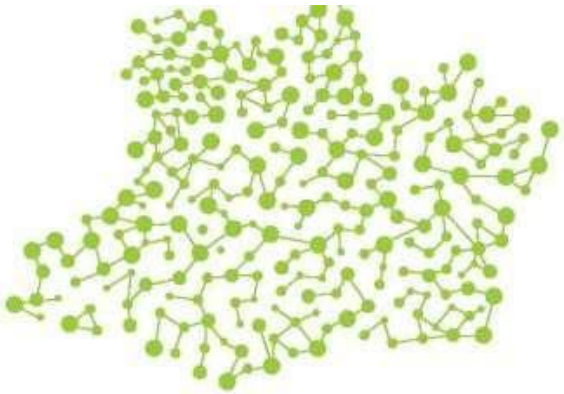
UNIVERSIDADE DO ESTADO DO AMAZONAS
FUNDAÇÃO DE MEDICINA TROPICAL DR. HEITOR VIEIRA DOURADO
PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA TROPICAL
DOUTORADO EM DOENÇAS TROPICAIS E INFECCIOSAS

HIV/AIDS EM CRIANÇAS NO ESTADO DO AMAZONAS: ANÁLISE DA TAXA DE TRANSMISSÃO VERTICAL E DE RESISTÊNCIA TRANSMITIDA

SOLANGE DOURADO DE ANDRADE

MANAUS
2017



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Tese apresentada ao Programa de Pós-Graduação em Medicina Tropical da Universidade do Estado do Amazonas em Convênio com a Fundação de Medicina Tropical Dr. Heitor Vieira Dourado**,** para obtenção grau de *Doutor em Doenças Tropicais e Infecciosas.*

Orientadora: **Prof.ª** Dra. Adele Schwartz Benzaken
Coorientadora: **Prof.ª** Dra. Meritxell Sabidó Espin
Coorientador: **Prof.** Dr. Amilcar Tanuri

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FOLHA DE JULGAMENTO

HIV/AIDS EM CRIANÇAS NO ESTADO DO AMAZONAS: ANÁLISE DA TAXA DE TRANSMISSÃO VERTICAL E DE RESISTÊNCIA TRANSMITIDA

SOLANGE DOURADO DE ANDRADE

"Esta Tese foi julgada adequada para obtenção do Título de Doutor em Doenças Tropicais e Infecciosas, aprovada em sua forma final pelo Programa de Pós-Graduação em Medicina Tropical da Universidade do Estado do Amazonas em convênio com a Fundação de Medicina Tropical Dr. Heitor Vieira Dourado".

Banca Julgadora:

_____
Presidente

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Membro

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Membro

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Membro

_____
Membro

DEDICATÓRIA

A todas as crianças e adolescentes
que um dia eu tive a chance de atender

AGRADECIMENTO

Acima de tudo, a Deus!

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EPÍGRAFE

"Luck in science smiles on prepared minds"

Louis Pasteur

RESUMO

Introdução: Crianças se infectam pelo HIV na maior parte dos casos, por transmissão da mãe para o filho que pode ocorrer em três situações: período gestacional; nascimento ou aleitamento materno. A taxa de transmissão vertical (TV) estimada no Brasil em 2001, era de 7.1%. A profilaxia preconizada para uso de medicação nos momentos de risco, consegue reduzir em quase 70% os casos de crianças infectadas. As crianças que porventura nascem infectadas, podem ter recebido formas resistentes do vírus, comprometendo a eficácia da terapia inicial. **Objetivo:** Caracterizar o perfil da epidemia de HIV/Aids em crianças, no estado do Amazonas, estimando a taxa de TV do HIV em crianças expostas e os fatores associados, no período de 1999 a 2011; determinar a taxa de resistência transmitida nas crianças infectadas pelo HIV entre 2010 e 2015, em um serviço de referência em infectologia pediátrica na cidade de Manaus, Brasil. **Metodologia:** Foi realizado levantamento de prontuários e fichas clínico-epidemiológicas das crianças expostas ao HIV nascidos entre janeiro de 1999 a dezembro de 2011 para estudo da taxa de transmissão vertical do HIV. Foram avaliados os exames de genotipagem de crianças virgens de TARVc e infectadas pelo HIV por transmissão materno infantil, entre 2010 e 2015, para verificação de resistência. **Resultados:** 1037 crianças foram incluídas no estudo. 68 crianças ficaram infectadas no período, resultando em taxa de transmissão vertical de 6,6 % (95% CI: 5,3 - 8,3). Entre as mães, 76,1% havia feito uso de terapia antirretroviral em algum momento da gestação. Ter sido amamentado estava relacionado com risco de infecção na criança OR 4,5 (95% CI: 2,19-9,50). Nos 117 exames genotípicos estudados, foi observada uma diversidade de HIV -1 circulantes e a cocirculação de subtipos puros como B (80,2%), C e F e de formas recombinantes únicas (BF1), incluindo mosaicos com subtipos como D e K. A prevalência encontrada de mutações de resistência foi alta, de 21,4%. A prevalência de resistência transmitida foi alta, sendo de 21,1% em crianças que receberam profilaxia para transmissão vertical e de 14,3% em crianças que não receberam profilaxia. **Conclusão:** Pelo que foi observado, as estratégias de prevenção apresentaram melhoria no estado, porém a TV ainda ocorre em taxas altas. Mais esforços são necessários para que mulheres e crianças consigam completar satisfatoriamente a cascata de cuidados de profilaxia da TV, com expansão de locais que ofereçam cuidados preventivos e fortalecendo acesso aos ARV. Pode ser observado um complexo padrão na epidemia no estado, com expansão do subtipo C. A variedade de padrões recombinantes de subtipos de HIV – 1, indica grande circulação de mosaicos no estado do Amazonas e sinaliza a necessidade de vigilância da diversidade na região. Baseado no estudo genotípico, observamos uma extensa proporção de crianças recém diagnosticadas, infectadas pelo HIV, carreando vírus resistentes, em particular à classe de inibidores de transcriptase reversa não nucleosídios. Atenção especial deve ser dada para as terapias a serem utilizadas nessa população que apresenta resistência transmitida.

Palavras chave: HIV, transmissão vertical, resistência, crianças

ABSTRACT

Introduction: Children are mostly infected by HIV through transmission from the mother, wich may occur in three situations: during pregnancy, birth or maternal breastfeeding**.** The vertical transmission rate estimated in Brasil in 2001, was 7.1%. If used, prophylaxis at three major peridos of risk, can reduce almost 70% the number of infected children born with HIV. Children that are born infected may have received resistent forms from the mother, wich may compromisse treatment efficacy. **Objective:** Caracterize the epidemic profile of HIV/Aids in children, in the state of Amazonas, estimating the mother to child transmission rate and factors associated to it, within the period from 1999 to 2011; determining the transmission resistance rate in infected children between 2010 to 2015, in a reference center for pediatric infectology, in the city of Manaus, Brazil. **Methodology:** Medical records and social epidemiologic data from hiv exposed children born between January 1999 and December 2011, were reviewd to study mother to child transmission data. Genotyping exames performed in naive treatment children infected by vertical transmission, between 2010 and 2015, were reviewed for resistance anlysis. **Results:** 1037 children were included in the sudy. 68 children became infected during this period of time, resulting in a vertical transmission rate of 6.6 % (95% IC: 5.3 – 8.3). Among the mothers, 76,1% had used some kind of prophylaxis in any moment of pregnancy. Being breastfed were related with infection in child, OR 4.5 (95% CI: 2,19-9,50). Over the 117 genotypic exams studied, a diversity of circulating HIV-1 subtypes and co circulation of pures, like B (80.2%), C and F subtypes, were observed. Recombinants unique forms (BF1), including mosaics with subtypes as D and K were also found. The prevalence founded of resistance mutations were high, 21.4%. The transmited resistance rate was also high, 21.1% in children that received prophylaxis and 14.3% for children that did not receive prophylaxis for mother to child transmission. **Conclusion:** Our observations indicate improvements in MTCT prevention strategies in the Amazonas, but MTCT continues to occur at a high rate. More efforts are needed leading to more women and babies successfully completing the PMTCT cascade, expanding sites that offer PMTCT of HIV and strengthening linkage to ART care. HIV-1 subtype showed a complex epidemic profile with expansion of subtype C. The variety of patterns of inter-subtype HIV-1 recombinants observed indicate a wide circulation of mosaic viruses in Amazonas state and highlight the need for surveillance of HIV-1 diversity in the region. Based on the genotyping study, our data show that an extensive proportion of newly diagnosed HIV-infected infants and young children carry resistant virus, in particular to NNRTI. Special attention should be addressed to the treatment to be used in this population with transmitted resistance.

Key words: HIV, vertical transmission, resistance, children

RESUMO LEIGO

A infecção pelo HIV acomete as crianças, na maioria dos casos, por transmissão da mãe para o filho. A infecção da criança pode acontecer enquanto ela está ainda na barriga da mãe, ou seja, durante a gestação ou na hora do parto. Após o nascimento, se a criança for amamentada também pode adquirir a infecção pelo HIV. Isso pode acontecer quando a mãe está grávida, tem HIV e não toma medicamentos para proteger seu filho da infecção. Esses medicamentos, chamados de profilaxia se tomados pela mãe e adotadas outras providências, como não amamentação, podem ajudar a proteger o bebê da infecção pelo HIV. Porém se mãe tomar medicação, mas de modo errado, pode acontecer de deixar o vírus resistente no seu sangue e ainda transmitir para o bebê esse tipo de vírus, que é mais difícil de tratar. Esse estudo, teve objetivo de analisar a taxa de transmissão do HIV da mãe para o filho no estado do Amazonas, no período de 1999 até 2011. Também teve objetivo de analisar o perfil de resistência que as crianças que nasceram infectadas, apresentaram quando iriam iniciar tratamento entre 2010 e 2015. O exame que possibilita verificar essa resistência é chamado de genotipagem. Foi então possível verificar que das 1037 crianças incluídas no estudo da transmissão vertical, 6,6 % delas ficaram infectadas. Essa taxa é considerada alta. E quando foram estudados os exames de genotipagem das crianças que iniciaram tratamento entre 2010 e 2015, observamos que 21.1% delas tinham recebido alguma resistência transmitida da mãe. Concluímos que são necessários maiores cuidados na região, para mães infectadas pelo HIV e seus bebês, para que possam receber a profilaxia e nascer sem o HIV. Observamos ainda, muitos subtipos circulantes de HIV no estado do Amazonas e formas resistentes nos bebês que se infectaram.

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LISTA DE ABREVIATURAS, SÍMBOLOS E UNIDADES DE MEDIDA

ABC	Abacavir
AIDS	Síndrome da Imunodeficiência Adquirida
ARV	Antirretroviral
AZT	Zidovudina
CCR5	Correceptor de quimiocina R5
CDC	*Center for Disease Control and Prevention* – Estados Unidos
CD4	Receptor de membrana presente no Linfócito T Helper e em outras células
CPR	Algoritmo de Resistência Populacional Calibrada
CV	Carga viral
CXCR-4	Correceptor de quimiocina X4
DNA	Ácido desoxirribonucleico
DRM	Mutações de resistência a drogas
ddI	Didanosina
DIAHV	Departamento de Vigilância, Prevenção e Controle das Infecções Sexualmente Transmissíveis, do HIV/Aids e das Hepatites Virais
d4T	Estavudina
EFZ	Efavirenz
ELISA	*Enzyme-linked immunosorbent assay*
FDA	*Food and Drug Administration*
HAART	*Highly active antiretroviral therapy*
ID	Número exclusivo
FMT- HVD	Fundação de Medicina Tropical Dr. Heitor Vieira Dourado
INI	Inibidor de Integrase
IP	Inibidor de Protease
ITRN	Inibidor de Transcriptase Reversa Nucleosídeo
ITRNN	Inibidor de Transcriptase Reversa Não análogo de Nucleosídeo
HIV	Vírus da Imunodeficiência Humana
LT- CD4	Linfócito T CD4+ (Linfócito T Helper)
HTLV	Vírus Linfotrópico Humano
NVP	Nevirapina

MS	Ministério da Saúde
OMS	Organização Mundial de Saúde
OPAS	Organização Panamericana de Saúde
PACTG	*Pediatric Aids Clinical Trial Group*
PCR	Reação em cadeia da polimerase *(polymerase chain reaction)*
PCDT	Protocolo clínico e diretrizes terapêuticas
PTV	Prevenção da transmissão vertical
PTMI	Profilaxia da transmissão materno infantil
PDR	Resistência à droga pré tratamento
PVHA	Pessoa vivendo com HIV*/*Aids
RAL	Raltegravir
Renageno	Rede Nacional de Genotipagem
RNA	Ácido ribonucleico
RENIC	Rede Nacional de Isolamento e Caracterização do HIV - 1
RN	Recém-nascido
SAE	Serviço de Assistência Especializada
SISGENO	Sistema de Controle de Exames de Genotipagem
SNC	Sistema Nervoso Central
SUS	Sistema Único de Saúde
SVS	Secretaria de Vigilância em Saúde
TAM	Mutações para os análogos de timidina
TARV	Terapia antirretroviral
TARVc	Terapia antirretroviral combinada
TDF	Tenofovir
TDR	Resistência transmitida à droga
TMI	Transmissão materno infantil
TR	Teste rápido
TV	Transmissão vertical
WB	Western Blot
3TC	Lamivudina

SUMÁRIO

1. INTRODUÇÃO

1.1 O Vírus da Imunodeficiência Humana (HIV) e a Aids

O HIV é um retrovírus não-citopático e não-oncogênico (Figura 1), que invade seletivamente células do sistema imune humano, incapacitando suas funções protetoras de modo irreversível. Circula na África Central desde 1970 tendo alcançado todos continentes e infectado milhões de pessoas.[1] [2]

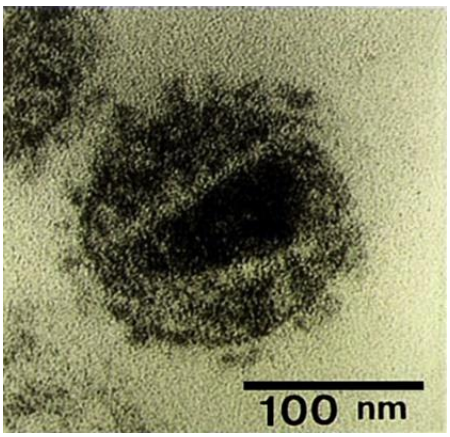


Figura 1: Microscopia eletrônica do vírus identificado em 1983, no Instituto Pasteur. Fonte: Science 1983

Pertencente à família *Retroviridae* e subfamília *Lentivirinae*, o HIV como os outros lentivírus, tem características genotípicas complexas que os diferem dos demais retrovírus. A maioria dos retrovírus possui três genes: *gag, pol e env*. No entanto, o HIV possui mais 6 genes em seu genoma de nove kilobases, intitulados *vif, vpu, vpr, tat, rev e nef*. É um vírus envelopado de formato icosaédrico, com 72 espículas externas. O core viral contém duas cópias de fitas simples de ácido ribonucleico (RNA). Associada ao genoma, encontra-se a enzima transcriptase reversa, responsável pela transcrição do RNA viral em fita dupla de ácido desoxirribonucleico (DNA), que o habilita a se integrar ao genoma do hospedeiro. Outras duas enzimas, integrase e protease também integram a unidade. O core tem em sua constituição, 4 proteínas do nucleocapsídio, p24, p17, p9 e p7. [3] [4]

A parte interna do nucleocapsídio é protegida principalmente por uma camada constituída pela p24 e a camada lipídica interna, pela p17, essa originada do hospedeiro infectado. O envelope externo além de conter várias proteínas do hospedeiro, contém ainda espículas formadas pelas proteínas virais gp120 e gp41. [3] [4] (Figura 2)

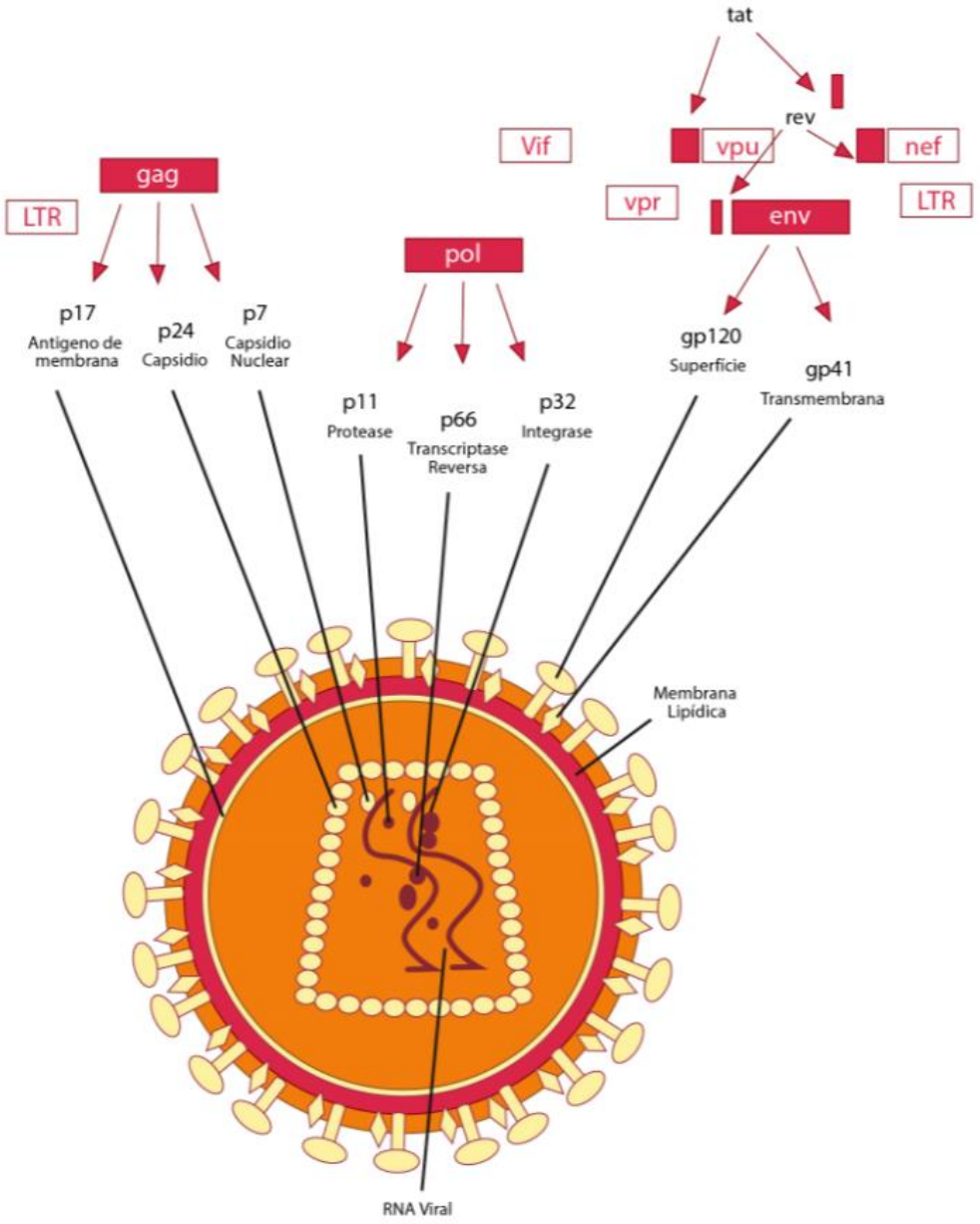


Figura 2: Desenho esquemático do HIV-1 com seus genes. Adaptado de Flossie

O gene *pol* codifica as enzimas transcriptase reversa, protease e integrase enquanto *gag* e *env* respectivamente, codificam polipeptídeos do nucleocapsídeo e proteínas de superfície. [4]

São dois os tipos de HIV, denominados HIV-1 e HIV-2. Isolado em 1983, a partir de células de linfonodo de um paciente com linfoadenopatia, o HIV-1, é o maior responsável pela pandemia atual e apresenta subtipos com distribuição mundial.[5] O HIV-2, uma variante menos patogênica e que acomete um número menor de indivíduos, foi isolado em 1986 de pacientes africanos e está mais concentrado na África. [6] [7] A homologia genética entre os dois tipos virais (40 a 45%), explica síntese de diversos produtos antigênicos semelhantes, residindo a diferença basicamente na codificação do envelope glicoproteico.[8]

Em março de 1984, o recém isolado vírus, então denominado HTLV III, pela similaridade com o vírus Linfotrópico Humano (HTLV), foi relacionado por um grupo de pesquisadores americanos, liderados por Robert Gallo, como agente causador da Síndrome da Imunodeficiência Adquirida (AIDS), descrita, cerca de dois anos antes, em 1981. O novo retrovírus, da família HTLV, havia sido identificado por Montagnier e Chermann, no Instituto Pasteur, na França, em 1983, e nomeado como LAV. [5] [9] Posteriormente, foi denominado AIDS-associated Virus (ARV) e Immunodeficiency Associated Virus (IDAV) e somente em 1986, recebeu o nome definitivo de Human Immunodeficiency Virus - HIV, pelo Comitê Internacional de Nomenclatura.[10]

Dentro da classificação genética, é possível distinguir quatro grupos de HIV-1: grupo M (Maior), grupo O (Outlier), grupo N (não M, não O) e o mais recente, grupo P, descoberto em 2009. Os grupos são ainda subdivididos em subtipos. O grupo M, responsável pela infecção de 95% dos pacientes no mundo, tem nove subtipos (A-D, F-H e K). O subtipo C é o mais disseminado globalmente enquanto subtipo B, predomina em países desenvolvidos. [11] [12] [13] [14]

Em suas primeiras observações, pesquisadores franceses, do Instituto Pasteur, identificaram que o novo vírus apresentava tropismo para linfócitos T CD4+ e assim exercia sua patogenicidade. [15]

Ao ingressar no organismo humano, o HIV-1, busca atingir células que apresentem em sua superfície, os marcadores CD4. A grande afinidade da gp120 presente no seu envelope, pelos receptores CD4, é determinante para iniciar o processo infeccioso. Tais receptores, são encontrados predominantemente nas superfícies dos linfócitos T Helper. Em menor quantidade, mas suficiente para infecção viral, estão presentes também, na superfície de células das linhagens monocitárias e macrofágicas do sangue periférico, células de Langerhans, células dendríticas foliculares e ainda outras células como da micróglia, integrantes do Sistema Nervoso Central (SNC). Através da ligação ao receptor CD4 e se utilizando de correceptores, CXCR-4 ou CCR-5, o vírus consegue se fundir à superfície celular e penetrar no citoplasma. Uma vez em ambiente citoplasmático, inicia processo de elaboração do seu mecanismo de domínio celular, a transcrição. O material genético viral originalmente composto de duas cadeias simples de RNA, é transcrito pela enzima transcriptase reversa, em cadeia de DNA, com objetivo de integração ao genoma humano. [16] [17] [18] [19]

Através da enzima intitulada integrase, uma das enzimas do HIV-1, o DNA recém-sintetizado, migra para o núcleo celular e é integrado ao genoma humano definitivamente, determinando assim infecção permanente. Ao ocorrer ativação da célula pelo sistema imune por qualquer fator, inicia-se também produção de RNA mensageiro, a partir do DNA proviral que migra para o citoplasma e sintetiza proteínas virais. Com citoplasma celular já contendo todas enzimas e proteínas virais essenciais, novos vírions são produzidos continuamente. Uma vez na corrente sanguínea, em processo de maturação, entra em ação a enzima viral protease e assim que maturados, os vírus recém produzidos, estão prontos para infectar novas células CD4+. [7] [2] (Figura 3)

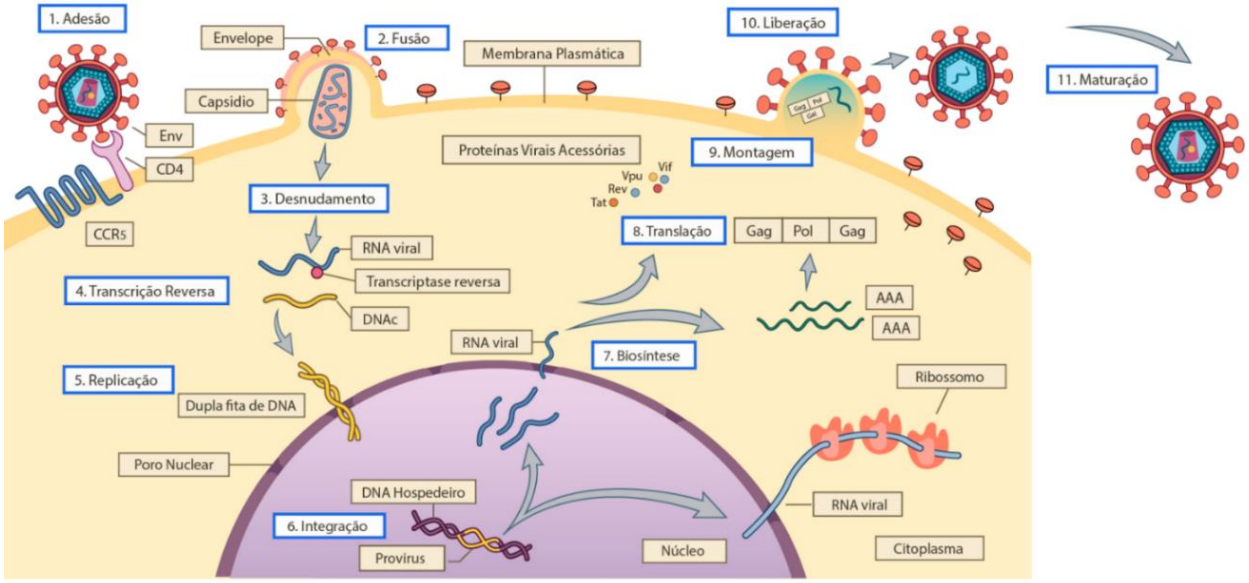


Figura 3: Ciclo do HIV-1dentro de célula CD4. Adaptado de www.kmph.matrik.edu.my

A partir de então, a replicação viral desencadeia resposta imune com ativação de toda cascata em especial células CD4+. Paradoxalmente, esta ativação, proporciona maior invasão viral por estimular que mais virions sejam produzidos. A intensa replicação viral ganha os tecidos linfoides e uma resposta imune robusta, porém ineficaz se estabelece. A carga de vírus no sangue pode reduzir nesta etapa, entretanto uma viremia persistente e crônica se instala. Clinicamente, na fase inicial, ocorre aumento das cadeias ganglionares e leve sintomatologia inespecífica, com febre, mialgia e possível esplenomegalia. Na sequência um longo período de latência clínica se segue até que níveis de CD4 estejam depletados a ponto de permitir surgimento de infecções oportunistas. [20] [21] [22] (Figura 4)

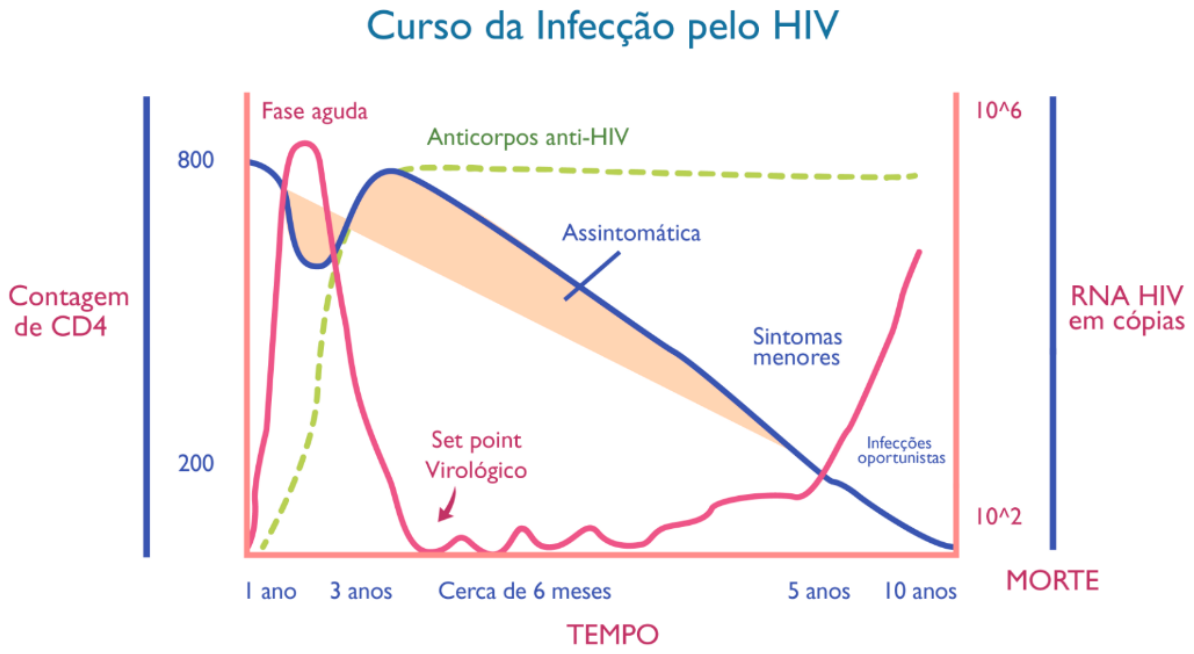


Figura 4: Curso clínico da infecção pelo HIV-1. Modificado de Jones 2003

1.2 Epidemiologia do HIV/Aids no mundo

Em 2016, segundo a Organização Mundial de Saúde (OMS) e Programa Conjunto das Nações Unidas sobre HIV/Aids (UNAIDS), 36,7 milhões de pessoas viviam com HIV no mundo.[23] [24] Dados globais da epidemia, segundo a UNAIDS, revelam que das pessoas vivendo com o HIV em 2016, 2,1 milhões eram crianças menores de 15 anos e 17,8 milhões , mulheres. [24] Embora a epidemia seja variável entre países e regiões, o número de pessoas infectadas pelo HIV no mundo vem apresentando declínio.[25] (Figura 5)

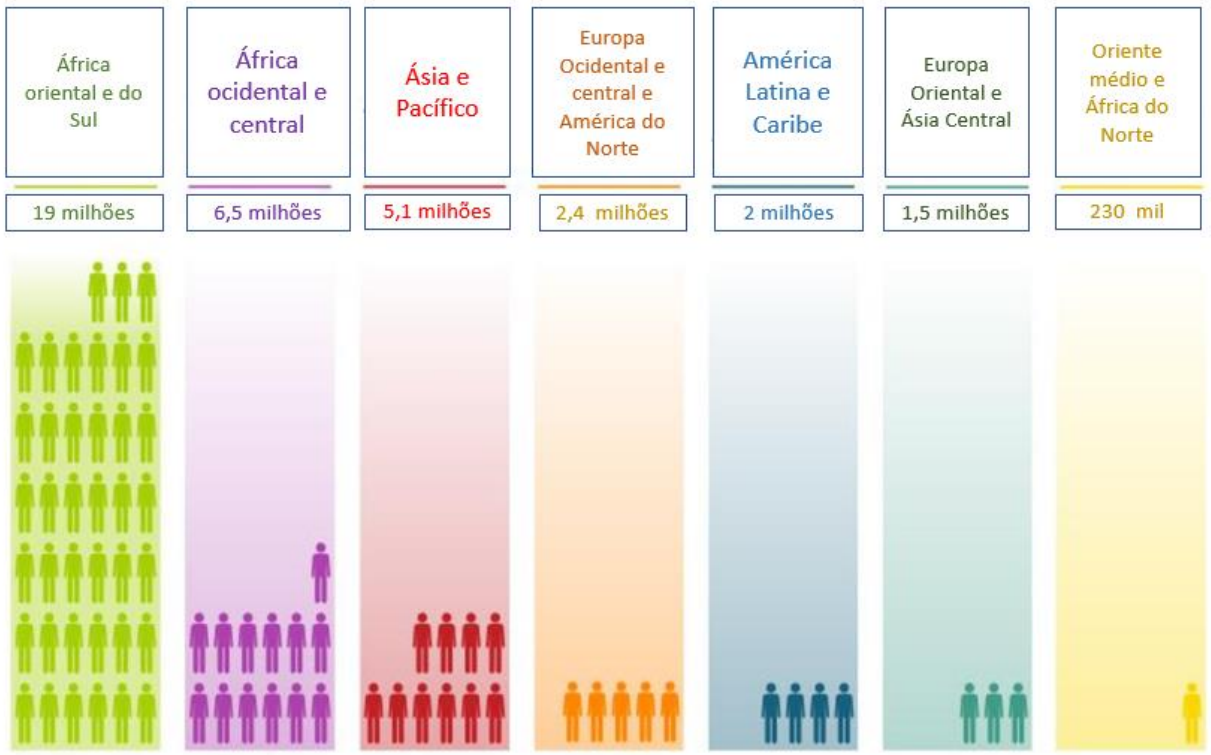


Figura 5: Quantidade de pessoas vivendo com HIV. Fonte Unaids 2016

Desde a descoberta dos primeiros casos registrados em 1981, aproximadamente 35 milhões (28,9 milhões – 41,5 milhões) de pessoas morreram de causas relacionadas à Aids.[26] Dados da UNAIDS mostram que o número de mortes por Aids está em declínio. Em 2005, o número de mortes foi cerca de 2,3 (2,1 – 2,6) milhões de pessoas e diminui para 1 milhão (830.000–1,2 milhões) em 2016.[27] [23] A África do Sul e oriental, onde moram mais da metade das pessoas infectadas globalmente , tiveram o número de mortes por Aids, reduzido, entre 2010 e 2016 , em 42%, sendo de 420.000 (350.000–510.000) em 2016. A América Latina teve um declínio no número de mortes de 12% no mesmo período, com 36.000 (28.000–45.000) em 2016 .[23]

A incidência global em 2016 apresentou cerca de 1,8 milhões (1,6 – 2,1 milhões) de novas infecções pelo HIV. Entre adultos, foi observada queda de 11%, desde 2010, quando 1,9 milhões de novos casos foram detectados comparados com 1,7 milhões (1,4 – 1,9 milhões) de novos casos em 2016. [23]

Em 2016, 76% das mulheres vivendo com HIV e grávidas tiveram acesso a medicamentos antirretrovirais para profilaxia da TV. [23]

Em crianças, a incidência também apresentou um declínio de 47% desde 2010, segundo dados apresentados num recente documento da UNAIDS, de 2017. [26] Novas infecções globais na infância, passaram de 300.000 (230.000–370.000) em 2010 para 160.000 (100.000–220.000) em 2016. [23]

Na América Latina, o Brasil responde por mais de 40% dos casos novos de infecção pelo HIV e lidera ranking de novas infecções na América Latina, ficando outros 41% distribuídos entre Argentina, Venezuela, Colômbia, Cuba, Guatemala, México e Peru.[28]

1.3 Epidemiologia do HIV/Aids no Brasil

O Brasil apresentava uma prevalência de 0.4% a 0,7% em 2014 e estimativa de 830.000 casos acumulados de pessoas vivendo com HIV e Aids (PVHA) em 2015, segundo dados da UNAIDS. [24] [28] No ano de 2015, foram notificados 32.321 novos casos de infecção pelo HIV-1. Esta incidência varia muito conforme a região. A Região Sudeste foi responsável pelo maior número de notificações, 40,4% do total, seguida pela região sul, com 22,5%.[29]

O país vem vivenciando uma epidemia de HIV-1, principalmente transmitida por via sexual, nos maiores de 13 anos de idade, de categoria heterossexual. Os homens ainda representam a maioria dos casos de HIV-1 com 17 homens infectados para cada 10 mulheres , na faixa etária de 13 a 19 anos , e 30 homens para cada 10 mulheres, entre 20 e 29 anos, no ano de 2015. A faixa etária mais afetada pela epidemia é a de indivíduos de 20 a 34 anos de idade e maior concentração de casos de aids, entre 25 e 39 anos, para ambos os sexos. [29]

Em média, foram notificados anualmente no país, nos últimos cinco anos, 41,1 mil novos casos de aids. A região Sudeste, com 16,8 mil casos, foi a que apresentou a maior concentração, seguida pela região Sul, com 8,7 mil casos. O Brasil como um todo, vem apresentando tendência de estabilização da taxa de detecção de aids, nos últimos 10 anos, com média de 20,7 casos /100 mil habitantes. A região Centro Oeste, a única região do país onde também é observada estabilização, tem média de 18,5 casos /100 mil habitantes, segundo Boletim Epidemiológico de HIV/Aids do Ministério da Saúde de 2016. Na figura 6 são demonstradas as taxas de detecção de aids em 2006 e em 2016, nos diversos estados brasileiros. [29]

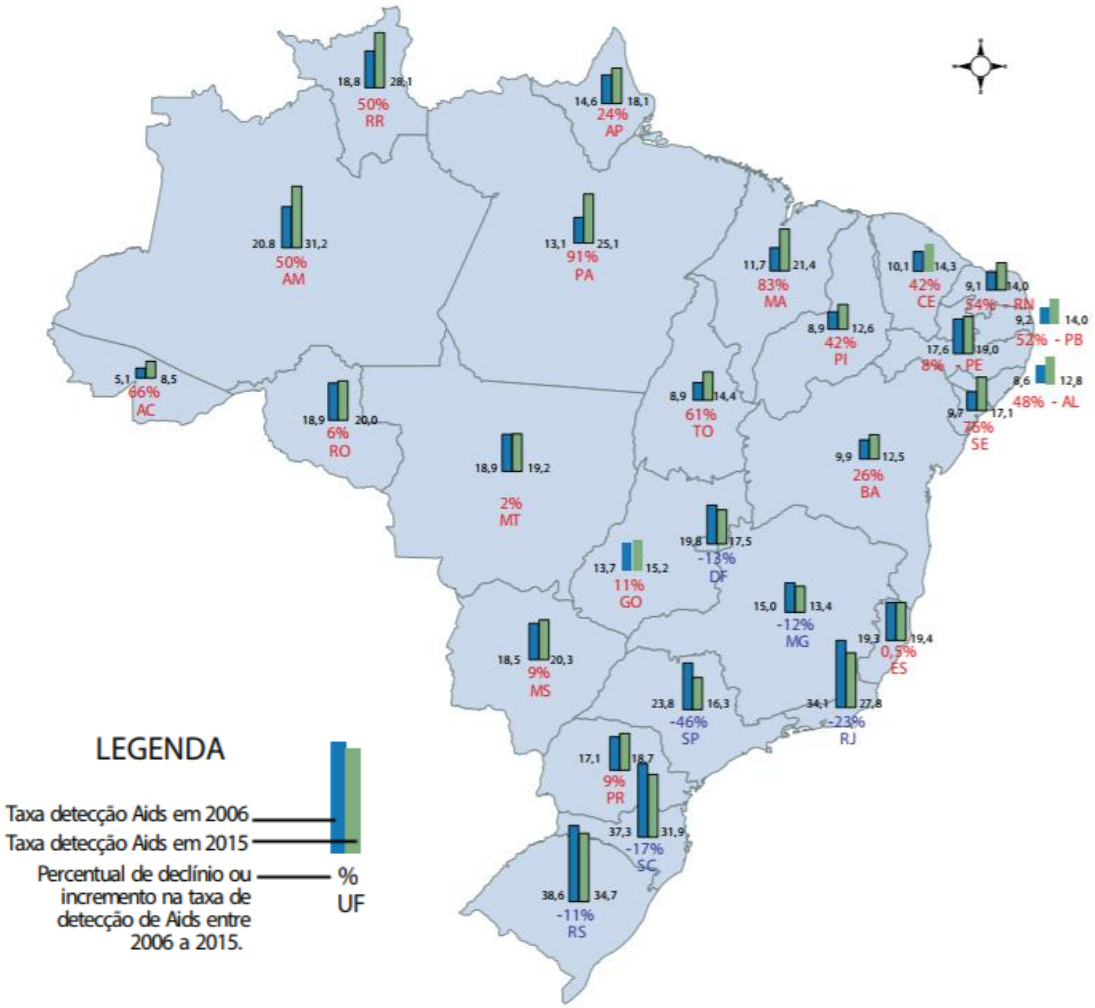


Figura 6: Taxa de detecção de HIV por estado. Fonte: Boletim Epidemiológico HIV/Aids, 2016

No período de 1980 ao final de 2015, 303.353 óbitos por aids, como causa básica, foram identificados no Brasil, incluindo todo período da epidemia nacional. O coeficiente de mortalidade, apresentou decréscimo, com queda de 5%, nos últimos 10 anos, chegando a 5,6 óbitos /100 mil habitantes, em 2015. No entanto, diferenças regionais foram observadas: regiões sudeste e sul com tendência de queda, 20,7% e 9,9 %, respectivamente. Regiões Norte e Nordeste apresentaram tendência de aumento de coeficiente de mortalidade no período. A Região Norte apresentou 56,2% de aumento e Região Nordeste , 34,3% ,em 2015, em relação ao ano de 2006, destacando estados do Amazonas e Pará, na região norte, com coeficientes de mortalidade superiores ao nacional.[29]

Segundo dados do Ministério da Saúde, a estimativa de número de casos de HIV-1 em gestantes, no Brasil é de aproximadamente 12.000 casos por ano.[30] No Brasil, do ano 2000 até junho de 2016 houve notificação de 99.804 gestantes infectadas com HIV-1, a maioria delas residindo na região Sudeste (39,8%), seguidas pela região Sul (30,8%), Nordeste (16,2%), Norte (7,4%), e Centro-Oeste (5,7%). [29]

Nos últimos 10 anos, a taxa de detecção de gestantes com HIV-1 no Brasil vem apresentando tendência de aumento , tendo chegado a 28,6%, neste período. Em 2006 a taxa foi de 2,1 casos por cada mil nascidos vivos, evoluindo para 2,7 em 2015. A faixa etária com maior número de gestantes infectadas está mantida entre 20 e 24 anos, desde 2006. A região norte foi a que apresentou maior incremento, sendo de 2,9 para cada mil nascidos vivos, seguida pela região nordeste, com 2,0 casos para cada mil nascidos vivos, em 2015. Seis unidades federadas, apresentaram taxa de detecção de HIV-1 em gestantes, superior à taxa nacional, no ano de 2015, entre eles, Rio Grande do Sul, Santa Catarina e Amazonas. [29]

No Brasil, a taxa de detecção de aids em menores de 5 anos, foi menor que 2,5 casos /100 mil habitantes, com uma tendência de queda (42,7%), nos últimos 10 anos, distribuídas nas diversas regiões do país. As regiões Norte e Sul mantêm taxa acima

nacional, segundo dados de 2015, do Ministério da Saúde. [29] Segundo a UNAIDS, em 2016, houve menos de 1000 novos casos de HIV-1 em crianças. [24]

Um estudo realizado no Brasil em 2003, evidenciou que o subtipo B era o predominante, seguido do subtipo C. Foram observadas formas de HIV-1 divergentes em 14,48% das amostras estudadas, baseado nas regiões genômicas de transcriptase reversa e protease. [31] A Rede Nacional de Isolamento e Caracterização do HIV - 1 (RENIC), mostrou que dados coletados entre 2013 e 2015 continuam apontando para predominância do subtipo B (66,8%) no território brasileiro, com exceção da região sul. Os subtipos C e F representaram 14,2% e 10% do total de total de amostras brasileiras analisadas conforme observado na Figura 7. [32] (Dados não publicados)

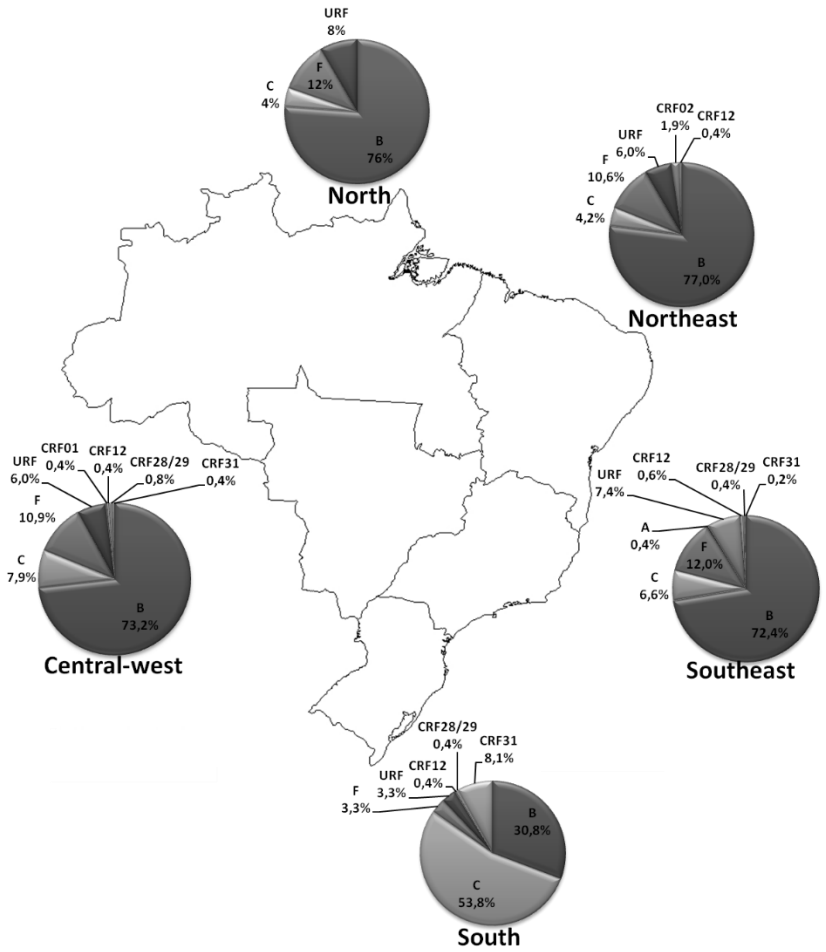


Figura 7: Subtipos virais distribuídos no Brasil. Fonte: Arruda et al, 2017

1.4 Epidemiologia do HIV/Aids no Amazonas

O Amazonas notificou 15.317 casos de aids desde o início da epidemia, em 1980.Tal como no resto do país, a notificação de casos de aids durante o período, mostrou uma concentração da doença entre os homens. A razão entre sexos no estado foi de 2,6 em 2015. Grande parte da taxa de detecção de aids em 2015, 34,6 /100 mil habitantes, foi em jovens com idades entre 15 e 24 anos. A principal via de transmissão foi sexual, sendo 48,2% dos casos classificada como transmissão heterossexual em 2015.[33]

No Amazonas, a taxa de mortalidade foi de 7,6 /100 mil habitantes em 2014, enquanto no Brasil era de 6,1 no mesmo ano. [34] Em 2015, o Amazonas foi um dos tres estados que apresentaram elevados coeficientes de mortalidade, com 8,7, acompanhado dos estados do Rio de Janeiro e Pará, com 8,7 e 8,6 respectivamente . Em 2013, com esse mesmo coeficiente de mortalidade , o Amazonas, havia sido o segundo maior do pais.[35]

O estado do Amazonas vem apresentando elevada taxa de detecção de HIV-1 em gestantes superior a media nacional, chegando em 2015, com 4 casos por 1.000 nascidos vivos. Já foram notificadas no estado, 2607 gestantes infectadas pelo HIV-1 no período de 2000 a junho de 2016. A maior parte dos casos em gestantes encontra – se na capital, Manaus, que tem taxa registrada de 6,2 por 1.000 nascidos vivos em 2015, notificados no Sinan. O Amazonas, juntamente com Roraima, Amapá, Alagoas, Maranhão e Mato Grosso do Sul são os estados que apresentaram tendencia de aumento nesta taxa nos últimos dez anos. [29]

Em 2015, o Amazonas passou a ocupar a décima colocação em taxa (3,1 /100 mil habitantes) de detecção de aids em crianças menores de 5 anos , entre os 27 estados federados. [29] No ano anterior, em 2014, ocupava a segunda colocação, com taxa de 7,1/100 mil habitantes. [35] (Figura 8)

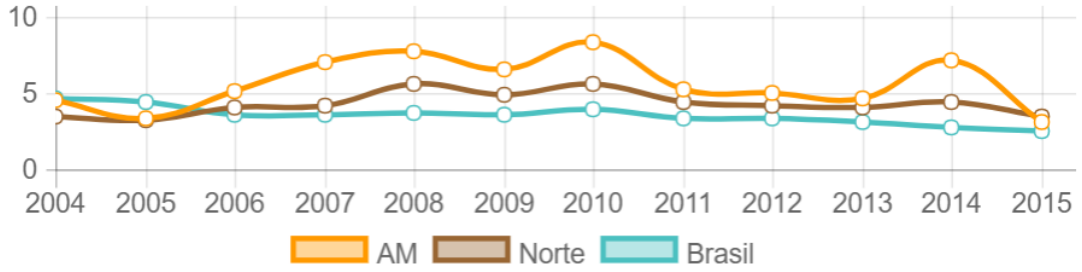


Figura 6: taxa de detecção de aids em menores de 5 anos /100 mil habitantes. Fonte: MS/SVS/DIAHV

1.5 HIV/Aids e a transmissão vertical

O HIV-1 pode ser adquirido pela criança por via sexual, transfusão sanguínea ou por transmissão vertical (TV) também chamada de materno-infantil (TMI). Em 2015, estudos realizados em 25 países, evidenciaram que entre jovens de 15 a 19 anos, 40% foi infectado por TV. [36] A transmissão vertical pode ocorrer pela passagem do vírus de mãe portadora do HIV-1 para o seu concepto, em três momentos: durante a gestação por via transplacentária; na hora do parto através de contato com sangue, secreções ou fluidos maternos ou pós-natal, durante o aleitamento. Os estudos realizados demonstram que a transmissão vertical ocorre em média em 25% a 30% das gestações de mulheres infectadas pelo HIV-1. Diversos fatores influenciam esse modo de transmissão, sejam eles maternos ou fetais e serão abordados a seguir. A amamentação acrescenta risco de 14% na aquisição da infecção para o lactente. [37] [38] [39]

A quantidade de vírus a que a criança é exposta, ou seja, presença de altas cargas virais materna, é hoje reconhecidamente o principal fator de risco de TV. Seja essa exposição intra – útero ou durante o momento do parto e até mesmo pela lactação, representa importante fonte de aquisição viral. Quanto maior o tempo de exposição, maior risco de infecção. Fatores imunes da criança como susceptibilidade a infecções e características virais, podem influenciar e predispor à infecção.[40]

Em um estudo do Zimbabwe, pesquisadores identificaram que 20% das infecções em crianças amamentadas ocorriam por aquisição materna do HIV-1 durante o período de lactação. Situações como essa são conhecidas como infecção incidental de HIV. Tal fator alerta para necessidade de triagem das mulheres durante lactação, mesmo que negativas para o HIV-1 na gestação. Na África, diferentemente do Brasil, mulheres com HIV-1 amamentam e recebem terapia antirretroviral combinada (TARVc) simultaneamente. [41]

1.6 Intervenções de prevenção de transmissão vertical no mundo

Em 1994, foi desenvolvido estudo do Pediátricas Aids Clinical Trial Group (PACTG) multicêntrico, chamado ACTG 076, com o objetivo de reduzir a TV. Utilizando o AZT (zidovudina), um inibidor de transcriptase reversa nucleosídeo, em 3 momentos (gestação e parto na mãe e pós-natal ao neonato), o protocolo permitia diminuir o risco da transmissão vertical do HIV. O estudo demonstrou que se aplicado adequadamente nas 3 situações mencionadas, era possível reduzir em quase 70% o risco de infecção do bebê.[42]

Diferentes estratégias de tratamento foram propostas para mulheres a fim de evitar a TV. A estratégia B^+ é a mais difundida atualmente e prevê tratamento de todas as mulheres, ao engravidar, independente de carga viral ou LT- CD4 ou critérios clínicos. Por essa diretriz, a mulher mantém TARVc mesmo após parto o que assegura redução de progressão de doença e risco de transmissão vertical em futuras gestações além de outros benefícios. [28]

A OMS em seus guidelines prévios, nomeou diretrizes terapêuticas voltadas para prevenção da TV, como opções A, B e B^+. No Guideline de 2013, as opções passaram a ser apenas B, quando a mulher gestante ou em lactação utilizava TARVc mesmo que não elegível para tal e Opção B^+, quando o tratamento era iniciado para todas gestantes e lactantes e mantido pela vida toda, independente de avaliação clínica ou imunológica. [41] (Figura 9)

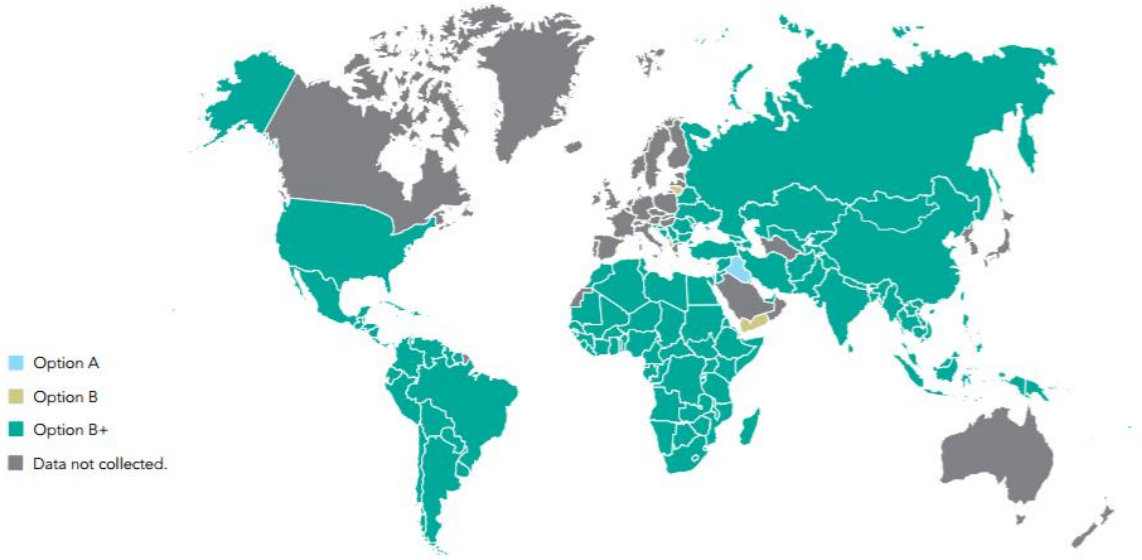


Figura 7: Regimes ARVs recomendados pela OMS para prevenção da Transmissão materno – infantil do HIV-1, 2016. Fonte: Prevention gap report – Unaids, 2016

A partir da terapia ARV universal para as gestantes, houve mudança de reconhecimento de risco para TV, com a carga viral maternal e tempo de uso de TARVc assumindo posição de determinantes de transmissão. Na era pré TARVc materna, o risco recaia sobre o tempo de ruptura de membranas, parto prematuro e baixo peso ao nascer. Algumas situações são atualmente consideradas como maior risco de TV. São elas: mulheres diagnosticadas ou expostas ao HIV-1 no parto ou período de amamentação; em TARVc por período inferior a 4 semanas na ocasião do parto e gestantes com carga viral conhecida superior a mil cópias, coletada ao longo das 4 semanas prévias ao parto. [41]

Em 2011 foi lançado pela UNAIDS, o Plano Global da Eliminação de novas infeções por HIV em crianças com a meta de reduzir as novas infecções em 90%, nessa faixa etária, até 2015. [43] Desde 2010, a Organização Panamericana de Saúde (OPAS), está comprometida com a eliminação da transmissão materno infantil do HIV-1. Critérios globais foram elaborados pela OMS em 2014 para o processo de validação da eliminação do HIV e sífilis. [44] Esse tema foi revisado no documento da OPAS voltado para as Américas em 2016. [45] Tais estratégias têm apresentado grande impacto com redução na

taxa da transmissão vertical de forma global. Em 2016, Armenia, Belarus, Cuba e Tailândia já foram certificados como livres de transmissão materno infantil do HIV-1, pela Organização Mundial de Saúde. [36]

Em 01 de dezembro de 2014, por ocasião da comemoração do dia Mundial da Aids, na cidade de Paris, foi assinada a Declaração de Paris coordenada pela Associação Internacional de Provedores de Cuidados em Aids (IAPAC) e UN – Habitat, com parceria de 26 cidades estratégicas. O objetivo estabelecido era atingir o que ficou conhecido como metas 90-90-90: que 90% das pessoas vivendo com HIV estejam diagnosticadas; que 90% destas, estejam em tratamento; e das pessoas em tratamento, 90% apresentem carga viral indetectável. O prazo determinado foi até 2020, focando em melhorias em ações sociais, em saúde, justiça e oportunidades econômicas. Lançado na 20a Conferência Internacional de AIDS, em Melbourne, na Austrália, no mesmo ano, ganhou adesão de mais de 200 cidades e municípios que aderiram e assinaram a declaração cujo objetivo final é acabar com a Aids como questão de saúde pública até 2030. Desde então o foco passou a ser não somente o acesso a terapia antirretroviral, mas a promoção de supressão viral em quem já vive com HIV. Tal abordagem possibilita estratégia de reduzir risco de transmissão do vírus além de proteger o indivíduo infectado. [26] A meta de tratamento para 15 milhões de pessoas foi atingida ao final de 2015. [46]

Novas infecções em crianças até 14 anos, vem diminuindo, como resultado de esforços globais de eliminação da transmissão vertical. [45] Houve queda de 51% em novas infecções, nesta faixa etária, em relação a dados de 2010, segundo UNAIDS. [36] O número de novas infecções em crianças diminuiu de 290.000 (250.000 – 350.000) em 2010 para 150.000 (110.000 – 190.000) em 2015 em todo globo. [45]

Tem sido demonstrado que um dos pilares na eliminação da transmissão vertical do HIV é diagnosticar gestantes e prover TARVc para pelo menos 95% das mulheres grávidas e lactantes. Dentro do grupo de países prioritários, cinco alcançaram essa meta em 2016: Botswana, Namíbia, África do Sul, Swaziland e Uganda. [26] Entretanto, em 2015, ainda 300.000 mulheres grávidas ou em amamentação deixaram de receber TARVc para

profilaxia TV. [28] No continente africano, as região norte e oriental foram as que apresentaram o pior desempenho, com quase um terço das mulheres infectadas transmitindo HIV para seus bebês. As regiões da África central e ocidental, também estiveram acima da média mundial de 10%. A Nigéria foi o país com pior performance em termos de novas infecções em crianças, no mundo. Nos países prioritários do Plano Global, houve redução desde 2009 de 60% em novos casos na infância enquanto na Nigéria, somente de 21% no mesmo período. [28]

Gestantes da América Latina e Caribe, foram testadas para o HIV em 2015, em 72%, representando um aumento em relação ao 2010. Houve incremento na oferta de TARVc entre as grávidas de 52% para 88% no mesmo período, na região. Consequentemente, as novas infecções em menores de 14 anos, estimadas em 2010 para 4.700 (3500 – 6400), reduziram para 2.100 (1600 – 2900) em 2015, representando uma queda de 55%. [28] A taxa de TV, na região, que era de 15% (11% - 20%) em 2010 foi para 8% (6% - 10%) em 2015, segundo estimativas da UNAIDS. O Caribe isoladamente apresentou desempenho em destaque, com redução de 83% de novos casos em crianças, pois reduziu para apenas 400 (200 – 700) em 2015, dos 2300 (1600 – 3000), em 2010. [28 45] (Figura 10)

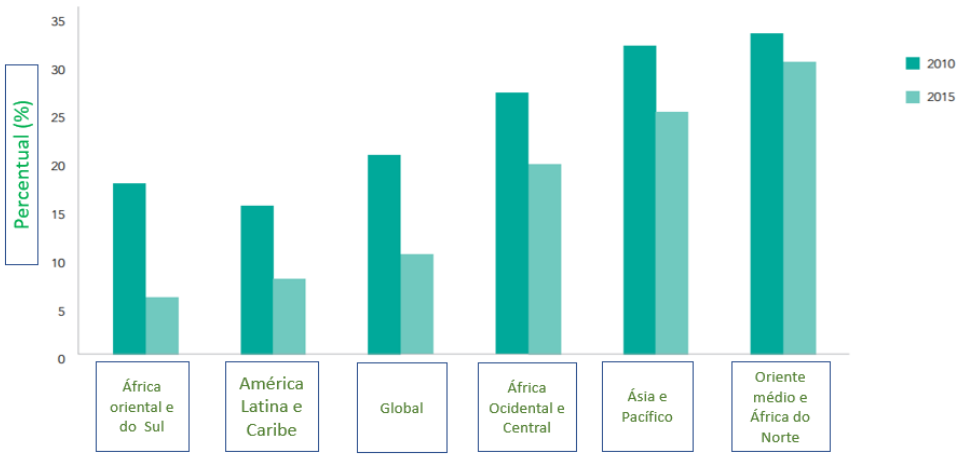


Figura 8: Taxa de transmissão materno infantil por região no globo 2010 – 2015. Fonte: Adaptado de Prevention gap report – Unaids, 2016

A Organização Panamericana de Saúde, estimou que em 2015, 22 países na região das Américas , reportaram dados sugerindo alcance de meta de eliminação da TV do HIV e 12 deles , estão próximo de alcançar a meta , estando com taxa de transmissão vertical entre 2 e 5%, com o Brasil situado neste grupo.[45] Alguns países no entanto, não tem alcançado a cobertura ideal de profilaxia da transmissão materno infantil do HIV (PTMI), como Venezuela com apenas 43%, precedida de Costa Rica e Guatemala , com respectivos 41% e 13%.[45] (Figura 11)

Brasil								
Total de bebês com exposição perinatal	Total de bebês sem diagnóstico definitivo	Crianças infectadas	Taxa de TMI HIV (%)	Total de bebês com exposição perinatal	Total de bebês sem diagnóstico definitivo	Crianças infectadas	Taxa de TMI HIV (%)	Taxa pediátrica por 1000 nascidos vivos
2012/2013				2014/2015				
6.876	102	307	4,05 (2012)	5.976	33	263	4,4 (2014)	0,09 (2014)

Figura 9: Lactentes expostos ao HIV-1, infectados pelo HIV-1 e taxas estimadas de TV 2010 – 2015 no Brasil. Fonte: Adaptado de Elimination of mother to child transmission of HIV – update 2016. WHO - OPAS.

Apesar do uso de profilaxia antirretroviral, algumas crianças se infectam e tal fato ocorre por provável infecção intrauterina, antes do diagnóstico materno e condutas profiláticas estabelecidas. A evolução clinica nesses casos é em geral mais rápida e o risco aumentado em três vezes de progressão para formas severas da doença. [47] Em 2016, foram revisadas as metas pela OMS tendo sido elaborado novo Plano de Ação para Prevenção e Controle do HIV e doenças sexualmente transmissíveis (2016 – 2021). [45]

1.7 Intervenções de prevenção de transmissão vertical no Brasil

O Brasil introduziu as primeiras recomendações de prevenção da transmissão vertical do HIV, em 1996. (Tabela 1). Essas recomendações foram revisadas em 2007, com a adoção de terapia combinada profilática para mulheres sem indicação de uso de ARV como tratamento. Em 2010 , as recomendações internacionais foram atualizadas passando a orientar início de TARVc para todas as gestantes HIV positivas independente da contagem de LT- CD4, de acordo com a estratégia B $^{+}$ já descrita anteriormente. [28]

O primeiro passo na prevenção da TV é a detecção de gestantes infectadas com risco de transmitirem o HIV para seus filhos. No Brasil, existe tendência de aumento nos últimos dez anos, na detecção de grávidas vivendo com HIV, em quase todas as regiões do país, exceto na região sudeste. Entre os estados brasileiros com maiores taxas de detecção de HIV em gestantes em 2015, superior à taxa nacional (2,7 casos/mil nascidos vivos), estão Rio Grande do Sul (10,1 casos/mil nascidos vivos), Santa Catarina (5,6) e Amazonas (4,0) seguido por Roraima (3,9), Amapá (3,6) e Rio de Janeiro (2,9). [29] O Brasil tem apresentado evoluções na provisão de ARV para mulheres grávidas para prevenir a transmissão mãe-filho, tendo sido 84 % a cobertura , em 2015. [45] (Figura 12)

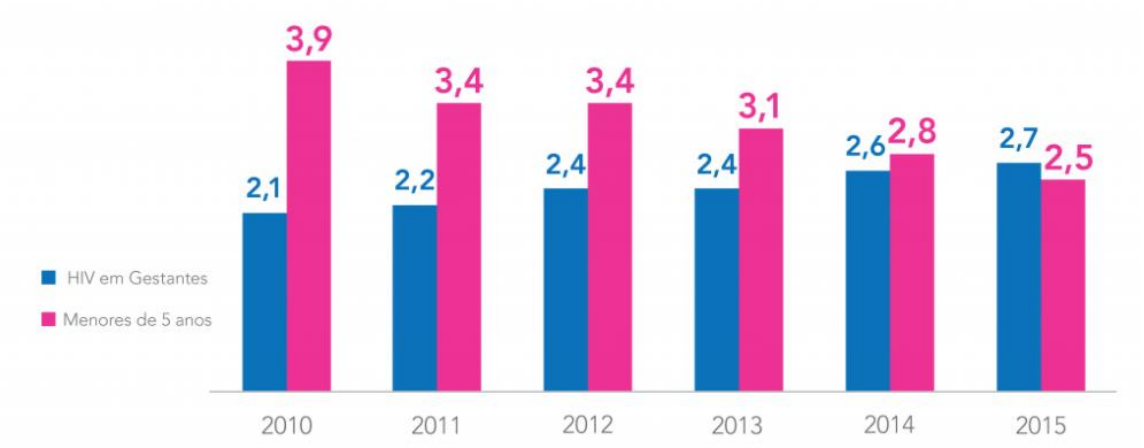


Figura 10: Taxa de detecção em gestantes por 1.000 nascidos vivos e em menores de 5 anos /100 mil habitantes. Fonte: MS/SVS/DIAHV 2015

A partir de 2013, houve uma mudança do paradigma da prevenção de forma global. Foi adotada pelo Ministério da Saúde, uma abordagem da prevenção que envolvia desde a promoção da saúde até o tratamento e a reabilitação. A prevenção foi ampliada buscando atingir todas possibilidades, envolvendo estruturas preventivas clássicas, estruturais e biomédicas. Com essa estratégia, a atenção se voltava para realidade dos sujeitos e para os seus contextos de vida. Nesta perspectiva, a identificação de gestantes positivas através da realização de exame de HIV no pré-natal, ganha destaque. [48] (Figura 13)

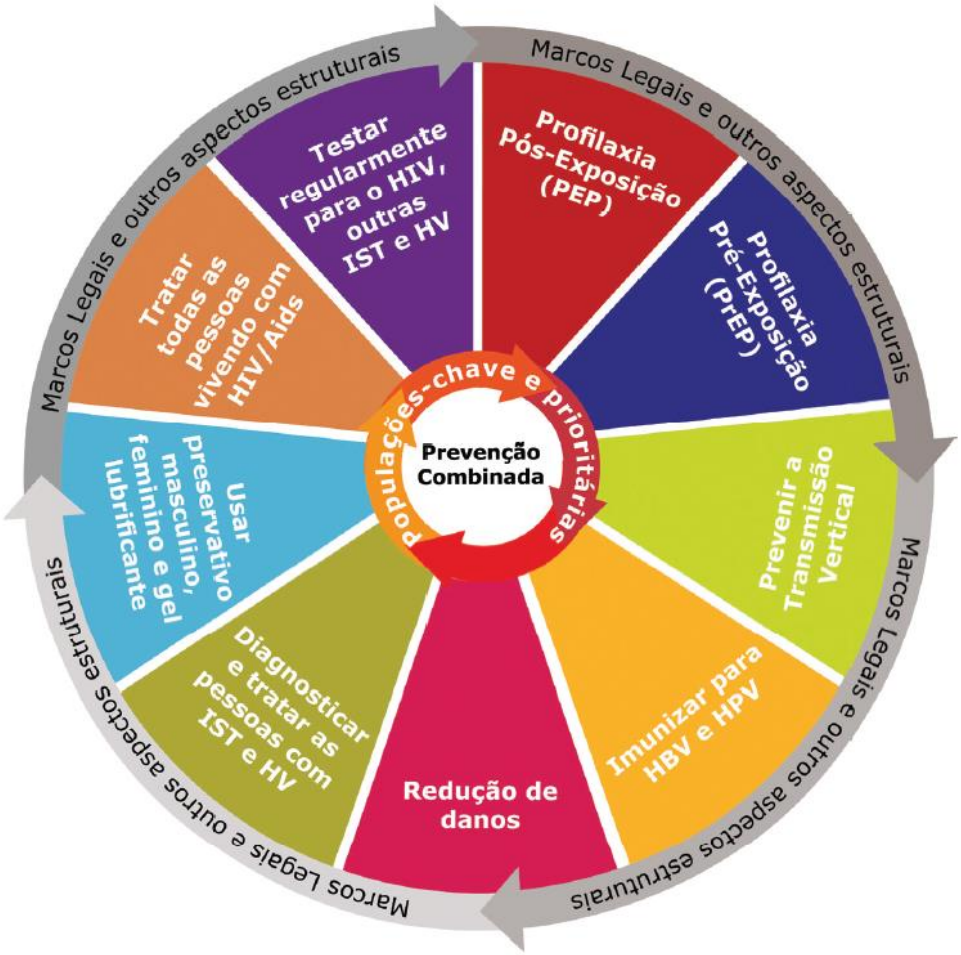


Figura 11: Mandala de prevenção combinada de HIV. Fonte: Ministério da Saúde, 2016

O último estudo multicêntrico nacional, que analisou a taxa de transmissão vertical, foi realizado em 2001, e reportou uma taxa de 7.1%, com algumas diferencias regionais.[49] Segundo dados da UNAIDS, menos de 500 casos de novas infecções em menores de 14 anos eram esperados no Brasil em 2015. [28] Tem se observado uma tendencia de queda na taxa de detecção de aids em menores de 5 anos, no Brasil como um todo. [29]

1.8 Diagnóstico de HIV na infância

Crianças menores de 18 meses, necessitam de ferramentas de diagnóstico mais complexas. É preciso lançar mão de exames moleculares realizados em períodos determinados para confirmação da infecção pelo HIV. O exame conhecido como carga viral, ou seja, quantificação de RNA, é então utilizado para este fim. A coleta aos 30 dias de vida seguida de uma segunda coleta após 4 meses de idade, praticamente selam o diagnóstio de exclusão de infecção em crianças sabidamente expostas e não infectadas, filhas de mães soropositivas para o HIV. A sorologia pelo método de ELISA, pode ser realizada após os 12 meses, opcionalmente. [38]

Em situações de detecção de presença de cópias virais no exame de carga viral, coletado no primeiro mês de vida, um segundo exame deve ser realizado imediatamente, para confirmação da infecção.[38] Em maiores de 18 meses, a sorologia por método ELISA pode ser usada como diagnóstico definitivo de infecção. [38]

1.9 Terapia antirretroviral

1.9.1. Os antirretrovirais

A terapia antirretroviral visa reduzir a carga de vírus circulantes e consequentemente melhorar os níveis de LT - CD4 e a capacidade funcional do sistema imune. Os medicamentos que compõem o esquema antirretroviral, são classificados conforme seu mecanismo de ação e visam atingir o HIV-1 durante seu ciclo replicativo. A utilização de 3 medicamentos de pelo menos duas classes distintas, caracteriza a chamada terapia de alta potência, em inglês *High Active Antiretroviral Therapy* (HAART). [41]

São 6 as classes atualmente utilizadas para compor a HAART ou TARVc, de modo

que pelo menos três medicamentos devam ser usados simultaneamente: [50]

- Classe de inibidores de transcriptase reversa análogos de nucleosídeos (ITRN)
- Classe de inibidores de transcriptase reversa não análogos de nucleosídeo e de nucleotídeo (ITRNN)
- Classe de Inibidores de Protease (IP)
- Classe de inibidores de fusão (IF)
- Classe de antagonistas de receptores de quimiocina CCR5 (Antagonistas de CCR5)
- Classe de inibidores de integrase (INI)

Nem todos ARVs estão liberados para uso pediátrico e muitas vezes não estão disponibilizadas formulações pediátricas, o que muito limita o uso em crianças menores. O primeiro ARV disponibilizado foi a zidovudina ou AZT e até os dias atuais ainda muito utilizado na pediatria. [38] [50]

1.9.2 Tratamento antirretroviral inicial

A TARVc tem determinado o aumento da sobrevida de pessoas que vivem com HIV-1 em especial de crianças. Um número crescente de crianças e adolescentes tiveram acesso a TARVc, em 2016. Cerca de 919.000 (810.000–956.000), 43% (30 – 54%) das crianças entre zero e 14 anos, que vivem com HIV-1 globalmente, quase o dobro dos 452.000 tratados em 2010. No entanto, com o ritmo atual do incremento de crianças em TARVc, a meta estabelecida para 2018, de tratar 1,6 milhões de crianças, corre risco de não ser alcançada. Até 2016 apresentava incremento anual de 10%, tendo sofrido queda para 6% no ultimo ano. [26] [28]

Crianças infectadas pelo HIV, demandam diagnóstico precoce e imediato início de terapia específica. [36] A preservação da função de células B, que se segue ao início precoce de TARVc e as restrições ao estabelecimento de reservatórios virais, tornam mandatórias as condutas de intervenção terapêutica precoce em recém-nascidos

expostos ao HIV. Resultados do estudo Children with HIV Early Antiretroviral Therapy (CHER), conduzido no continente africano, evidenciou benefícios como 76% de redução no risco de morte e 75% de redução em progressão para estágios mais avançados da doença, com introdução precoce de TARVc. [51]

Os reservatórios virais são locais onde o acesso dos antirretrovirais é limitado, o que permite a presença do vírus livre da pressão farmacológica. Os reservatórios são também denominados santuários. Estes podem ser anatômicos, como o sistema genital masculino e feminino além do SNC, e ainda celulares, como células de memória, linfócitos CD4, macrófagos e células dendríticas. Tais células carregam o DNA viral em forma de provírus (integrada ao genoma) ou mesmo virions extracelulares no caso das células dendríticas.[52]

A partir do conhecimento da capacidade viral do HIV de se instalar em células inativadas como linfócitos T e assim permanecer inatingível pelas drogas circulantes, conseguir eliminar tais santuários passou a ser meta de pesquisa na busca da cura para pessoas infectadas pelo HIV. Os antirretrovirais possibilitam uma queda nos níveis plasmáticos da viremia assim que iniciados em pacientes virgens de terapia, de forma bifásica. A primeira e mais marcante redução se refere às células CD4 em atividade e aos virions livres circulantes. Essa etapa gira em torno de 2 a 4 semanas e elimina 99% da carga de vírus na circulação. A segunda etapa, mais lenta, atinge células macrofágicas e dendríticas, carreadoras de DNA viral não integrado. [52]

Um único caso havia sido relatado de cura do HIV-1 em adulto. Um paciente do sexo masculino, que recebeu transplante de medula de doador com alteração em alelo, sendo homozigoto para CCR5 delta32 e que a partir de então se mostrou soronegativo para o HIV-1.[53] [54] Em 2013, um bebê nascido nos Estados Unidos, de parto vaginal e diagnosticado logo após o nascimento, como infectado pelo HIV-1, recebeu precocemente, com 30 horas de vida, ARVs, da classe de ITRN e ITRNN. Após 18 meses de adesão, abandonou tratamento e quando novamente testado para HIV-1 aos 23 meses, não apresentava qualquer resposta positiva de infecção. [55] Este caso ficou conhecido como Bebê de Mississipi. Anos mais tarde ficou evidente que não havia

remissão permanente, pois, a quantificação plasmática voltou a positivar reforçando a idéia de que santuários com reservatórios de vírus podem ser responsáveis pela reativação da doença na ausência da ação inibidora das drogas. A cura funcional ainda não está bem esclarecida e permanece inatingida. [56]

A Organização Mundial de Saúde, preconiza condutas de tratamento universal para todas as crianças e adolescentes em seu Guideline de 2016, independente de cargas virais ou situação clínica imunológica. A classe de inibidores de integrase foi inserida como tratamento alternativo em locais de recursos limitados além de apresentação diferenciada do Efavirenz, com melhor tolerabilidade. [57]

As formulações pediátricas existentes são restritas em comparação com as disponibilizadas para os adultos. O Food and Drug Administration (FDA) é o órgão americano responsável por regulamentar uso de novas drogas nos Estados Unidos. Grande parte do arsenal em terapia antirretroviral, ainda não está liberado pelo FDA para uso em crianças americanas, por diversos fatores. Algumas das drogas já disponibilizadas para faixa etária pediátrica, não o são para extremos de idade como período neonatal. A nevirapina por exemplo, ainda tem estudos em andamento sobre dose e segurança para este período da vida. O Efavirenz, outra droga da classe de não nucleosídeos, usada no arsenal de primeira linha, não deve ser utilizado em menores de 3 anos. Ambos são metabolizados pelo CYP2B6, e a presença de polimorfismos podem impactar na farmacocinética. [46 59 60]

No Brasil, critérios de tratamento são determinados pelos Protocolos Clínicos elaborados frequentemente pelo Departamento de Vigilância, Prevenção e Controle das Infecções Sexualmente Transmissíveis, do HIV/Aids e das Hepatites Virais (DIAHV), Ministério da Saúde, para início de terapia em crianças no território nacional. São disponibilizadas três classes de medicamentos para este fim: inibidores de transcriptase reversa análogo de nucleosídeo (ITRN), inibidor de transcriptase reversa não análogo de nucleosideo (ITRNN) e inibidor de protease (IP). Mais recentemente, em 2017, uma nova classe também está sendo disponibilizada para início de terapia, com melhor atuação

sobre o vírus, que é a classe de inibidor de integrase (IT), apresentados na Tabela 1. [38] [58]

Tabela 1: Drogas usadas na terapia antirretroviral inicial em crianças e adolescentes

FAIXA ETÁRIA	INÍCIO DE TRATAMENTO			
	PREFERENCIAL		ALTERNATIVO	
	ITRN	3º ARV	ITRN	3º ARV
14 dias a 3 meses	AZT + 3TC	LPV/r	AZT + 3TC	NVP
3 meses a 2 anos	ABC[(a)] + 3TC	LPV/r	AZT + 3TC	NVP
2 anos a 3 anos	ABC[(a)] + 3TC	RAL	AZT + 3TC	NVP
3 anos a 12 anos	ABC[(a)] + 3TC	RAL	AZT + 3TC TDF[(b)] + 3TC	EFZ
Acima de 12 anos[(c)]	TDF[(b)] + 3TC	DTG	ABC[(a)] + 3TC AZT + 3TC	EFZ

Fonte: Protocolo clinico e diretrizes terapêuticas para manejo da infecção pelo HIV-1 em crianças e adolescentes - MS/SVS/DIAHV 2017

1.10 Diversidade genética e Resistência viral

1.10.1. Diversidade genética do HIV-1

Diversos fatores são responsáveis pela diversidade genética do HIV-1. A falta de atividade revisora da transcriptase reversa, leva a uma alta taxa de mutação durante sua replicação nas celulas humanas. A cada genoma transcrito, ocorrem em média 5 a 10 erros. [62] Somado a isto, o HIV-1 tem uma replicação muito ativa levando a produção que chega a mais de 10 bilhões de particulas virais diariamente, em um paciente cronicamente infectado, o que aumenta a chance de aparecimento de virus mutantes que podem ser selecionados. Ainda, a presença de certas mutações, leva a vantagem seletiva. O resultado final são populações virais bem diversificadas geneticamente, que compõe um fenômeno chamado de “quasiespecies”. Com tanta diversidade genética, o HIV-1 tem êxito na infecção, pois fica assim mais capacitado para escapar do sistema imune. [62] [63] [64] [65] [50]

A coinfecção, com a presença de duas ou mais cepas num mesmo indivíduo, tem sido descrita e leva a recombinações e grande diversidade viral. O caráter diploide do HIV-1, com duas moléculas genômicas de RNA, permite a produção de virions heterozigotos com a recombinação ocorrendo durante a transcrição reversa. Isso é possível quando uma célula está coinfectada por dois vírus diferentes. Algumas dessas formas recombinantes se disseminaram na população humana e têm sido classificadas como formas recombinantes circulantes (CRF). [50] [13] [66]

1.10.2. Resistência do HIV**-1** aos antiretrovirais

Desde a identificação em um paciente em 1995, a seleção de resistência aos ARVs tem sido um dos fatores limitantes para o sucesso da terapia. [67] A resistência viral (HIVDR), ocorre quando uma mutação altera a configuração genética do HIV-1. A partir desta mutação, locais de ação da TARVc passam a estar desconfigurados e a droga perde sua efetividade em bloquear a replicação viral. A falha terapêutica de muitos pacientes infectados pelo HIV-1, está relacionada a não adesão ao tratamento e a replicação elevada de vírus resistentes aos fármacos administrados aos pacientes, sendo esta a maior problemática na aplicação clínica dos quimioterápicos inibidores da protease e da transcriptase reversa viral. [68]

Segundo a OMS, podem ser identificados tres tipos de resistência: a resistência adquirida, transmitida e a anterior ao uso de TARVc. A resistência adquirida é desenvolvida em pessoas em uso de TARVc. A resistência transmitida é quando um indivíduo infectado virgem de tratamento é detectado com virus resistente, tendo recebido a resistência de seu comunicante. A resistência à droga anterior ao uso de TARVc ou pré tratamento (PDR), é detectada antes de inicio de tratamento podendo ter sido transmitida (de mãe para filho) ou adquirida (em indivíduos que tenham feito uso de profilaxia com ARVs). [68] A presença de vírus com mutações de resistência em indivíduos virgens de tratamento, não é oriunda da emergência natural do HIV-1 e sim exclusivamente da transmissão de cepas resistentes. [65]

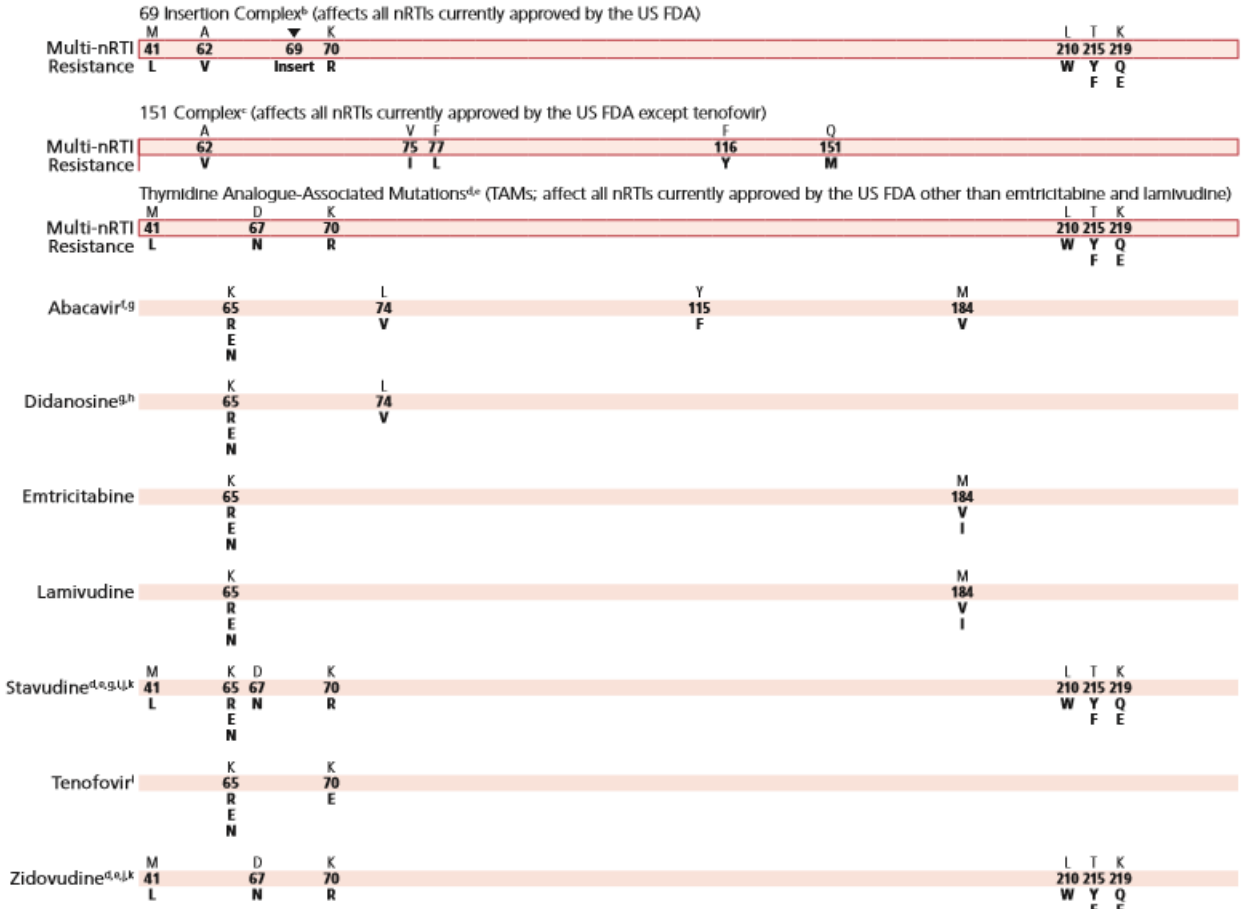
A resistência viral é então a consequência direta da diversidade genética do HIV-1 e da taxa de erro da transcriptase reversa somada à pressão seletiva exercida pelas drogas anti-retrovirais.[69] Todas as drogas antirretrovirais correm risco de serem totalmente ou parcialmente inativadas pela emergência de vírus resistentes. Vale ressaltar que as resistências adquiridas por pressões de drogas, são resistências que se fixam, ou seja, se mantém quando passadas para outro indivíduo e tem papel fundamental na resistência transmitida. [68] [66]

Cada ARV seleciona mutações específicas em códons reconhecidos e geram interpretação a partir de exames de genotipagem que guiam as condutas médicas. Com o acúmulo de mutações de resistência, a susceptibilidade às drogas diminui, reduzindo progressivamente a potência do esquema terapêutico. Desse modo, esquemas terapêuticos sem a potencia desejada, adesão subótima, absorção limitada, podem permitir a emergência de vírus resistentes. Esta emergência de resistência origina um ciclo vicioso de falha terapêutica, deixando o tratamento ainda mais difícil de manejar.[70]

Mutações de resistência, de modo geral, impactam na capacidade de replicação do vírus, ou seja, no *fitness* viral. Mutações adicionais, podem ajudar o vírus a recuperar sua capacidade replicativa, em especial quando ocorrem na protease. [65]

Nas Figuras 14 e 15 podem ser observadas as principais mutações de resistência geradas pelo uso de ARVs das classes de ITRN, ITRNN e IP.

Inibidores de Transcriptase reversa Análogos de Nucleosídeo e de Nucleotídeo



Inibidores de Transcriptase Reversa Não-Análogos de Nucleosídeo

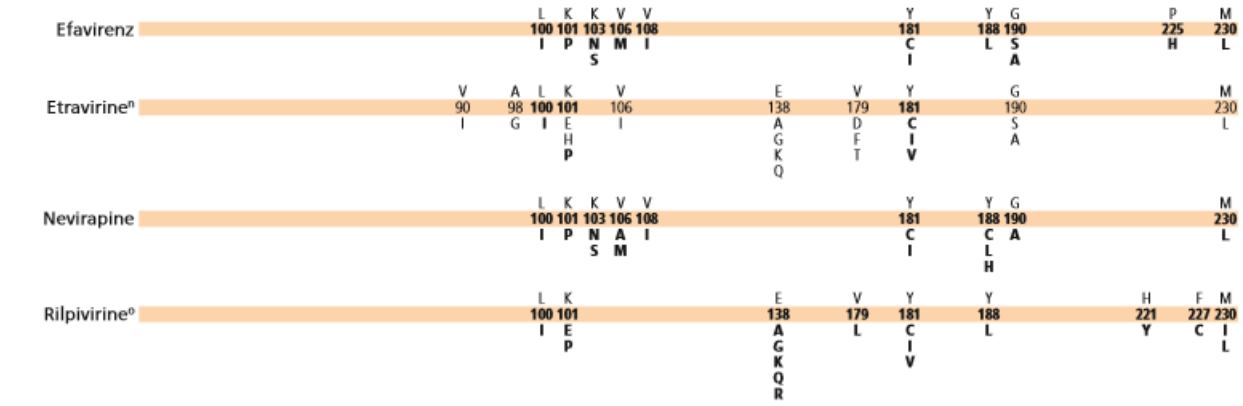


Figura 12: Mutações mais freqüentemente encontradas no gene da TR do HIV-1 que conferem resistência às drogas ITRN e ITRNN. Fonte: International AIDS Society 2017

Inibidores de Protease

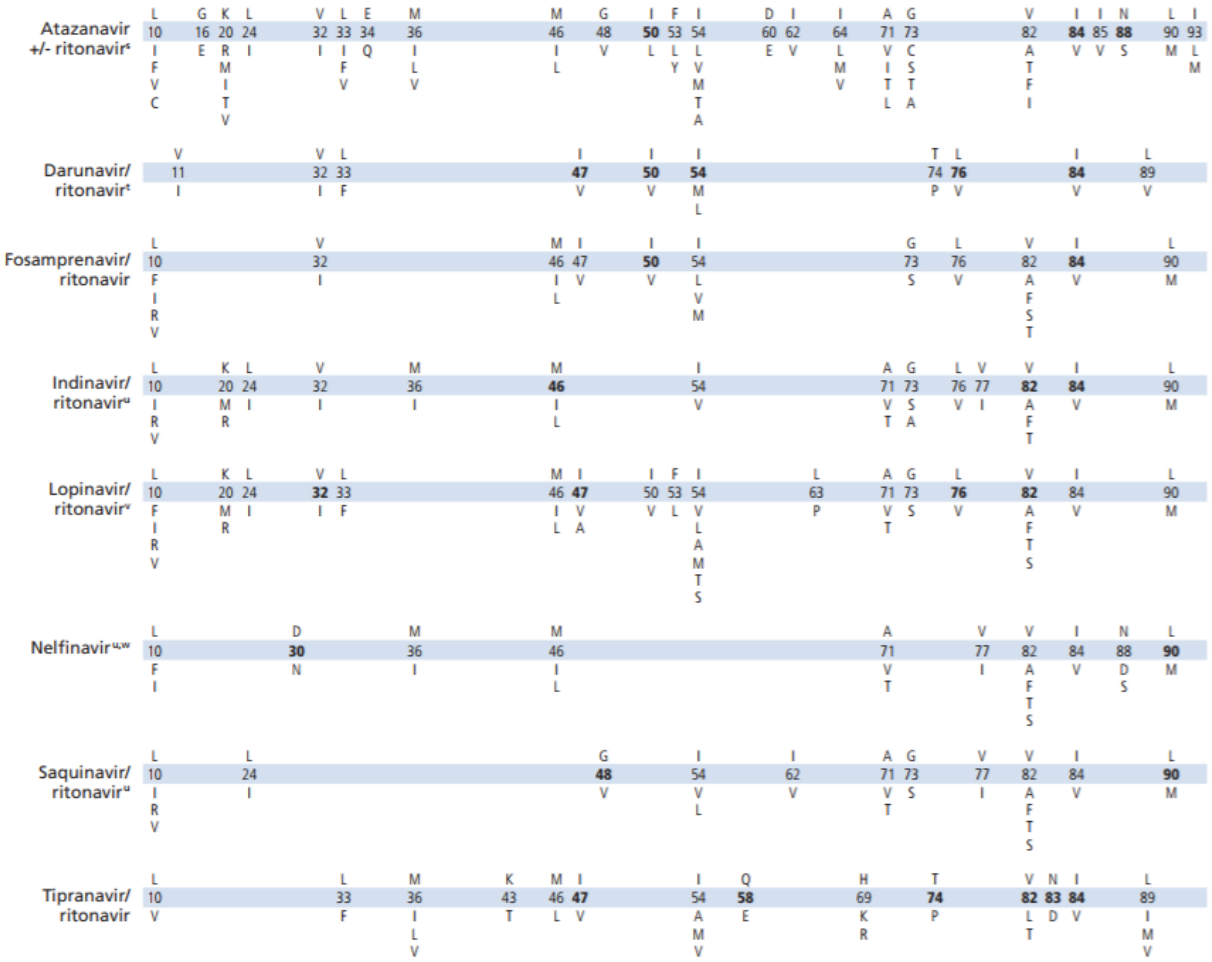


Figura 13: Mutações mais freqüentemente encontradas no gene da protease do HIV-1 que conferem resistência às drogas IP. Fonte: International AIDS Society, 2017

1.10.3. As enzimas virais e mutações de resistência

Duas são as enzimas, foco de ação dos ARVs, que vem compondo o esquema combinado inicial de terapia antiretroviral para crianças, desde 1995 -1996. São elas, a enzima transcriptase reversa e enzima protease. [38]

A enzima transcriptase reversa é um heterodímero composta por duas subunidades, p66 e p51. A subunidade 66 tem subdomínios incluindo dedos, palma e polegar e constitui o sítio ativo da TR, que participa da polimerização e conexão. [71] (Figura 14)

A protease é uma enzima aspártica composta de dois monômeros idênticos estruturalmente e ligados por associação não covalente. Contém uma região flexível que se fecha sob o sítio ativo após ligação do substrato. É a enzima responsável pelo processamento pós-tradução das poliproteínas virais gag e gag-pol para produção de proteínas estruturais e enzimas do vírus. Ela reconhece e cliva 9 diferentes sequências peptidicas. [72] [73]

O HIV-1 pode desenvolver mutações e por vezes em grupos de mutacoes especificas. Quando isso ocorre, esses grupos são chamados de vias mutacionais. Uma via mutacional é um grupo de mutacoes especificas selecionadas por um medicamento. Um ARV pode selecionar mutacoes por varias vias mutacionais distintas *in vitro* e *in vivo*, porém em geral, somente uma via é adotada. As vias mutacionais podem implicar em resistência cruzada a medicamentos da mesma classe, o que impacta na terapia como um todo. [65]

Os medicamentos antirretrovirais que atuam no sítio de ação da TR, podem ser ITRN ou ITRNN. Os ITRN mimetizam nucleosidios utilizados na formação da cadeia de DNA viral. Como próprio nome já diz, os não análogos, apesar de atuarem no mesmo local, não mimetizam nucleosídios. [71]

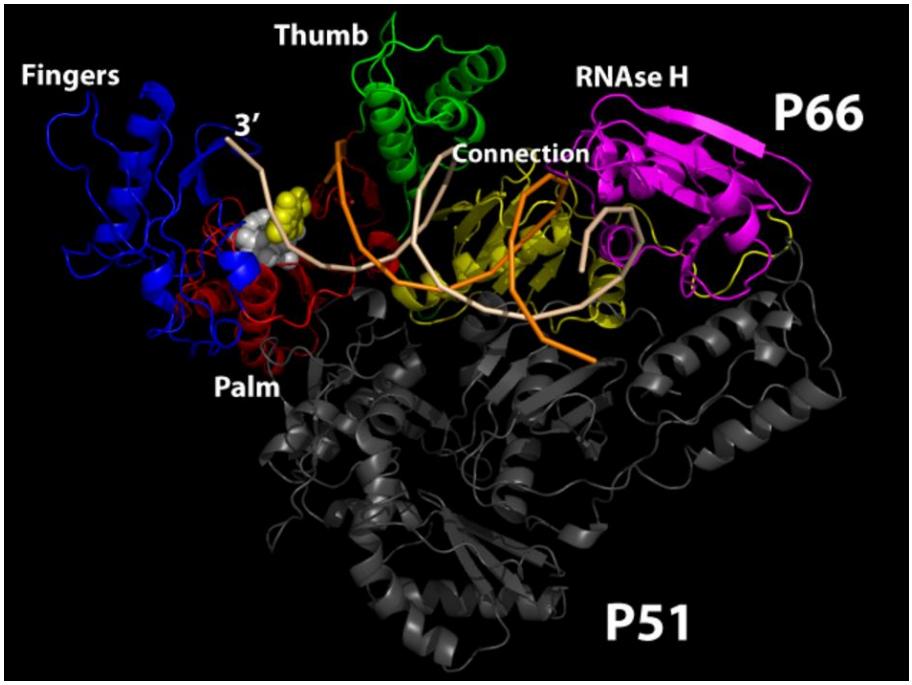


Figura 14: Representação cristalográfica estrutural da enzima transcriptase reversa. Fonte: Stanford, 2017

Dois mecanismos bioquímicos de geração de mutações de resistência podem ocorrer em ITRN. Um mecanismo é mediado por mutações que permitem que a enzima TR, identifique, ou seja, discrimine, um ITRN e não permita que seja adicionado à cadeia de DNA em formação. São as mutações discriminatórias, apresentadas na Figura 15. O outro, é mediado por mutações que aumentam a remoção hidrolítica do terminal do ITRN. São selecionadas pelos análogos timidínicos, AZT e Estavudina e são denominadas, mutações dos análogos da timidina (TAMS). [71]

As TAMs são mutações não polimórficas, que possuem duas vias ou padrões de acúmulo de mutações, a primeira via (tipo 1) inclui mutações nos códons 41, 210, e 215, enquanto a segunda via (tipo 2) inclui as mutações nos códons 67, 70 e 219. [74] As do tipo I, tem efeito de impacto negativo maior na resposta aos ARVs, Abacavir, ddI ou TDF

do que as do tipo 2. A seleção de mutações do tipo M184V, atenua os efeitos das TAMs sobre AZT, d4T e TDF, mas reduz a susceptibilidade ao ABC e ddI. [75]

Dependendo do subtipo viral, existe seleção para um tipo ou outro de via mutacional. A via tipo 1 (TAM1) é a mais frequentemente selecionada pelo subtipo B. A via tipo 2 (TAM2), é a mais selecionada pelo subtipo C. No caso do subtipo F, ambas podem ser selecionadas. Na sequência, após a seleção de uma das vias, pode ocorrer um acumulo progressivo de mutacoes, e em alguns pacientes muito experimentados pode haver acúmulo de 5 ou até de 6 TAMs. [65]

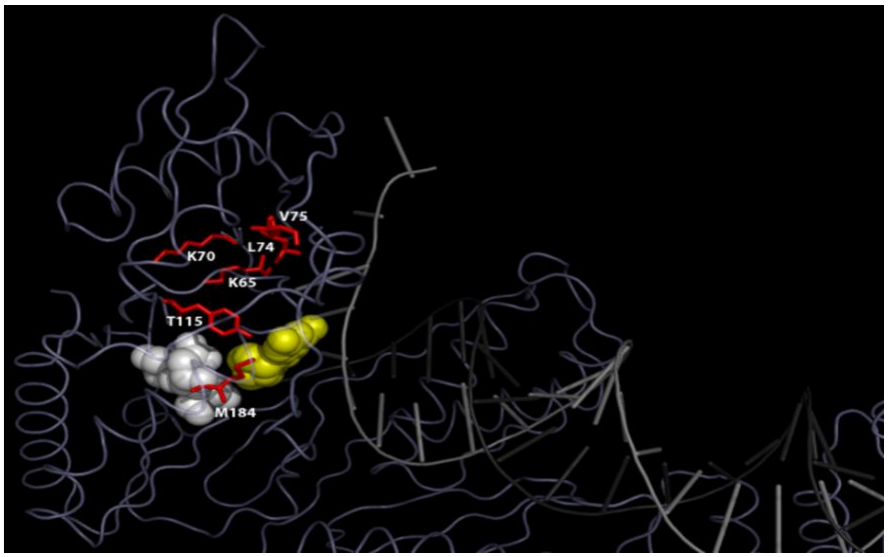


Figura 15: Posições das mutações discriminatórias mais frequentes na TR do HIV-1 que conferem resistência às drogas ITRN: K65, K70, L74, V75, Y115 e M184V. Fonte: Stanford, 2017

Os códons selecionados por cada uma das duas vias TAMs, são: M41L, L210W e T215Y, para TAM1 e os codons D67N, K70R e K219Q/E/M, para TAM2 conforme observado na Figura 16. A seleção para uma das vias, leva a respostas diferentes em relação aos ARVs. A via mutacional 1, compromete em maior grau o uso de Tenofovir, por resistência cruzada. [65]

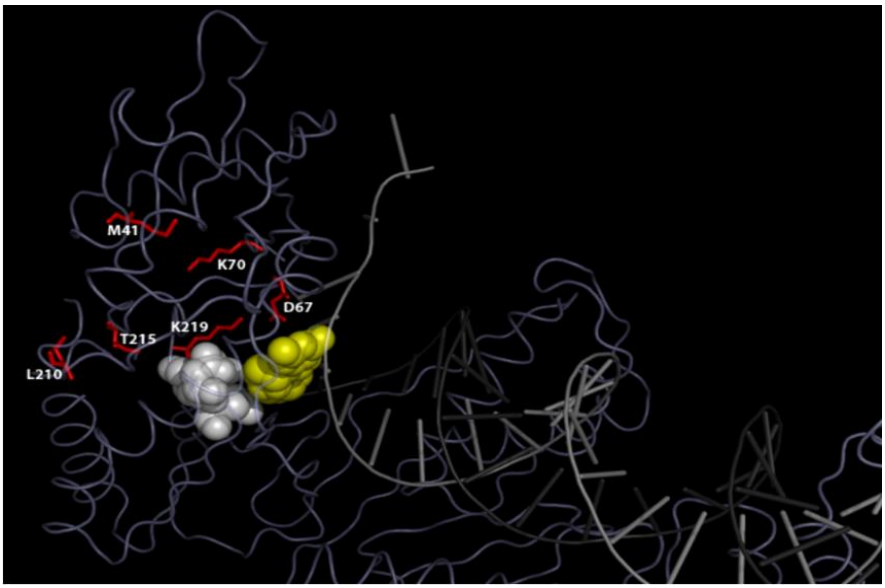


Figura 16: Posições das TAMs mais frequentemente encontradas na TR do HIV-1 que conferem resistência às drogas ITRN. Fonte: Stanford, 2017

Os inibidores de transcriptase reversa não análogos de nucleosídeo, promovem sua ação alostéricamente através da adesão a um bolso hidrofóbico, abaixo do sítio ativo da TR. Posições associadas com resistência aos ITRNN e que compõem o bolso de conexão central são: L100, K101, K103, V106, V108, V179, Y181, Y188, G190, F227. Posições adicionais incluem E138 e M230, L234, P236, K238, L318, A98 e P225. A maioria constituindo uma espécie de bolso extensivo. Na Figura 17, estão demonstradas a nevirapina acoplada (em rosa) e as posições associadas com mutações. [71]

A presença da mutacao K103N, leva a falha ao EFV e pode vir associada das mutacoes L100I e P225H. A resistência relacionada a NVP vem normalmente associada da mutacao Y181C, e esta pode estar acompanhada das mutacoes K101E e G190A.[71] Existe uma grande quantidade de códons comuns a vários integrantes da classe. A presença de mutações em determinados códons, leva ao comprometimento de quase todos os ARVs pertencentes à mesma, em especial de primeira geração, como EFV e NVP. [65]

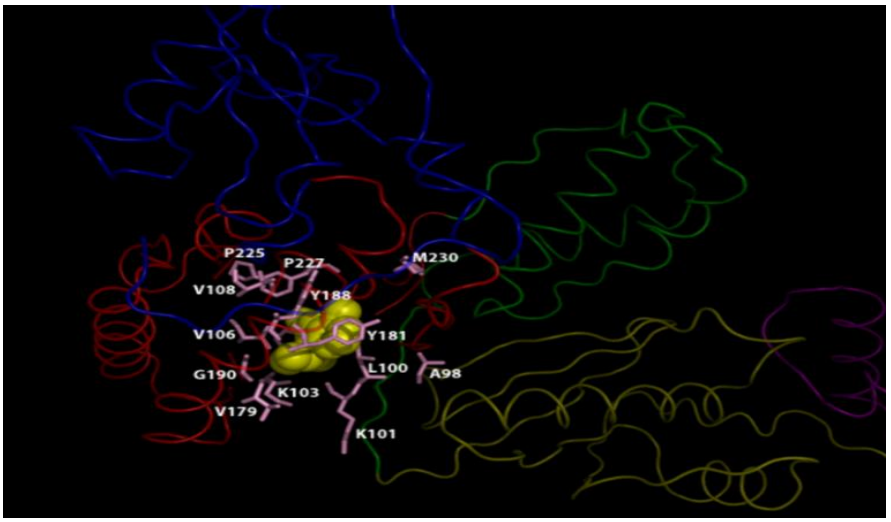


Figura 15: Regiões da subunidade p66 da TR com o ITRNN. Fonte: Stanford, 2017

Os IPs selecionam mutações no genoma, levando a uma alteração na estrutura proteica da protease. Essas mutações podem comprometer em maior ou menor grau o *fitness* viral. As chamadas mutações primárias ou principais, ocorrem mais precocemente, muito impactam no *fitness* e dimimuem de modo importante a susceptibilidade ao IP em uso. Em contrapartida, as mutações secundárias ou acessórias, não apresentam tanto comprometimento na efetividade do ARV, porém ajudam a recuperar o *fitness* viral.[65] (Figura 18) (Figura 19)

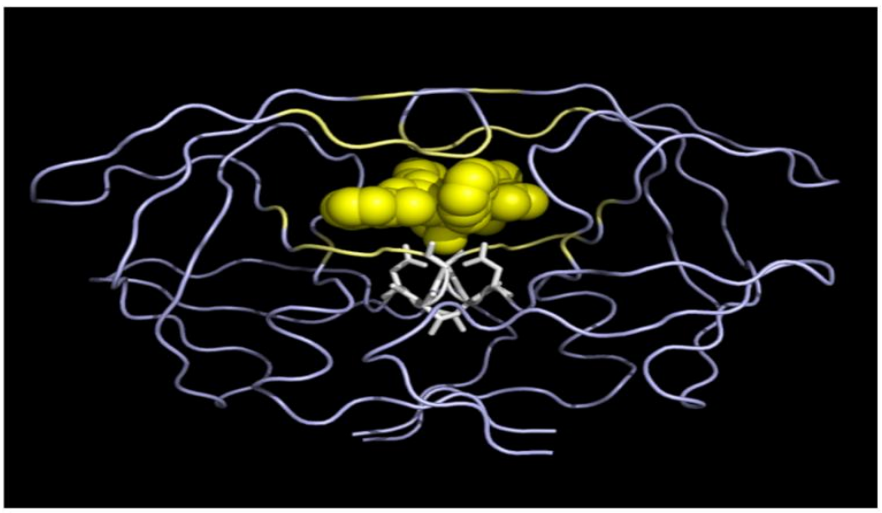


Figura 16: Estutura cristalográfica da protease do HIV 1 acoplada ao Lopinavir. Fonte: Stanford, 2017

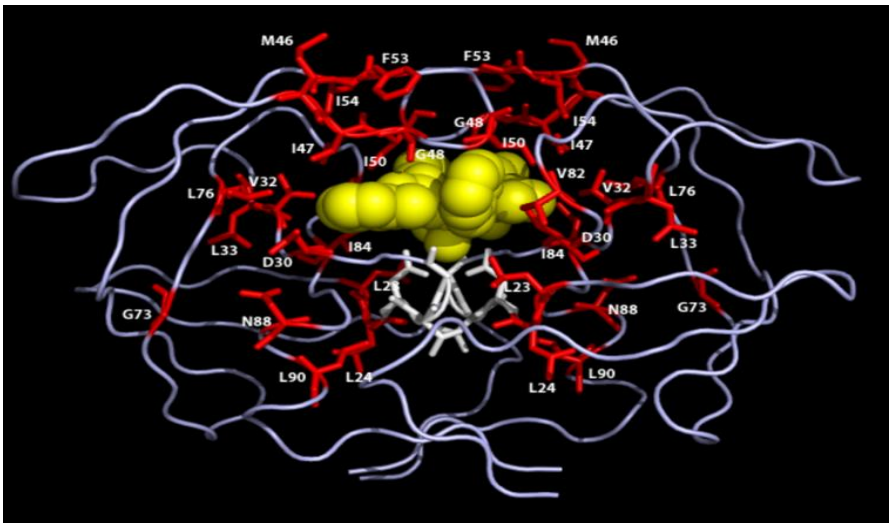


Figura 17: Posições das resistências maiores para protease. Fonte: Stanford, 2017

1.10.4. Resistência transmitida

A transmissão de vírus resistentes para uma pessoa, compromete a efetividade da TARVc a ser utilizada e representa maior risco de falência virológica e desenvolvimento de resistência. A vigilância da resistência transmitida, portanto, é primordial para suportar políticas de saúde que permitam evitar o desenvolvimento e a transmissão de resistência bem como a adoção de uso racional de TARVc. [76]

A fim de facilitar as comparações de dados epidemiológicos, gerados nos diversos programas de vigilância no globo, a OMS criou em 2007, uma lista de mutações do HIV-1. Para tal padronização, foram elaborados critérios para que as mutações fossem consideradas na vigilância genotípica, de resistência transmitida. [76]

A exposição a níveis insatisfatórios de ARV's para a prevenção da transmissão vertical (PTV) também tem sido causa de preocupação. A Nevirapina (NVP) era o terápico mais usado nos programas de PTV do vírus de HIV-1, sendo também usado como parte de um esquema terapêutico combinado nos países em desenvolvimento.[77] Estudos revelaram presença de mutações de resistência (20 a 60%) a NVP em mulheres

que receberam uma dose única durante o trabalho de parto e entre 40% a 80% das crianças que usaram NVP como profilaxia para TV. [78] Uma revisão sistemática, realizada com dados publicados entre 2014 e 2017, documentou altas taxas de resistência (49%) a ITRNN entre crianças que iniciavam TARVc em especial naquelas que haviam feito uso de PTMI. Nestas, alguns estudos mostraram resistência acima de 50% para ITRNN. [68] [79]

Nas crianças que adquirem a infecção por transmissão vertical de mãe portadora de vírus já resistentes, a formação de reservatório poderá se dar com esse tipo viral. Este fato representa impacto na terapia inicial bem como na sobrevida, sendo necessárias drogas mais potentes para sucesso terapêutico. [65] [80] (Figura 20).

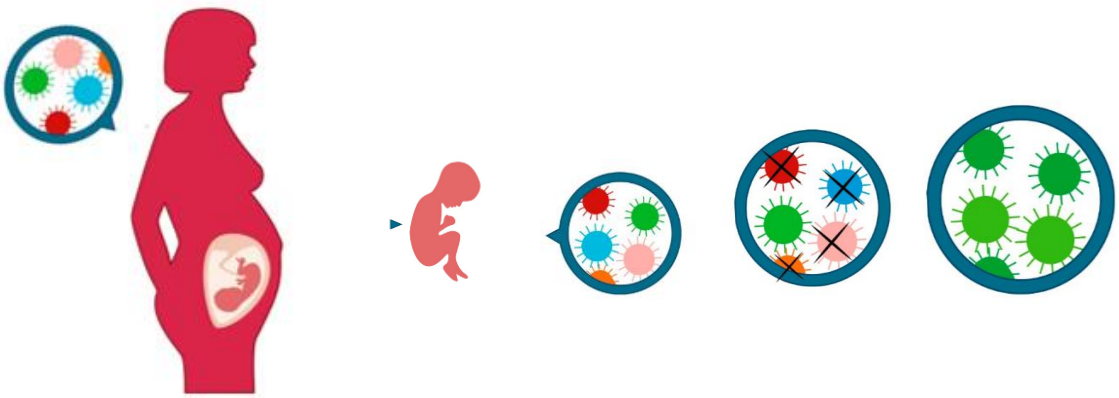


Figura 18: Transmissão de HIV de mãe para filho. Fonte: Adaptado de Science Museum of Minnesota

Guidelines de 2017 da OMS. recomendam que em regiões onde a resistência pré tratamento aos medicamentos ITRNN, atinge 10%, estes não devem ser utilizados como terapia inicial. Em vários países, PDR alcançou ou superou 10% como resultado da expansão do uso de TARVc, nos últimos anos. [68] (Figura 21)

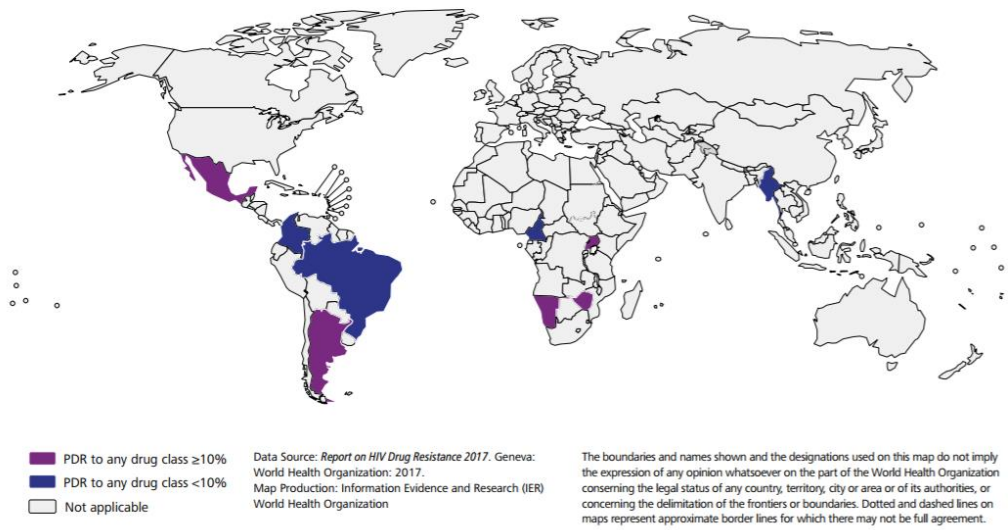


Figura 19: Locais pesquisados com resistência a ARV pré tratamento 2014 – 2016. Fonte: Global Action Plan – OMS, 2017

Em 2009 Inocêncio e colaboradores mostraram que as mutações de resistência transmitida mais encontradas no Brasil, em população de adultos, foram a K103N e Y188L/I (ITRNN), seguidas por M41L, T69D, M184V/M, T215C/S/E, K219R (ITRN) e M46I e L90M (IP). Estes achados revelam um problema sério no tratamento, principalmente para o uso dos ITRNNs como primeira linha de tratamento. A incidência global de resistência transmitida foi de 8,1%, o que é considerada prevalência intermediária pelos padrões da OMS. Segundo a OMS, prevalencia de resistência transmitida inferior a 5% é definida como baixa; entre 5 e 15%, intermediária e elevada quando superior a 15%. [81] [82]

No Brasil desde 2008, é recomendado exame de genotipagem nas gestantes e prévio ao tratamento em todas as crianças. A prevalência de resistência à droga transmitida (TDR) em crianças tem sido documentada neste período e com dados variados em todo território nacional. No entanto, os trabalhos mostram quantidades pequenas de casos, variando de 10 a 55 crianças analisadas. As taxas descritas variaram de TDR 0 a 19,2% para ITRNN, 0 a 11,5% para ITRN e 0 a 8% para IPs. Há pouco

estudos sobre a presença de DRM na população infantil e apesar de bem documentada em populações adultas, não se conhece bem os padrões e o impacto na faixa etária pediátrica. [83] [84] [85]

Conhecer a realidade da epidemia de HIV-1 em cada região brasileira é de suma importância para o correto desenhar de estratégias específicas, que busquem soluções para as questões locais. O Amazonas é um dos estados com maiores índices de transmissão vertical, do país. O conhecimento da dinâmica da epidemia em especial nas gestantes e o reflexo para as crianças expostas ao HIV-1, permite traçar ações para redução do agravo nos bebês filhos de mulheres HIV-1 positivas.

Estudar a epidemiologia molecular do HIV-1 através da determinação da prevalência de resistência primária é primordial para o sucesso terapêutico. A realidade cada vez mais presente de vírus resistentes, exige uma abordagem de tratamento mais agressiva, no enfrentamento do HIV-1, quando este alcança os lactentes por transmissão vertical.

2. OBJETIVOS

2.1 Geral

Caracterizar o perfil da epidemia de HIV/Aids em crianças, no estado do Amazonas, estimando a taxa de TV do HIV-1 em crianças expostas e os fatores associados, no período de 2000 a 2011 em um serviço de referência em infectologia pediátrica na cidade de Manaus, Brasil.

2.2 Específicos

- Examinar a tendência da taxa de transmissão vertical do HIV-1 em relação às diferentes estratégias de prevenção como uso de ART durante a gestação, parto, profilaxia neonatal e à não amamentação.

- Identificar fatores maternos e neonatais associados à transmissão vertical do HIV-1.
- Determinar a taxa de resistência transmitida nas crianças infectadas pelo HIV-1.

3.0 METODOLOGIA

3.1 Tipo de estudo

O desenho é de um estudo de coorte.

- Exposição: filhos de mães soropositivas para o HIV-1 (crianças expostas ao HIV)
- Desfecho 1: infecção pelo HIV-1
- Desfecho 2: resistência aos ARV nas crianças infectadas

3.2 Local do estudo

O estudo foi realizado em um serviço de infectologia pediátrica de referência para HIV/Aids no estado do Amazonas. Encontra-se localizado na Fundação de Medicina Tropical Doutor Heitor Vieira Dourado (FMT-HVD), na cidade de Manaus, Brasil. Esse hospital universitário fornece atenção e tratamento para pacientes com HIV/Aids dentro do sistema público de saúde e atende o maior número de casos de HIV/Aids no Estado do Amazonas. O serviço de infectologia pediátrica acompanha crianças nascidas de mães infectadas pelo HIV-1 referenciadas de todos os serviços de atenção pré-natal e maternidades do Estado. Grande parte das mães é acompanhada e recebe terapia antirretroviral no mesmo hospital.

3.3 População do estudo e critérios de inclusão

Crianças nascidas de mães infectadas pelo HIV-1 referenciadas ao serviço de infectologia pediátrica oriundas de todos os serviços de atenção pré-natal do estado. Critérios de inclusão: Crianças nascidas de mães soropositivas para o HIV-1, entre os anos de 1999 e 2011 em Manaus e que se registraram no serviço de infectologia

pediátrica da FMT-HVD antes dos vinte e quatro meses de idade, foram incluídos no estudo. Critérios de exclusão: As crianças cujas mães não tiveram confirmação do diagnóstico do HIV-1 no prazo de três meses do registro da criança no serviço de pediatria, foram excluídas.

O manejo de crianças expostas ao HIV-1, seguiu as diretrizes nacionais brasileiras e as atualizações pertinentes. As crianças expostas ao HIV-1 foram acompanhadas até pelo menos 18 a 24 meses de idade.

3.4 Tamanho da população do estudo

Foram elegíveis para o componente de taxa de transmissão vertical, todas as crianças expostas ao HIV-1, nascidas entre os anos de 1999 e 2011, que foram registradas no serviço de infectologia pediátrica da FMT- HVD. Para o componente de resistência transmitida foram elegíveis todas crianças recém diagnosticadas com modo de transmissão vertical de HIV-1 e sem histórico de uso de ARV, que tiveram coleta de genotipagem, prévia à TARVc, entre janeiro de 2010 e dezembro de 2015.

3.5 Componente 1: Determinaçao da taxa de transmissão vertical

3.5.1 Coleta de dados

Todos os dados foram obtidos mediante levantamento de prontuários e fichas clínico-epidemiológicas das crianças expostas ao HIV nascidos entre janeiro de 1999 a dezembro de 2011. Os dados sociais foram obtidos da Casa Vhida, Associação de Apoio a Criança com HIV, organização não governamental que presta assistência social a estes pacientes. As informações epidemiológicas obtidas, foram baseadas nas respostas verbais dos responsáveis e sempre que possível, confirmadas por documentação (carteira de identidade da mãe, cartão de nascimento da criança entre outros). Como parte da rotina clínica, as mães foram perguntadas sobre a idade e os seguintes dados obstétricos: atenção pré-natal e tipo de parto, momento do diagnóstico da infecção pelo

HIV, uso de ART (ARV no momento da concepção da gravidez, ART recebidos durante a gravidez), profilaxia no momento do parto, profilaxia pós-natal da criança e amamentação. Nos casos em que a mãe tivesse falecido ou estivesse ausente por outro motivo, estas informações foram obtidas a partir de outro membro da família ou dos cuidadores da criança.

Foram considerados como tendo recebido atenção pré-natal, qualquer consulta médica realizada pelas mulheres infectadas pelo HIV, durante a gestação. O aleitamento materno foi considerado quando a criança foi amamentada em qualquer momento e por qualquer período de tempo. Todos os dados foram ingressados em um banco de dados Excel pela médica pediatra que atendeu as crianças expostas ao HIV.

3.5.2 Exames diagnósticos utilizados

Os exames de carga viral (quantificação de RNA viral) foram realizados com ensaio NucliSens HIV-1QT (bioMe´rieux, Boxtel, The Netherlands) até dezembro de 2010 e com Abbott Real-Time PCR usando 2000sp (Abbott Molecular Inc., Des Plaines, IL, USA), após essa data. A contagem de LT- CD4 foi realizada por citometria de fluxo, com fluxometro FACScalibur, (Becton Dickinson Biosciences, San Jose, CA, USA)

3.5.3 Medida de resultado

As crianças foram consideradas infectadas quando apresentaram resultados positivos / reagentes em pelo menos um dos testes a seguir, tomados em momentos diferentes:

1) teste para anticorpos do HIV por enzima-imunoensaio e Western blot realizado após o 12 º mês de vida, ou

2) duas quantificações de RNA viral com resultado acima do nível de detecção, pelas metodologias acima mencionadas, em amostras de sangue separadas obtidas em ≥ 2 meses de idade (até 2008) e em ≥ 1 mês de idade (após 2008).

Foram consideradas não-portadoras do HIV, as crianças que tiveram resultado negativo para testes de anticorpos anti-HIV realizados após 18 meses de vida, ou 1) duas quantificações de RNA viral com resultados abaixo do nível de detecção, sendo pelo menos, um deles tomado após o quarto mês de vida, ou 2) uma quantificação do RNA viral com resultados abaixo do nível de detecção e teste de anticorpos anti-HIV-1 negativo ou não reativo após 12 meses de vida. As crianças nas quais não foi possível definir o status da infecção pelo HIV-1 (infectado ou não infectado) até o final do período de coleta de dados, foram consideradas perdidas para o seguimento e excluídos da análise estatística.

3.5.4 Análise estatística

Os dados foram descritos usando porcentagens e medianas com intervalos interquartil, conforme apropriado. Foram comparadas as características das crianças perdidas durante o seguimento com as crianças com estado da infeção para o HIV-1 conhecido para excluir qualquer potencial viés de seleção. A taxa de transmissão vertical foi calculada como a proporção de crianças infectadas pelo HIV entre aquelas crianças nascidas de mães infectadas pelo HIV. A taxa de transmissão vertical anual foi expressa como uma proporção e o intervalo de confiança de 95 %. A proporção das características das estratégias de prevenção da mãe e da criança foram descritas por ano. A análise de tendência de variáveis dicotômicas foi realizada utilizando o teste do qui-quadrado de dois colas.

As características maternas e infantis foram comparadas entre as crianças infectadas pelo HIV e as não-infectadas usando o teste do qui-quadrado para variáveis categóricas e teste t de Student para variáveis contínuas. Foi realizado análise univariada e multivariada por regressão logística para avaliar o efeito das variáveis maternas e

infantis na taxa de transmissão vertical do HIV. Todas as variáveis associadas com o resultado (transmissão vertical do HIV) na análise univariável (p valor <0,25), foram incluídas na análise multivariada.

Para obter o modelo final, foi usada uma estratégia “backward-stepwise” mantendo no modelo aqueles fatores associados à transmissão vertical do HIV de forma significativa. O nível de significância estabelecido foi o valor de p <0,05. Os resultados do modelo foram expressos com a odds ratio (OR) ajustada e o IC 95% como medidas de associação. Os dados foram analisados utilizando Stata 10.0 (StataCorp, CollegeStation, TX, EUA).

3.6 Componente 2: Taxa de resistência transmitida

3.6.1 Coleta de dados

Os dados foram obtidos mediante levantamento de prontuários e laudos de exames de genotipagens, constantes dos prontuários médicos, realizados entre 2010 e 2015, de crianças sabidamente infectadas pelo HIV-1. Foram coletados retrospectivamente, dados, em crianças que realizaram genotipagem na FMT-HVD até 2014 e prospectivamente, entre maio e dezembro de 2015. Todas genotipagens foram coletadas préviamente ao início de TARVc nessas crianças. As mutações encontradas na genotipagem do HIV-1 isoladas das crianças infectadas foram obtidas do sistema SISGENO, acessados pelo site http://sisgeno.aids.gov.br, com descrição detalhada dos diversos códons encontrados nas amostras de cada paciente. A interpretação da resistência a cada droga antiviral foi feita através do algoritmo localizado no web site da Stanford University (hivdb.stanford.edu). Os dados foram inseridos no mesmo banco de dados pela pesquisadora principal.

3.6.2 Exames de genotipagem utilizados

Os exames de genotipagem foram realizados utilizando TrueGene^{TM} kit (Siemens HealthCare Diagnostics, USA) e os testes de qualidade foram realizados usando painel de qualidade externa distribuído pelo Ministério da Saúde. As mutações foram atribuídas pelo Algoritmo de Resistência Populacional Calibrada (CPR), baseada na lista de Mutações de Resistência a Drogas (DRM) da OMS para resistências transmitidas de HIV-1. Análises adicionais foram conduzidas para verificar resistências pré tratamento usando o Programa HIVdb de Stanford, versão 7 (Stanford University, Palo Alto, CA, USA).

3.6.3. Medida de resultado

As mutações com potenciais de resistência aos antirretrovirais das classes de análogos de nucleosídeos e de não análogos de nucleosídeos e ainda da classe de inibidores de protease foram analisadas baseado na interpretação do programa de análise de mutação do HIV-1 localizado no website da Stanford University (hivdb.stanford.edu). Para predizer efeitos dos DRM identificados na susceptibilidade das drogas, as sequências que foram identificadas pelo algoritmo, foram classificadas como sensíveis (Stanford nível 1 nível 2), baixo nível de resistência (Stanford nível 3), intermediário (Stanford nível 4) e alto nível de resistência (Stanford nível 5). Somente foram considerados como resistentes, os perfis mutacionais com níveis intermediários ou de alta resistência no programa da Stanford University, para as classes de ARVs ou drogas específicas.

Os subtipos de HIV-1 foram definidos de acordo com o instrumento de subtipagem de HIV-1, Rega, versão 2.0 (http://www.bioafrica.net/ rega-genotype/html/subtypinghiv.html) e pelo HIVdb Program (Stanford University HIV Drug Resistance Database – http://sierra2.stanford.edu/si erra/servlet/JSierra).

3.6.4. Análise estatística

Os dados obtidos tanto na análise das gestantes e suas intervenções assim como os de resistência transmitida foram colocados numa base de dados excel e depois analisadas as taxas de transmissão vertical e resistência e seus intervalos de confiança usando o programa EPIINFO versão 7.

3.7 Gerenciamento dos dados (dos 2 componentes)

Todos os dados gerados no estudo foram coletados pela pesquisadora principal do estudo, que também é a médica especialista em infectologia pediátrica que atende as crianças expostas ao HIV na FMT-HVD. Os dados foram levantados dos prontuários clínicos e os dados sociais coletados da Casa Vhida e ingressados em formato eletrônico num banco Excel totalmente anonimizado. Os pacientes foram identificados mediante um identificador numérico gerado automaticamente pelo sistema. Esse banco foi armazenado no computador do serviço de infectologia pediátrica da FMT, protegido sob senha. Somente a pesquisadora principal e a estatística do estudo tiveram acesso à senha ao banco. Os dados foram gerenciados pela pesquisadora principal do estudo, e pela copesquisadora encarregada da análise estatística (pesquisadora visitante sênior). O resto da equipe da pesquisa somente teve acesso aos resultados do estudo e não aos dados.

O banco eletrônico já anonimizado foi copiado em um pen-drive criptografado pela pesquisadora principal e entregue para a copesquisadora encarregada da análise estatística. A pesquisadora principal do estudo foi responsável pela geração de uma cópia dos dados eletrônicos em um computador protegido por senha, de uso exclusivo para o estudo. Somente a pesquisadora principal do estudo tem acesso às senhas.

A análise estatística foi gerada no programa STATA. O computador usado na análise de dados foi protegido por senha. Todos os arquivos e bancos de dados somente estavam acessíveis para pessoal do estudo autorizado (pesquisadora principal e

estatística). Em conformidade com a legislação brasileira, os dados eletrônicos do estudo serão armazenados por cinco anos após a conclusão do estudo com proteção com chave ou senha e em seguida, serão apagados/destruídos sob a responsabilidade do investigador principal do estudo.

3.8 Aspectos de biossegurança e aspectos éticos

O protocolo foi submetido ao Comitê de ética da FMT-HVD e aprovado sob número 1054945.

Trata-se de uma análise retrospectiva de dados coletados durante a rotina da atenção clínica do serviço de infectologia pediátrica da FMT – HVD e do atendimento social na Casa Vhida entre os anos 2000 a 2014 e prospectiva para os dados gerados em 2015. Não foram pedidas outras informações adicionais além daquelas solicitadas durante a prática clínica habitual. Os dados dos prontuários foram ingressados num banco de dados Excel anonimizado criado pela médica especialista em infectologia pediátrica que atende as crianças expostas ao HIV.

Este banco foi anonimizado completamente pela médica especialista em pediatria e posteriormente e entregue para a co-pesquisadora encarregada da análise estatística e transferido a um software estatístico para análise. Neste software, os sujeitos do estudo foram identificados por um número exclusivo (ID) assignado de forma automática. Não há forma de vincular este ID com o nome ou outro dado identificador do banco Excel, nem o banco de dados Excel com o prontuário ou outro identificador da criança. Os dados eletrônicos são protegidos por senha (ver sessão “Gerenciamento de Dados” acima).

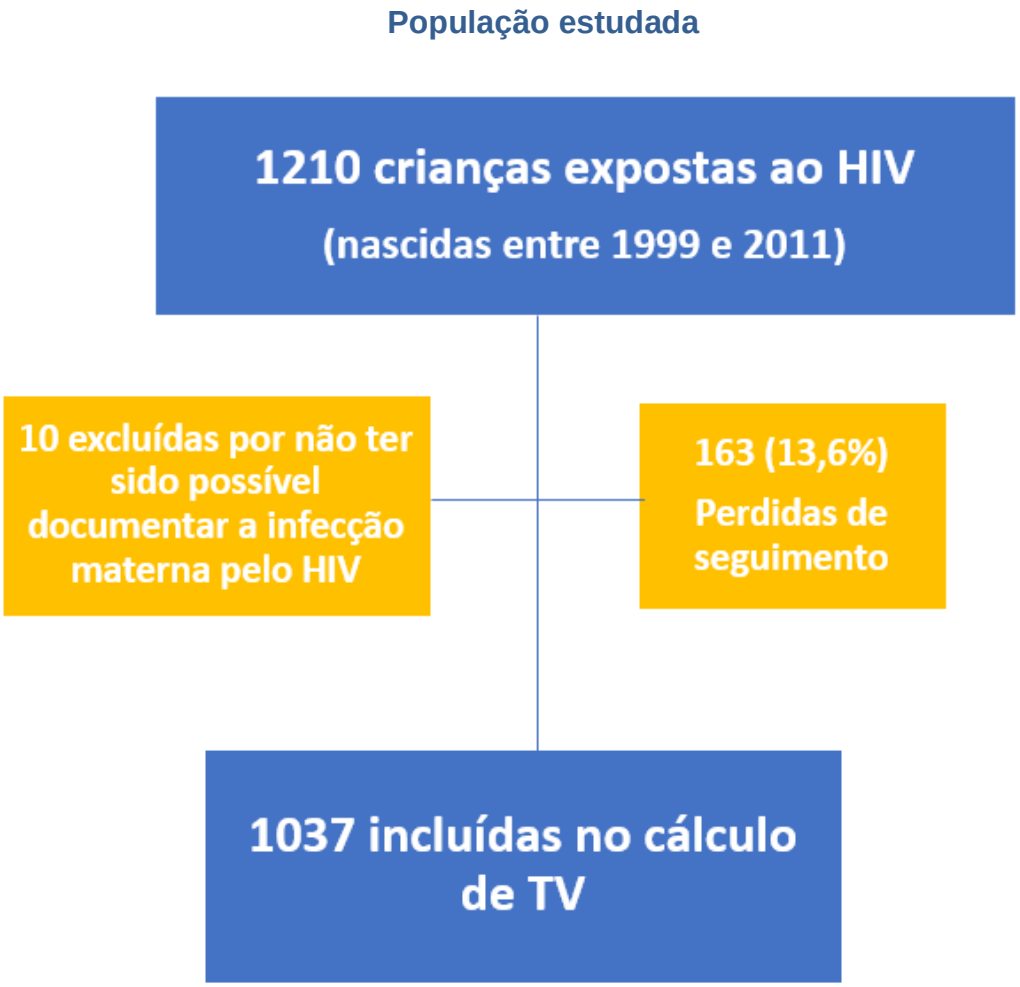
O único risco do estudo seria a quebra do sigilo. No entanto, por estarem anonimizados os dados, não seria possível estabelecer um link entre o banco de dados Excel e prontuários ou fichas clinicas e dados no STATA.

4. Resultados

4.1. Artigo 1:

Mother-to-child Transmission of HIV From 1999 to 2011 in the Amazonas, Brazil

Risk Factors and Remaining Gaps in Prevention Strategies



Mother-to-child Transmission of HIV From 1999 to 2011 in the Amazonas, Brazil

Risk Factors and Remaining Gaps in Prevention Strategies

Solange Dourado de Andrade, MD,† Meritxell Sabidó, MD, PhD,*‡ Wuelton Marcelo Monteiro, PhD,* Luiz Canellas, MD,* Vania Prazeres, MD,* and Adele Schwartz Benzaken, MD, PhD*§*

Background: The purpose of the study was to estimate rates of mother-to-child transmission (MTCT) of HIV in the Amazonas, Brazil, and to identify the associated factors.

Methods: This was a retrospective cohort study of 1210 children born to HIV-infected women between 1999 and 2011 and enrolled before age of 18 months in a reference HIV/AIDS pediatrics service in Manaus. We used multivariable logistic regression to assess the effect of maternal, obstetric and prophylactic interventions on MTCT of HIV.

Results: Ten children were excluded because of undocumented maternal HIV status. Among 1200 children, 163 (13.6%) were lost to follow-up. We included in the analysis 1037 children with known HIV status. Of those, 68 children were HIV infected, resulting in a MTCT rate of 6.6% [95% confidence interval (CI): 5.3–8.3]. Among mothers, 76.1% had received antiretroviral therapy during pregnancy, 59.3% elective caesarean, and 9.7% were breastfed. Factors associated with lower odds of MTCT of HIV were antiretroviral therapy during pregnancy [odds ratio (OR): 0.26; 95% CI: 0.12–0.58], elective caesarean (OR: 0.48; 95% CI: 0.23–0.98) and with MTCT: being breastfed (OR: 4.56; 95% CI: 2.19–9.50). Transmission decreased from 7.5% in 2007–2008 to 3.2% in 2011, while breastfeeding decreased from 30.8% in 1999–2000 to 3.9% in 2011–2012.

Conclusions: The HIV rate of MTCT is still high in the Amazonas and challenges for its prevention prevail including lost to follow-up and gaps in critical strategies such as antiretroviral use during pregnancy. More efforts are needed to increase the number of women and babies who successfully complete the prevention of MTCT cascade and work toward elimination of MTCT of HIV.

Key Words: epidemiology, risk factors, HIV, mother-to-child-transmission, Amazon

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Mother-to-child transmission (MTCT) of HIV in Brazil is the main source of HIV infection in children. In 2013, 14,352 children younger than 13 years were living with HIV in this country, 92.6% of whom had acquired HIV through MTCT.[1] The most recent estimate of the prevalence of HIV among pregnant women in Brazil is 0.41%.[2] The incidence rate of HIV in children younger than 5 years fell from 5.0 per 100,000 in 2001 to 3.4 per 100,000 in 2012.[1] The rate of MTCT has also decreased from 7.1% in 2001[3] to 6.4% in 2004.[4] However, MTCT rates vary across the country, with the most recent estimates being higher for northern Brazil, ranging from 9.2% to 9.9%,[5,6] compared with the south of the country, at 4.9%.[7]

Although prevention of MTCT (PMTCT) is among the highest priorities of the Ministry of Health in Brazil, the country still falls short of the MTCT elimination goal of 2% or less by 2015.[8] Translating this target into clinical practice is a difficult challenge. Important progress has been made in antenatal care coverage, which stands at 96%,[9] and in HIV testing for all pregnant women, currently at 83.5%.[10] MTCT services have been expanded but still only 64% of HIV-infected pregnant women receive antiretroviral therapy (ART), and among infants born to mothers with HIV, only 68% receive antiretroviral (ARV) prophylaxis and only 37% will be tested within 2 months of birth.[11]

Well-known prevention interventions to reduce MTCT of HIV include a combination of ARV prophylaxis, elective caesarean delivery and avoidance of breastfeeding. Brazil issued the first recommendations on PMTCT in 1996 (Table 1).[12,13] These recommendations were revised in 2007 with the adoption of combination ARV prophylaxis for those women not eligible for lifelong ART.[14] In 2010, the guidelines were updated to offer lifelong ART to all HIV-infected pregnant women regardless of their CD4 count.[15]

Substantial reductions in new pediatric infections can be achieved as a result of high coverage with effective interventions for PMTCT of HIV. PMTCT programs are particularly ineffective in remote areas of the Amazonas with limited services and uncoordinated referral networks.[20] The State of Amazonas has experienced increasing HIV incidence in children 0–5 years, as well as in women.[20] However, there is a lack of information from cohort studies regarding temporal evolution of the MTCT of HIV burden in the Amazon region.

The objective of the study was to estimate rates of MTCT of HIV in the State of Amazonas, Brazil, in the years 1999–2011, in children followed by a pediatric reference service in the city of Manaus, and to identify maternal and newborn factors associated with HIV MTCT.

MATERIAL AND METHODS

Study Setting and Design

A retrospective cohort study was conducted at the reference HIV/AIDS pediatrics service of Fundação de Medicina Tropical Doutor Heitor Vieira Dourado (FMT-HVD) in the city of Manaus, Brazil. This teaching hospital provides HIV care within

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From the *Fundação de Medicina Tropical Doutor Heitor Vieira Dourado (FMT-HVD), Manaus, Amazonas, Brazil; †Universidade do Estado do Amazonas, Manaus, Amazonas, Brazil; ‡TransLab., Department of Medical Sciences, Universitat de Girona, Catalunya, Spain; and §Departamento de DST, Aids e Hepatites Virais, Secretaria de Vigilância em Saúde, Ministério da Saúde, Brazil.

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Address for correspondence: Meritxell Sabidó, MD, PhD, Fundação de Medicina Tropical Doutor Heitor Vieira Dourado, Avenida Pedro Teixeira 25, CEP: 69040-000 Manaus, Amazonas, Brazil. E-mail: xellsabido@gmail.com.

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TABLE 1. History of Interventions to Prevent MTCT of HIV in Brazil

Year	Intervention
1996	ZDV prophylaxis. Since 1996, intrapartum prophylaxis with ZDV, postpartum infant prophylaxis with ZDV until age 6 weeks and counseling to avoid breastfeeding.[12,13]
2000	Compulsory notification of HIV-positive pregnant women cases and infants exposed to the risk of HIV transmission (Decree no. 933 of September 4, 2000)[16]
2002	Formula freely available for replacement feeding
2007	Short-term triple-drug ARV prophylaxis (starting as early as 14 weeks of gestation until delivery) in women with CD4 cell count of <200 cells/mL and plasma viral load of >1000 copies/mL[14]
2010	Triple ARV prophylaxis initiated during pregnancy (from week 14 or as soon as possible thereafter and continued until after finishing breastfeeding (Option B)[15]
2011	Stork Network to strengthen ANC and to expand the number of services that offer PMTCT actions
2012	Triple ARV starting as soon as HIV is diagnosed and continued lifelong, regardless of CD4 count (Option B+)[11]
2013	Adoption of TaSP in Brazil[17] Advisory Committee on MTCT[11] Municipal MTCT Committees[11]
2014	Guidelines for Qualification of Care Lines of MTCT of HIV, Hepatitis B and Syphilis[18] Expanded ARV prophylaxis using 3 doses of nevirapine for infants born to women who received no ARV during pregnancy[19]

ANC indicates antenatal care; TaSP: treatment as prevention.

the public health system and attends the highest number of cases of HIV/AIDS in the Amazon state. The pediatric service follows up children born to mothers with HIV infection referred from all the state antenatal care services. The mothers receive follow-up and ART within the same hospital. The study was approved by the Ethical Institutional Review Board of FMT-HVD with waiver of consent.

Study Population

All children born to mothers with HIV infection between January 1, 1999 and December 31, 2011 and who were enrolled into the HIV pediatric service before 18 months of age were included in the study. Children were excluded if mothers could not document HIV infection within 3 months of admission of the child. HIV management followed Brazilian national guidelines, as regularly updated (Table 1). Children were followed up until they were at least 18 months old.

Data Collection

As part of routine practice, mothers were asked about their age, obstetric data such as prenatal care and mode of delivery, timing of their HIV-infection diagnosis, use of ART (beginning ART before or after becoming pregnant), intrapartum prophylaxis, postnatal infant prophylaxis and breastfeeding. When possible, this information was obtained from a family member for women who had died or moved away. Prenatal care was considered as any medical or pediatric consultation that women had during gestation. Maternal breastfeeding at any time and for any duration was defined as maternal breastfeeding present. All data were entered into a database by the pediatrician.

HIV Status of Children

Children were considered infected if they were positive on at least 1 of the following tests, taken at different times: (1) test for HIV antibodies by enzyme-linked immunosorbent assay and Western blot carried out in children at age 18 months or older or (2) 2 plasma quantitative viral RNA results above the detection level on separate blood specimens obtained at ≥2 months of age (until 2008) and at ≥1 month of age (after 2008). Children were considered not infected if they tested negative by (1) tests for HIV antibodies carried out after 18 months of life, (2) 2 plasma quantitative viral RNA tests, at least 1 of which was taken after the fourth month of life or (3) 1 plasma quantitative viral RNA test and 1 test for HIV antibodies after the 12th month of life. Those children for whom it was not possible to define HIV-infection status (infected or not infected) by the end of the data collection period (June 2014) were considered lost to follow-up (LTFU) and were excluded from the analysis.

Statistical Analysis

Data were described using percentages and medians with interquartile ranges, as appropriate. We compared characteristics of children LTFU with those in whom HIV status could be determined to exclude any potential selection bias. We categorized the standard ART used during pregnancy for PMTCT in Brazil according to the recommendation period: 1999–2006 [zidovudine (ZDV) prophylaxis], 2007–2009 (triple-drug ARV prophylaxis according to CD4 cell count) and from 2010 triple-drug ART regardless of CD4 count. We further categorized type of delivery into having received elective caesarean (yes or no).

MTCT rate was calculated as the proportion of HIV-infected children among those born to HIV-infected women and who had determined HIV status as of June 2014, expressed with the confidence interval (CI) at 95%.

We compared maternal and obstetric characteristics and strategies for PMTCT between HIV-infected and noninfected children using the χ^2 test for categorical variables and Student's t test for continuous variables. Univariable and multivariable analyses using logistic regression were conducted to assess the effect of maternal and child variables on HIV MTCT rates. All variables associated with the outcome in univariable analysis (Wald P value < 0.25) were included in the multivariable analysis. A backward-stepwise strategy was then applied to obtain the final model that retained only those factors significantly associated with MTCT of HIV. The level of significance was established at P value <0.05. The individual contribution of each risk factor was assessed by a likelihood ratio test. Crude and adjusted odds ratios (AOR) with 95% CI were used as measures of association. Data were analyzed using Stata 10.0 (StataCorp LP, College Station, TX).

RESULTS

HIV-exposed Children

From 1999 to 2011, a total of 1210 HIV-exposed children were enrolled in the pediatric service. Among the enrolled children, there were 7 sets of twins. Ten children were excluded because the HIV-infection status of their mothers could not be confirmed. Among 1200 children, 163 (13.6%) were LTFU before the HIV-infection status could be established. The analysis, therefore, included 1037 children with complete follow-up, 528 (50.9%) of whom were male (Table 2). These children were more likely to have a mother who was older than 25 years, received her HIV diagnosis during pregnancy, had prenatal care, received ART during pregnancy regardless of CD4 count, underwent elective caesarean and received intrapartum and postnatal prophylaxis with ZDV. The 2 groups were similar regarding child's sex and presence of breastfeeding.

TABLE 2. Infant, Maternal, Obstetric Characteristics and Strategies for PMTCT of Children Exposed to HIV, According to Whether Their Follow-up Was Complete, Amazonas, Brazil, 1999–2011

Characteristics	Complete Follow-up (N = 1037), n (%)	LTFU (N = 163), n (%)	*P* Value*
Infant sex			
Male	528 (51.0)	72 (44.2)	0.11
Female	508 (49.0)	91 (55.8)	
Maternal age, yr			
<25	432 (46.9)	32 (69.6)	0.003
≥25	490 (53.2)	14 (30.4)	
Timing of HIV infection diagnosis			
Before pregnancy	297 (32.3)	13 (28.3)	
During pregnancy	449 (48.9)	15 (32.6)	0.007
During labor	67 (7.3)	8 (17.4)	
Postpartum	106 (11.5)	10 (21.7)	
Prenatal care			
Yes	890 (94.7)	43 (86.0)	0.01
No	50 (5.3)	7 (14.0)	
ARVs received during pregnancy			
Yes	724 (76.1)	44 (60.3)	0.003
No	228 (23.9)	29 (39.7)	
Type of ARVs received during pregnancy			
ZDV prophylaxis	420 (40.5)	77 (47.3)	<0.001
Triple ART if CD4 <200 cells/mL	335 (32.3)	66 (40.5)	
Triple ART regardless of CD4 count	282 (27.3)	20 (12.3)	
Mode of delivery			
Vaginal	200 (20.9)	25 (34.3)	
Emergency caesarean	190 (19.8)	11 (15.1)	0.03
Elective caesarean	569 (59.3)	37 (50.7)	
Intrapartum prophylaxis with ZDV			
Yes	765 (80.6)	49 (69.1)	0.02
No	184 (19.4)	22 (30.9)	
Postnatal infant prophylaxis with ZDV			
Yes	865 (89.5)	56 (80.0)	0.02
No	102 (10.6)	14 (20.0)	
Breastfeeding			
No	878 (90.3)	45 (83.3)	
Yes	94 (9.7)	9 (16.7)	0.10

*Using Pearson χ^2 test. Statistically significant ($P < 0.05$).

Characteristics of Children With Known HIV Status

Mean age of mothers at the time of delivery was 25.4 years (standard deviation: 5.5; Table 2). The diagnosis of HIV infection for the mothers was made before pregnancy for 297 (32.3%) children, during pregnancy for 449 (48.9%), during labor for 67 (7.3%) and 106 (11.5%) in postpartum. Most (890, 94.7%) women attended antenatal care during their recent pregnancy, and 724 (76.1%) started ART during pregnancy. ZDV prophylaxis was received by 420 (40.5%) women, triple ART when CD4 <200 cells/mL by 335 (32.3%) and triple ART regardless of CD4 count by 282 (27.3%). Delivery was vaginal in 200 (20.9%) women, by emergency caesarean in 190 (19.8%) and elective caesarean in 569 (59.3%). At delivery, 765 (80.6%) women received intrapartum prophylaxis with ZDV. Among infants, 865 (89.5%) received postnatal prophylaxis with ZDV, and 94 (9.7%) were breastfed.

Risk Factors Associated With MTCT of HIV

Bivariate analysis of children according to their HIV status (Table 3) revealed that the variables significantly associated with an increased odds of MTCT were maternal HIV diagnosis in the postpartum period, not receiving prenatal care, not receiving intrapartum prophylaxis with ZDV, not receiving postnatal infant prophylaxis with ZDV and being breastfed. Variables significantly associated with a decreased odds of MTCT were receiving ART during pregnancy and elective caesarean.

In the multivariable model (Table 3), receiving ART during pregnancy (AOR: 0.26; 95% CI: 0.12–0.58; $P = 0.001$) and elective caesarean (AOR: 0.48; 95% CI: 0.23–0.98; $P = 0.04$) remained associated with a significantly lower odds of MTCT after adjustment. Being breastfed (AOR: 4.56; 95% CI: 2.19–9.50; $P < 0.001$) was significantly associated with transmission of HIV infection after adjustment.

MTCT Rates During the Study Period

Sixty-eight of the 1037 children with known infection status were HIV infected according to virologic or serologic testing, corresponding to an overall MTCT rate of 6.6% (95% CI: 5.0–8.1) for the cohort of children in this study born from 1999 to 2011. Assuming that children with unknown HIV-infection status were all uninfected or all infected, the lower and upper estimates of MTCT were 5.7% (95% CI: 4.4–7.0) and 19.3% (95% CI: 17.0–21.5).

During the study period, the rate of HIV MTCT decreased from 7.5% in 2007–2008 to 3.2% in 2011 (Fig. 1). The proportion of mothers who received ART during pregnancy varied from 70.8% in 1999–2000 to 79.4% in 2011, whereas the proportion of children breastfed decreased during the study period from 30.8% in 1999–2000 to 3.9% in 2011. Neonatal and postnatal prophylaxis followed a similar increasing trend overtime, as did the proportion of children born by elective caesarean that increased since 2003.

TABLE 3. Mother-to-child Transmission of HIV According to Infant, Maternal, Obstetric Factors and Strategies for PMTCT of HIV, Amazonas, Brazil, 1999–2011 (Bivariable and Multivariable Analysis)

Factors	HIV-infected Infants (N = 68), n (%)	HIV-uninfected Infants (N = 968), n (%)	Crude OR (95% CI)	*P* Value*	Adjusted OR (95% CI)	*P* Value*
Infant sex						
Male	29 (5.5)	499 (94.5)	1			
Female	39 (7.7)	469 (92.3)	1.43 (0.87–2.35)	0.16	—	—
Maternal age, yr						
<25	22 (5.1)	410 (94.9)	1			
≥25	39 (7.9)	451 (92.1)	1.61 (0.94–2.76)	0.08	—	—
Timing of HIV infection diagnosis						
Before pregnancy	13 (4.4)	284 (95.6)	1			
During pregnancy	13 (2.9)	436 (97.1)	0.65 (0.30–1.42)	0.28	—	—
During labor	5 (7.5)	62 (92.5)	1.76 (0.61–5.13)	0.30	—	—
Postpartum	30 (28.3)	76 (71.7)	8.62 (4.29–17.33)	<0.001	—	—
Prenatal care						
Yes	52 (5.8)	838 (94.2)	1			
No	7 (14.0)	43 (86.0)	2.62 (1.13–6.12)	0.03	—	—
ARVs received during pregnancy						
No	43 (18.9)	185 (81.2)	1		1	
Yes	17 (2.4)	707 (97.7)	0.10 (0.06–0.19)	<0001	0.26 (0.12–0.58)	0.001
Type of ARVs received during pregnancy						
ZDV prophylaxis	17 (6.0)	265 (94.0)	1			
Triple ART if CD4 <200 cells/mL	20 (6.0)	315 (94.0)	0.99 (0.51–1.93)	0.98	—	—
Triple ART regardless of CD4 count	31 (7.4)	389 (92.6)	1.24 (0.68–2.29)	0.49	—	—
Elective caesarean						
No	42 (10.8)	348 (89.2)	1		1	
Yes	14 (2.5)	555 (97.5)	0.21 (0.11–0.34)	<0.001	0.48 (0.23–0.98)	0.04
Intrapartum prophylaxis with ZDV						
Yes	25 (3.3)	740 (96.7)	1			
No	36 (19.6)	148 (80.4)	7.20 (4.20–12.35)	<0.001	—	—
Postnatal infant prophylaxis with ZDV						
Yes	34 (3.9)	831 (96.1)	1			
No	26 (25.5)	76 (74.5)	8.37 (4.77–14.67)	<0.001	—	—
Breastfeeding						
No	34 (3.9)	844 (96.1)	1		1	
Yes	32 (34.0)	62 (66.0)	12.81 (7.41–22.15)	<0.001	4.56 (2.19–9.50)	<0.001

*Using Pearson χ^2 test. Statistically significant ($P < 0.05$).

DISCUSSION

To our knowledge, this is the largest Amazonas cohort study of HIV MTCT that includes all HIV-exposed children who attended a pediatric HIV referral center of that state. The overall HIV transmission rate at 6.6%, although still high, was lower than the one reported for 2 recent cohorts from northeastern Brazil, estimated at 9.2% in Pernambuco[6] and 18.9% in Sergipe.[21] Importantly, in the state of Amazonas progress has been made in reducing MTCT rate over time, from an estimated 7.2% in 2001–2002 to an estimated 3.2% in 2011. This most recent MTCT rate is similar to the country reported, modeled HIV transmission rate for 2013, at 3.6%.[9] Brazil is within range of MTCT of HIV >2% to ≤5%, positioning the country close to meeting the elimination goal (<2% of MTCT of HIV rate).[9]

The uptake of PMTCT interventions improved over time, although we identified gaps in critical interventions, notably in use of ART during pregnancy, elective caesarean and avoidance of breastfeeding.

The use of ART during pregnancy significantly reduced the odds of MTCT, consistent with other Brazilian studies.[6,22,23] The fact that only 75% of HIV-infected women used ARV drugs during pregnancy might be related to receiving a late diagnosis of HIV infection. Prenatal care was high at 94% but in 18.8% of women HIV was diagnosed during labor and the postpartum period. Among 168 women who were tested during labor or postpartum, 79% did not have any prenatal care visits. Another reason for late testing might be a lack of availability of HIV rapid tests,[24] although since 2012 the Stork Network Program has made substantial efforts to expand antenatal HIV and syphilis rapid testing in the Amazonas.[11] Since 2014, the Brazilian guidelines for the management of HIV infection in children and adolescents support the use of expanded ARV prophylaxis to high-risk infants of mothers who received no ARVs during pregnancy.[10] The provision of 3 doses of nevirapine prophylaxis associated with ZDV for infants born to mothers identified late in pregnancy successfully documented a reduction in the overall HIV transmission rate (in utero + intrapartum), compared with ZDV alone (7.1% vs. 11.1%, $P = 0.034$).[25] Even when successfully tested, the challenge of poor availability of and access to PMTCT services in the Amazonas remains a significant barrier for ART initiation. Indeed, a recent meta-analysis estimated that 49% of HIV-infected pregnant women in sub-Saharan African PMTCT programs are lost between registration and delivery,[26] and other studies showed up to 80% of infected women are lost by 6 months postpartum.[27,28]

Breastfeeding, which is known to increase the risk of MTCT of HIV,[3,6,29,30] was also found to be significantly associated with postnatal HIV infection in our study. Despite national guidelines recommending avoidance of breastfeeding and the provision of free formula for babies born to HIV-infected mothers, 10% of children were breastfed. This might be partly attributable to late HIV diagnosis. In our cohort, of the 88 breastfeeding women with available information about the timing of HIV diagnosis, 73 (83.0%) were diagnosed only after delivery, and therefore, they might breastfed without being aware of their HIV status. Sociocultural norms,

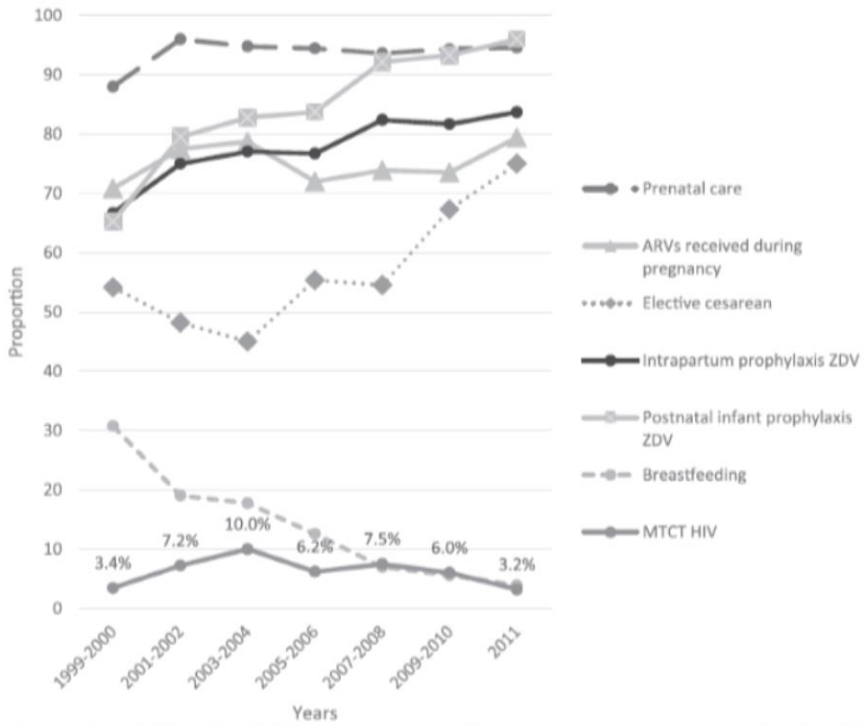

FIGURE 1. Trends in MTCT of HIV and in PMTCT strategies according to year of birth, Amazonas, Brazil, 1999–2011.

stigma and socioeconomic conditions profoundly influence the ability of women to follow messages from healthcare providers.[31] Therefore, interventions to support safe infant feeding practices to prevent transmission through breastfeeding must reach communities.[32] In Brazil, with the introduction of lifelong ART for pregnant women regardless of CD4 cell count (Option B+),[15] more pregnant and lactating women with HIV are eligible for ART. This advance should prevent further postnatal HIV transmission through breastfeeding.

The association of mode of delivery and MTCT has varied among different studies. In our cohort, elective caesarean reduced the odds of MTCT in the multivariable analysis. Elective caesarean is an efficacious intervention for the prevention of MTCT among HIV-1–infected women not taking ARVs or taking only ZDV.[33] Therefore, it is a relevant intervention in the Amazonas, where 25% of HIV-infected pregnant women did not receive ARV. We have observed that up to 19.3% women underwent emergency caesarean probably because they arrived late in labor and delivered shortly after. This would also result in a missed opportunity for the timely provision of intrapartum prophylaxis with ZDV.

We observed a LTFU of 13.6% at 18 months, which was slightly lower than that reported in 2 cohorts from Pernambuco, at 15% in HIV-exposed children[6] and at 16.2% among a small cohort (n = 195) of HIV-infected children and adolescents.[34] A recent systematic review found that in African cohorts, the proportion of HIV-infected patients younger than 18 years who died or were LTFU during 2008–2013 ranged from 2.6% to 44.6%.[35] Interventions to reduce pediatric LTFU are urgently needed to improve long-term success of ART programs. Early infant diagnosis (EID) is a key strategy to retaining HIV-exposed infants through the end of the exposure period, as it provides an opportunity to offer early clinical care and continuous follow-up.[36] Early mortality is a major problem when EID fails.[37] One strategy to identify these children earlier is to expand testing to other routine contact with the healthcare system, such as immunization visits.[38] Other innovative outreach activities to enhance the identification of women with HIV, such as home-based counseling and testing for HIV using point-of-care (POC) test, were successful in reaching untested individuals in the interior of the Amazonas.[39] POC technologies are an innovative strategy to improve access to EID[37] that might be well suited for the Amazon region. POC devices allow results to be returned during the same patient visit, minimizing problems related to transportation to healthcare facilities, which has been identified as an underlying factor of poor infant follow-up.[34,40] Patients referral isolated areas in the interior of the Amazonas to a reference laboratory, added to the processing of the sample and delivery of results represent considerable obstacles to the provision of timely and quality care to HIV-infected children in the region which are all avoided with implementation of field use of POC.

This study had some limitations. We reviewed only pediatric medical records, and although they usually include prenatal information, there were missing data for maternal clinical characteristics, HIV viral load, CD4+ T-lymphocyte counts, duration of ART, adherence to ART and duration of breastfeeding. Nevertheless, HIV-exposed children in our study are representative of HIV-exposed children in the Amazonas. Fluctuations in HIV transmission rates over time may partially occur because of the small sample size of HIV-exposed children.

Our observations indicate improvements in MTCT prevention strategies in the Amazonas in recent years, but MTCT continues to occur at a rate that is unacceptably high. More efforts are needed leading to more women and babies successfully completing the PMTCT cascade, expanding sites that offer PMTCT of HIV to the interior of the Amazonas, strengthening linkage to ART care and improving EID through strategies such as POC testing and case finding of HIV-exposed children in other routine health services.

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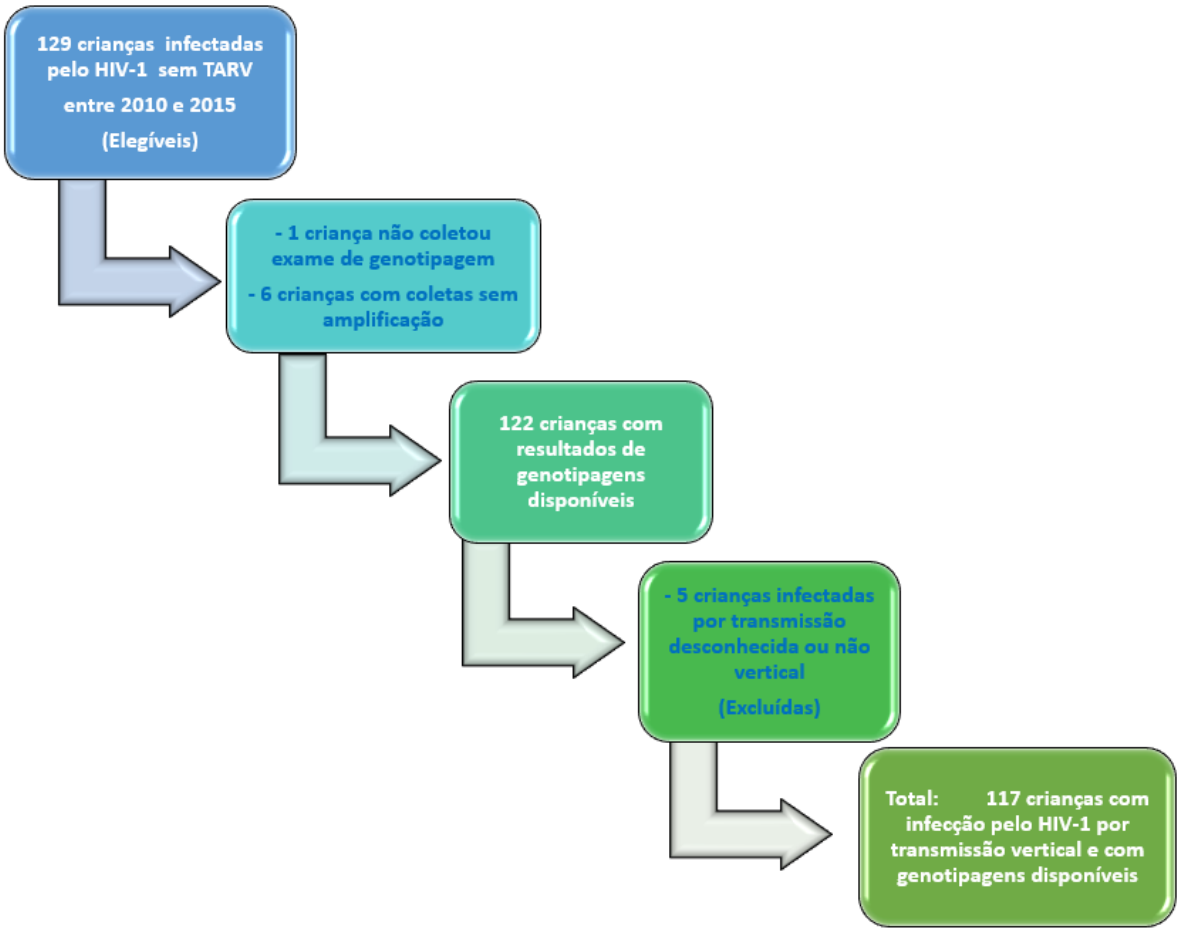
4. 2. Artigo 2:

Drug resistance in antiretroviral-naive children newly diagnosed with HIV-1 in Manaus, Amazonas

População estudada



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Drug resistance in antiretroviral-naive children newly diagnosed with HIV-1 in Manaus, Amazonas

Solange Dourado de Andrade[1,2], Meritxell Sabidó[1,3,4]*, Wuelton Marcelo Monteiro[1], Adele Schwartz Benzaken[1,2,5] and Amilcar Tanuri[1]

[1]Tropical Medicine Foundation Doctor Heitor Vieira Dourado (FMT-HVD), Av. Pedro Teixeira 25, Manaus, Amazonas, CEP: 69040-000, Brazil; [2]Universidade do Estado do Amazonas, Avenida Djalma Batista, Manaus, Amazonas, CEP: 358-69005-010, Brazil; [3]TransLab, Department of Medical Sciences, Universitat de Girona, Emili Grahit 77, Catalonia 17071, Spain; [4]CIBER of Epidemiology and Public Health (CIBERESP), Av. Monforte de Lemos 3–5, Pabellón 11, Planta 0, Madrid 28029, Spain; [5]Department of STI, AIDS and Viral Hepatitis, Secretary for Health Surveillance, Ministry of Health Brazil, Brasilia, Brazil

*Corresponding author. Tropical Medicine Foundation Doctor Heitor Vieira Dourado (FMT-HVD), Av. Pedro Teixeira 25, Manaus, Amazonas, CEP: 69040-000, Brazil. Tel/Fax: +55-9221273498; E-mail: xellsabido@gmail.com

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Objectives: To determine the prevalence of drug resistance mutations (DRM), the prevalence of drug susceptibility [transmitted drug resistance (TDR)] and the prevalence of HIV-1 variants among treatment-naive HIV-infected children in Manaus, Amazonas state, Brazil.

Methods: Children born to HIV-infected mothers and diagnosed with HIV in an HIV reference service centre and with available *pol* sequence between 2010 and 2015 prior to antiretroviral initiation were included. TDR was identified using the Calibrated Population Resistance Tool. HIV-1 subtypes were defined by Rega and phylogenetic analyses.

Results: One hundred and seventeen HIV-infected children with a median age of 3.7 years were included. Among them, 28.2% had been exposed to some form of prevention of mother-to-child transmission (PMTCT). HIV DRM were present in 21.4% of all children. Among PMTCT-exposed children, 3% had NRTI mutations, 15.2% had NNRTI mutations and 3% had PI mutations. Among PMTCT-unexposed children, 1.2% had NRTI mutations, 21.4% had non-NNRTI mutations and 1.2% had PI mutations. The most common DRM was E138A (8.5%). The prevalence of TDR was 16.2%; 21.1% among PMTCT-exposed children and 14.3% among PMTC-unexposed children. The analysis of HIV-1 subtypes revealed that 80.2% were subtype B, 6.0% were subtype C, 3.4% were subtype F1 and 10.3% were possible unique recombinant forms (BF1, 4.3%; DB, 4.3%; BC, 0.9%; KC, 0.9%).

Conclusions: We report a high prevalence of DRM in this population, including in almost a quarter of children with no reported PMTCT. The high prevalence of TDR observed might compromise ART effectiveness. Results show extensive HIV-1 diversity and expansion of subtype C, which highlights the need for surveillance of HIV-1 subtypes in Amazonas state.

Introduction

In Brazil there are about 15 900 children under the age of 13 infected with HIV-1, 92.8% of whom have acquired HIV through mother-to-child transmission (MTCT).[1] The most recent estimate of HIV-1 prevalence in pregnant women is 0.4%.[2] The rate of MTCT is 3.6% in the country[3] and 6.6% in Amazonas state.[4] There is currently nationwide access to free combination ART. Although access to ART has expanded in recent years, only 61% of HIV-infected people that knew their HIV status were receiving it at the end of 2014.[5]

The use of antiretrovirals for treatment in mothers and as prophylaxis in newborns for prevention of MTCT (PMTCT) can lead to selection of HIV drug resistance in infected infants and is likely to be the source of primary drug resistance in this population.[6–8] The threat of increased onward transmission of drug-resistant strains to newly infected individuals, i.e. primary drug resistance, has the potential to compromise the effectiveness of first-line ART regimens,[9–11] increasing the rate of viral failure or the length of first-line antiretroviral regimens.[12] Brazilian paediatric treatment guidelines recommend drug resistance testing in all treatment-naive infants, children and adolescents to guide individual therapy choices.[13] Additionally, Brazilian treatment guidelines were updated in 2014 to recommend providing ART to all HIV-infected children below 12 months of age regardless of their CD4 cell count, and after that age following clinical and CD4 cell count criteria.[13]

As first-line treatment, children receive a regimen consisting of two NRTI plus either an NNRTI or a PI.[13]

In Brazil, the prevalence of primary resistance has been estimated and reported to be very variable in children newly diagnosed with HIV.[14] The number of subjects analysed has been small, ranging from 10 to 55, with TDR rates from 0% to 19.2% for NNRTI, 0% to 11.5% for NRTI and 0% to 8% for PI.[15–18] However, wider use of ART could result in increased primary resistance to NRTI and especially to NNRTI, as has been reported in high-income[11,19] and low-income countries.[20,21]

The genetic diversity in HIV-1 subtypes and recombinants might affect the emergence of resistance[22,23] and has implications for resistance monitoring. In Brazil, there are reports of increasing HIV-1 genetic diversity in adults[24–26] as well as in children with multiple subtypes and 21.7% harbouring non-B subtypes.[27]

Although the clinical significance of HIV drug resistance has been well documented in adult populations, there are few studies on the prevalence and patterns of drug resistance mutations (DRM) in paediatric HIV-infected populations.[14] Transmitted drug resistance (TDR) monitoring of paediatric HIV-1-infected populations is needed to optimize treatment success and preserve future treatment options.[28] The aim of this study was to describe the prevalence and profile of DRM, of TDR and of HIV-1 genetic variants among newly diagnosed, antiretroviral-naive children infected with HIV-1 in a paediatric HIV reference service centre in Manaus, Amazonas state, Brazil.

Methods

Study design and subjects

A retrospective cohort study was conducted at the reference HIV/AIDS paediatric service of Tropical Medicine Foundation Doctor Heitor Vieira Dourado (FMT-HVD) in the city of Manaus, Amazonas state, Brazil. This tertiary hospital is a reference centre that provides HIV care and services for PMTCT of HIV within the public health system and deals with the highest number of cases of HIV/AIDS in Amazonas state.

Eligible participants were children who attended the HIV/AIDS service from all age groups who were newly diagnosed with HIV, with perinatally acquired HIV infection, and ART naive. They were included if they had at least one available genotypic resistance profile between January 2010 and December 2015. Children with previous prophylactic use of antiretrovirals (zidovudine) for PMTCT were included. Children older than 2 years were mainly self-referrals brought in by parents or guardians, or referrals from other healthcare facilities. Diagnosis of HIV-1 infection was confirmed by positive HIV RNA in children aged less than 18 months and by two serial positive serological assays in children older than 18 months.

Data collection

A paediatrician extracted routine sociodemographic, clinical and laboratory data recorded in medical records and entered these into a database. Sociodemographic data included diagnosis age, sex and transmission mode. Clinical data included obstetric variables from the mother such as prenatal care and mode of delivery, timing of their HIV infection diagnosis, use of ART (beginning ART before or after becoming pregnant) and intrapartum prophylaxis, postnatal infant prophylaxis and breastfeeding. For recorded values of CD4 cell count and HIV RNA viral load, the most recent measurement before the date of enrolment was defined as the pretreatment count. CD4 cell counts were assessed with a FACSCalibur flow cytometer (Becton Dickinson Biosciences, San José, CA, USA) and viral loads with the NucliSens HIV-1 QT assay (bioMérieux, Boxtel, The Netherlands) until December 2010 and with the Abbott Real-Time PCR assay using m2000sp (Abbott Molecular Inc., Des Plaines, IL, USA) thereafter. Immunological category definition was based on CD4 count or percentage according to age, following Brazilian guidelines.[13] Children were classified into: no evidence of suppression; evidence of moderate suppression; or severe suppression. If the CD4+ count and the CD4+ percentage indicated different classification categories, the child was classified into the more severe category.

Genotypic analyses

HIV genotypic tests were run with a TrueGene™ kit (Siemens HealthCare Diagnostics, USA) and the quality of the test was ensured by using an external quality proficiency panel distributed by the Brazilian Ministry of Health. Resistance mutations were assigned by the Calibrated Population Resistance (CPR) algorithm,[29] which is based on the WHO DRM list for surveillance of transmitted HIV-1 drug resistance.[30] Additional analyses were conducted in order to verify pretreatment drug resistance, using the Stanford HIVdb Program, version 7.0 (Stanford University, Palo Alto, CA, USA).[31] To predict the effect of the identified DRM on drug susceptibility, sequences identified by the CPR algorithm were classified as susceptible (Stanford level 1 or 2), low-level resistant (Stanford level 3), intermediate-level resistant (Stanford 4) or high-level resistant (Stanford level 5) to the drug classes and specific drugs. HIV-1 subtypes were defined according to the Rega HIV-1 subtyping tool, version 2.0 (http://www.bioafrica.net/rega-genotype/html/subtypinghiv.html) and the HIVdb Program (Stanford University HIV Drug Resistance Database – http://sierra2.stanford.edu/sierra/servlet/JSierra).

Outcomes

The main outcomes of interest were the prevalence of HIV-1 subtype diversity, and the presence of DRM and TDR. The presence of sequences containing at least one DRM to any drug class (i.e. NRTI, NNRTI and PI) was considered to constitute DRM. The presence of mutations in two of these drug classes (NRTI + NNRTI, NRTI + PI or NNRTI + PI) was considered double resistance, and triple resistance was the presence of DRM in all three classes. TDR was defined as the presence of mutations associated with any level of impaired drug susceptibility. Sequences identified by the CPR algorithm as having surveillance DRM (SDRM), but classified as susceptible or potentially low-level resistant by the HIVdb Program, were not considered resistant for transmitted resistance rate estimation. The presence of low-, intermediate- or high-level resistance was considered as resistance in our analysis. For PI, only major mutations were considered. In this analysis, major PI mutations were defined as those DRM with a score of 30 or more in the Stanford HIVdb Program.[32]

In addition, the prevalence of any DRM was calculated using the WHO criteria from the pretreatment HIV drug resistance surveillance that is based on relevant antiretrovirals used in the country.[33] According to this definition, any HIV drug resistance is defined with respect to one or more of the following drugs or drug classes: nevirapine, efavirenz, any NRTI, darunavir/ritonavir, lopinavir/ritonavir or atazanavir/ritonavir. Sequences classified as low-, intermediate- or high-level resistant according to the Stanford HIVdb Program were aggregated as 'HIV drug resistant'.

Data analysis

The prevalence was expressed as a percentage with the 95% CI. Data were described using percentages, and medians with IQRs, as appropriate. The prevalence of any DRM, any DRM based on the WHO criteria and any TDR were also analysed in the sample of children <18 months of age with genotype available between 2010 and 2015, as recommended by the WHO for surveillance of initial drug-resistant HIV-1 in children newly diagnosed with HIV.[34] Baseline characteristics were compared between those who had received PMTCT and those who had not, using the χ^2 test (categorical

variables) and the Mann–Whitney *U*-test (continuous variables). Data were analysed using Stata SE 10.0 (StataCorp LP, College Station, TX, USA).

Ethics

The study was approved by the Ethical Institutional Review Board of FMT-HVD with waiver of consent (reference number 1.054.945).

Results

Study population selection

A total of 129 children infected with HIV-1 in the 2000–15 period were ART naive and eligible for inclusion in this study. Drug resistance was tested between 2010 and 2015 in 128 children (99.2%) (1 had no blood collected to perform the assay). Among those tested ($n = 128$), 122 had results available. Four samples were excluded due to unknown HIV transmission route and one due to sexual violence as the transmission route. Thus, 117 children (90.7%) were included in the analysis.

Characteristics of the sample

The characteristics of the study sample are shown in Table 1. Children were mainly female (60.7%), native residents of Manaus (94.0%) and the median age of the study sample when tested was 3.7 years (IQR = 0.9–7.9 years). Viral load measurements before treatment had a value of >50 000 copies/mL in 53.8% of the children and 73.5% of the children presented evidence of moderate or severe immunosuppression. Most children (64.3%) had a history of breastfeeding.

Among the 117 children (Table 1), 33 (28.2%) had been exposed to maternal and/or infant PMTCT. Only 16.2% of the mothers had been diagnosed with HIV before or during pregnancy. Among PMTCT-exposed children, 10 of the mothers (30.3%) had received antiretrovirals during pregnancy, 51.5% had a history of intrapartum prophylaxis with zidovudine and all children received postnatal infant prophylaxis with zidovudine.

Characteristics of HIV-1 variants

Most children had HIV-1 subtype B (80.2%), followed by 6.0% subtype C, and several unique recombinant forms [BF (4.3%), DB (4.3%) and F (3.4%)]. In addition, two children had other unique recombinant forms [BC (0.9%) and KC (0.9%)].

Prevalence of DRM in our cohort

Table 2 shows all samples with detected DRM and specifies which ones were considered for TDR analysis. Twenty-five of the 117 patients had viruses with at least one mutation in genes associated with resistance to one or more drug families indicative of DRM. Thus, the overall DRM prevalence was 21.4% (95% CI = 13.8–28.9) in this study population (Table 3). That included 1.7% (95% CI = 0.6–4.0) resistance associated with NRTI, 19.7% (95% CI = 12.3–27.0) resistance associated with NNRTI and 1.7% (95% CI = 0.6–4.0) resistance associated with PI.

Mutations were found in 22.6% of children with no previous PMTCT exposure and in 18.2% of those with PMTCT exposure (Table 3). The frequencies of mutations associated with resistance to PI and NRTI were higher in PMTCT-exposed children (3.0%) than in PMTCT-unexposed children (1.2%). Mutations associated with resistance to NNRTI were detected in 21.4% of children with no previous PMTCT and in 15.2% of PMTCT-exposed children.

Dual-class DRM involving NRTI and NNRTI was observed only in two children (Table 3). Of the 31 sequences that presented DRM, 20 sequences had a single drug mutation (64.5%) (Table 4). The most common NNRTI mutation was E138A (37.0%; 10 out of 27 sequences), for NRTI the only DRM found was M184V ($n = 2$) and for PI the two DRM observed were M46IM ($n = 1$) and M46L ($n = 1$) (Figure 1).

Among children <18 months of age with genotype available between 2010 and 2015 ($n = 39$), the prevalence of any DRM was 20.5% (95% CI = 7.3–33.8) (Table S1, available as Supplementary data at *JAC* Online). Among those <18 months with no previous PMTCT exposure ($n = 9$), the prevalence of any DRM was 22.2% (95% CI = 11.7–56.1).

Prevalence of TDR in our cohort

Based on WHO criteria, six children presented with any DRM, resulting in a prevalence of 5.1% (95% CI = 1.1–9.2) (Table 3). Nineteen children harboured virus with TDR, i.e. presented mutations associated with any level of impaired drug susceptibility (Table 3). Thus, global prevalence (i.e. to any antiretroviral drug class) was 16.2% (95% CI = 9.5–23.0). The prevalence of TDR for each of the available drugs was: 13.7% (95% CI = 7.4–20.0) for NNRTI, 1.7% (95% CI = 0.6–4.1) for NRTI and 3.4% (95% CI = 0.8–6.8) for PI. The prevalence of TDR was higher among PMTCT-exposed children than in PMTCT-unexposed children, overall and by drug class. However, the prevalence of TDR to any NNRTI in PMTCT-unexposed children (13.1%) was similar to that of PMTCT-exposed children (15.2%) (*P*: 0.77).

Among children <18 months of age with genotype available between 2010 and 2015 ($n = 39$), the prevalence of any DRM based on WHO criteria was 5.1% (95% CI = 0.2–1.2) and the prevalence of any TDR was 20.5% (95% CI = 7.3–33.8) (Table S1).

Based on current Stanford drug-specificity in the classification of NNRTI mutations, 5 children were resistant to efavirenz (4.3%), 3 were resistant to etravirine (2.6%), 4 were resistant to nevirapine (3.4%) and 13 were resistant to rilpivirine (11.1%) mainly associated with the E138A mutation. Four children were resistant to the PI nelfinavir (3.4%) and two showed resistance to the NRTI lamivudine (1.7%). None of the patients showed resistance to relevant PI (darunavir/ritonavir, lopinavir/ritonavir or atazanavir/ritonavir). There was no association between PMTCT exposure and resistance to antiretrovirals (data not shown).

Discussion

The results of this study conducted among ART-naive children in Manaus, the capital of Amazonas state, indicate that a complex epidemic pattern is in place. Coupled with extensive prevalence of DRM and TDR, we observed unprecedented HIV-1 diversity characterized by the co-circulation of pure subtypes B, C and F, and diverse unique recombinant forms, including new mosaic sequences composed of subtypes such as D and K.

We found that the prevalence of DRM was high (21.4%) and overall our estimate was higher than rates reported in children and adolescent populations in Brazil, which range from 0%

Table 1. Baseline characteristics among 117 newly diagnosed HIV-infected children, according to whether or not they received PMTCT, Amazonas state, Brazil

Characteristic	Total ($N = 117$)	No PMTCT ($N = 84$)	PMTCT ($N = 33$)	P^a
Female, *n* (%)	71 (60.7)	53 (74.6)	18 (25.4)	0.39
Age when tested (years)				
median (IQR)	3.7 (0.9–7.9)	6.1 (3.4–9.5)	0.4 (0.3–1.1)	<0.001
<1, *n* (%)	32 (27.4)	8 (25.0)	24 (75.0)	
≥1 to <5, *n* (%)	34 (29.0)	26 (76.5)	8 (23.5)	<0.001
≥5, *n* (%)	51 (43.6)	50 (98.1)	1 (1.9)	
Origin, *n* (%)				
Manaus	110 (94.0)	78 (70.9)	32 (29.1)	
interior of the Amazonas	5 (4.3)	5 (100.0)	0 (0.0)	0.18
non-Amazon Brazil/other countries	2 (1.7)	1 (50.0)	1 (50.0)	
Immunological classification, *n* (%)				
no evidence of immunosuppression	31 (26.5)	18 (58.1)	13 (41.9)	
evidence of moderate immunosuppression	46 (39.3)	32 (69.6)	14 (30.4)	0.04
evidence of severe immunosuppression	40 (34.2)	34 (85.0)	6 (15.0)	
Viral load (log HIV RNA copies/mL), *n* (%)				
<1500	0 (0.0)	0 (0.0)	0 (0.0)	
1500–50 000	54 (46.2)	47 (87.0)	7 (13.0)	<0.001
50 001–100 000	15 (12.8)	15 (100.0)	0 (0.0)	
>100 000	48 (41.0)	22 (45.8)	26 (54.2)	
Timing of HIV infection diagnosis, *n* (%)				
before pregnancy	9 (7.7)	1 (11.1)	8 (88.9)	
during pregnancy	10 (8.5)	1 (10.0)	9 (90.0)	<0.001
during labour	9 (7.7)	1 (11.1)	8 (88.9)	
post-partum	86 (73.5)	78 (90.7)	8 (9.3)	
unknown	3 (2.6)	3 (100.0)	0 (0.0)	
Antiretrovirals received during pregnancy, *n* (%)				
yes	10 (8.5)	0 (0.0)	10 (100.0)	<0.001
no	104 (88.9)	81 (77.9)	23 (22.1)	
unknown	3 (2.6)	3 (100.0)	0 (0.0)	
Intra-partum prophylaxis with ZDV, *n* (%)				
yes	17 (14.5)	0 (0.0)	17 (100.0)	
no	97 (82.9)	81 (83.5)	16 (16.5)	<0.001
unknown	3 (2.6)	3 (100.0)	0 (0.0)	
Postnatal infant prophylaxis with ZDV, *n* (%)				
yes	33 (28.2)	0 (0.0)	33 (100.0)	
no	80 (68.4)	80 (100.0)	0 (0.0)	<0.001
unknown	4 (3.4)	4 (100.0)	0 (0.0)	
Breastfeeding ($N = 112$), *n* (%)				
no	40 (35.7)	11 (27.5)	29 (72.5)	
yes	72 (64.3)	68 (94.4)	4 (5.6)	<0.001
HIV-1 subtype ($N = 116$), *n* (%)				
BB	93 (80.2)	66 (71.0)	27 (29.0)	
CC	7 (6.0)	7 (100.0)	0 (0.0)	
FF	4 (3.4)	2 (50.0)	2 (50.0)	0.17
BF	5 (4.3)	3 (60.0)	2 (40.0)	
DB	5 (4.3)	5 (100.0)	0 (0.0)	
BC	1 (0.9)	0 (0.0)	1 (100.0)	
KC	1 (0.9)	1 (100.0)	0 (0.0)	

ZDV, zidovudine.
[a]Using Pearson χ^2 test. Statistical significance: $P < 0.05$.

Table 2. Genotypic resistance results of samples with detected DRM and those considered for TDR analysis in HIV-infected children in Manaus, Amazonas state, Brazil

Patient ID	Age at genotyping test (years, unless indicated)	Gender	Most recent CD4 cell count (cells/mm^3)[a]	Viral load (log HIV RNA copies/mL)	Subtype	DRM found			Resistance to antiretroviral drugs
						NRTI	NNRTI	major PI	
24AM09013	4	F	1368	147 994	B	none	V90I	none	none
24AM100063	5	F	190	12 068	B	none	**E138A**	none	RPV/r
24AM100075	7	F	1020	12 641	B	none	V106I	none	none
24AM100083	2	F	1178	none	B	none	**V108IV**	none	NVP
24AM110040	7	M	725	9029	C	none	**E138A**	none	RPV/r
24AM110047	6	F	1098	4381	B	none	V106I, **G190E**	none	EFV, ETV, NVP, RPV/r
24AM110069	3	M	757	280 053	B	none	**E138A**	none	RPV/r
24AM110087	11	F	98	38 892	C	none	**E138A**	none	RPV/r
24AM110165	19 months	F	459	138 730	B	none	**E138A**	none	RPV/r
24AM120046	5 months	M	2597	499 668	B	none	none	none	EFV, NVP
24AM120053	3	M	58	200 000	B	none	V106I	none	none
24AM120084	6	F	64	68 126	B	none	**K103E**	none	none
24AM120089	16 months	M	1349	463 294	B	none	V106I, V179T	none	none
24AM120152	7	F	228	12 107	B	none	V90I, **Y188NY**	none	none
24AM120205	3	F	775	4886	B	none	V179D	none	none
24AM130189	1	M	1551	4819	DB	none	none	**M46L**	NFV
24AM130202	5 months	F	5533	161 814	B	none	none	**M46IM**	NFV
24AM130211	12	M	687	42 698	B	**M184V**	**K103IN**, V106I, **V108IV**, M230LM	none	3TC, ABC, FTC, EFV, ETV, NVP, RPV
24AM130224	13	M	839	33 148	C	none	**E138A**	none	RPV/r
24AM140040	4 months	M	1059	2 205 244	B	none	**E138A**	none	RPV/r
24AM140058	7	F	160	37 320	B	none	**E138A**	none	RPV/r
24AM140116	4 months	M	1680	205 544	B	none	**E138A**	none	RPV/r
24AM140312	12	F	331	7655	C	none	**K103N**	none	EFV, NVP
24AM150040	10	F	826	11 900	B	none	**E138A**	none	RPV/r
24AM150568	4 months	F	1967	651 701	F	none	none	none	NVP[b]
24AM150898	1	M	1914	20 355	B	**M184V**	**Y181V**	none	3TC, ABC, TDF/3TC, FTC, EFV, ETV, NVP, RPV, NFV
24AM40073	8 months	F	2291	422 512	B	none	V179D	none	none

F, female; M, male; RPV, rilpivirine; /r, /ritonavir; NVP, nevirapine; EFV, efavirenz; ETV, etravirine; 3TC, lamivudine; ABC, abacavir; FTC, emtricitabine; TDF, tenofovir; NFV, nelfinavir.
Patients' HIV-1 sequences with one or more DRM by drug class (NRTI, NNRTI and PI).
Mutations considered for TDR analysis are shown in bold.
[a]Within 6 months of viral load/resistance testing.
[b]Presents only T74S as a PI-selected accessory mutation that is polymorphic.

to 12.8%.[16,17,27,35,36] However, studies from Bahia have reported a much higher prevalence of DRM in ART-naive children (27.0%–33.0%).[18,37] Likewise, the prevalence of TDR was high, at 16.2%, which compromises future treatment options. This prevalence was comparatively higher than that in naive and treated HIV-infected children in Bahia.[37] An ART-naive children cohort in São Paulo, Brazil did not report any TDR.[27] As reported in some earlier studies,[38] the majority of patients with TDR had viruses with singleton mutations. The definition of any DRM based on the WHO criteria takes into consideration relevant DRM, i.e. those that affect antiretroviral drugs that are included in the national guidelines and commonly used in clinical practice.[34] The prevalence of any DRM with this criteria was 5.1% given that the mutation E138A, which greatly affects DRM prevalence, is not considered a relevant NNRTI, as rilpivirine is not used in clinical practice in Brazil in children.[13]

The high prevalence of DRM in Manaus can be partly explained by the fact that suboptimal monotherapy with zidovudine for PMTCT was used in Brazil until 2007,[39] a regimen that is strongly associated with DRM development in women and transmission of drug-resistant virus to the children.[40] Thereafter, the Brazilian Ministry of Health guidelines recommended temporary triple-antiretroviral prophylaxis during pregnancy and delivery for those women not eligible for lifelong ART.[41,42] This recommendation prevailed until the adoption of the intervention option B+, i.e. lifelong ART for all adult patients starting as soon as HIV is diagnosed

Table 3. Mutation patterns in ART-naive children, stratified by previous PMCTC exposure

	Total (n = 117)		Number of patients without PMTCT (n = 84)		Number of patients with PMTCT (n = 33)	
	number of children with mutations	% (95% CI)	number of children with mutations	% (95% CI)	number of children with mutations	% (95% CI)
Prevalence of DRM[a]						
any DRM	25	21.4 (13.8–28.9)	19	22.6 (13.5–31.8)	6	18.2 (4.3-32.1)
PI related	2	1.7 (0.6–4.0)	1	1.2 (0.1–3.6)	1	3.0 (0.3-9.2)
NRTI related	2	1.7 (0.6–4.0)	1	1.2 (0.1–3.6)	1	3.0 (0.3-9.2)
NNRTI related	23	19.7 (12.3–27.0)	18	21.4 (12.5–30.4)	5	15.2 (2.2-28.1)
to two classes	2	1.7 (0.6–4.0)	1	1.2 (1.0–3.6)	1	3.0 (0.3-9.2)
to three classes	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)
Any DRM (WHO criteria)[b]	6	5.1 (1.1–9.2)	4	4.8 (0.1–9.4)	2	6.1 (0.3-14.7)
Prevalence of TDR[c]						
any TDR	19	16.2 (9.5–23.0)	12	14.3 (6.6–21.9)	7	21.2 (6.5-35.9)
to PI	4	3.4 (0.8–6.8)	1	1.2 (1.1–3.6)	3	9.1 (1.3-19.4)
to NRTI	2	1.7 (0.6–4.1)	1	1.2 (1.1–3.6)	1	3.0 (0.3-9.2)
to NNRTI	16	13.7 (7.4–20.0)	11	13.1 (5.7–20.5)	5	15.2 (2.2-28.1)
to two classes	2	1.7 (0.6–4.1)	1	1.2 (1.1–3.6)	1	3.0 (0.3-9.2)
to three classes	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)

[a]Number of children with at least one DRM among 117 children.
[b]Any HIV drug resistance is defined with respect to one or more of the following drugs or drug classes: nevirapine, efavirenz, any NRTI, darunavir/ritonavir, lopinavir/ritonavir or atazanavir/ritonavir. Sequences classified as low-, intermediate- or high-level resistant according to the Stanford HIVdb Program are aggregated as 'HIV drug resistant'.
[c]Number of children with at least one TDR among 117 children, i.e. impact on drug susceptibility.

regardless of the CD4 cell count at the end of 2013.[43] Temporary antiretroviral exposure associated with long-term universal distribution of antiretroviral drugs is known to favour selection of resistance mutations, which can compromise the effectiveness of antiretroviral prophylaxis for MTCT and future therapeutic options.[44] However, only 28.2% of the children in our study had been exposed to some form of PMTCT intervention. The high DRM presence found could also be due to suboptimal antiretroviral regimens in mothers for whom prophylaxis is not always well implemented.

NNRTI-associated mutations were predominant. Interestingly, NNRTI-associated mutations were observed in 21.4% of newly diagnosed, treatment-naive children with no reported or recorded PTMCT exposures. The finding of DRM among PMTCT-unexposed children suggests prior and unreported use of antiretrovirals or transmission of resistant viruses to these mothers. Thus, PMTCT could be an inadequate means of ruling out pretreatment drug resistance in our settings. The current WHO ART guidelines (2013)[45] recommend lopinavir-based regimens for all children <3 years of age irrespective of PMTCT exposure. However, current Brazilian guidelines still have NNRTI-based regimens as one of the options for preferred first-line treatment for children.[13]

In our study, 1.7% of treatment-naive children harboured viruses with resistance to two drug classes and 1.2% had not received PMTCT. Four other countries (Cameroon, Cuba, India and Spain) have reported naive paediatric subjects harbouring viruses with resistance to two drug classes.[14]

The most common mutation was E138A and its prevalence (8.5%) was higher than that reported in other Brazilian (4.6%)[36] and international studies (up to 7.5%).[46] E138A occurs in up to 5% of ART-naive patients, depending on the virus subtype.[46] This mutation can be considered a natural polymorphism and may compromise new NNRTI such as rilpivirine or etravirine. Etravirine is currently available as a third-line option and is recommended only for adults in whom it is unlikely to cause viral suppression even with the use of darunavir/ritonavir or raltegravir.[47] In this situation drug activity may be limited due to class recycling. The most common NRTI drug mutation was M184V (1.7%), which confers resistance to lamivudine, emtricitabine and abacavir, but delays resistance to zidovudine and stavudine.[48,49]

As expected, the epidemic in this region was composed mainly of subtype B, which is in agreement with the predominance of HIV-1 subtype B infections in Brazil[27,50] and in the rest of Latin America.[14] However, subtype C was the second most prevalent form of infection. In Brazil, subtype C prevails in the southern region,[51] although recent studies have shown an unexpectedly high prevalence in central western Brazil.[27,36,52] Subtype C was first described in Amazonas state in 2006,[53] and its analysis showed high similarity to sequences from the south of Brazil, suggesting that HIV subtype C might have disseminated to the north following a south to north gradient pattern.[53] Pure subtype F1 isolates had a lower prevalence than BF1 unique recombinant forms, suggesting the co-circulation of HIV-1 subtype B and subtype F1 in the Amazonas has likely favoured its inter-subtype recombination.[54]

Table 4. Characteristics and susceptibility of HIV strains with single and multiple mutations and their impact on drug susceptibility

Patients with a single drug mutation

Number of patients	NNRTI mutation	Predicted susceptibility			
		EFV	NVP	ETV	RPV
10	E138A	S	S	S	R
1	K103N	R	R	S	S
1	K103E	S	S	S	S
1	V108IV	S	R	S	S
2	V179D	S	S	S	S
1	V90I	S	S	S	S
2	V106I	S	S	S	S

		Predicted susceptibility							
	PI related	ATV/r	DRV/v	FPV/r	IDV/r	LPV/r	SQV/r	TPV/r	NFV/r
1	M46IM	R	S	R	R	R	S	S	R
1	M46L	S	S	R	R	R	S	R	R

Children with multiple drug mutations

Number of patients	Year of diagnosis	CD4 count (cells/mm^3)	Subtype	NRTI	NNRTI	Number of fully active drugs		
						NRTI (8)	NNRTI (4)	PI/r (8)
1	2012	1914	BB	M184V	K103IN, V106IV, V108IV, M230LM	5	0	8
1	2009	1098	BB		V106I, G190E	8	0	8
1	2012	1349	BB		V106IV, V179T	8	4	8
1	2012	228	BB		V90I, Y188NY	8	4	8
1	2011	687	BB	M184V	Y181V	4	0	8

EFV, efavirenz; NVP, nevirapine; ETV, etravirine; RPV, rilpivirine; ATV, atazanavir; /r, /ritonavir; DRV, darunavir; FPV, fosamprenavir; IDV, indinavir; LPV, lopinavir; SQV, saquinavir; TPV, tipranavir; NFV, nelfinavir; R, resistant; S, susceptible.

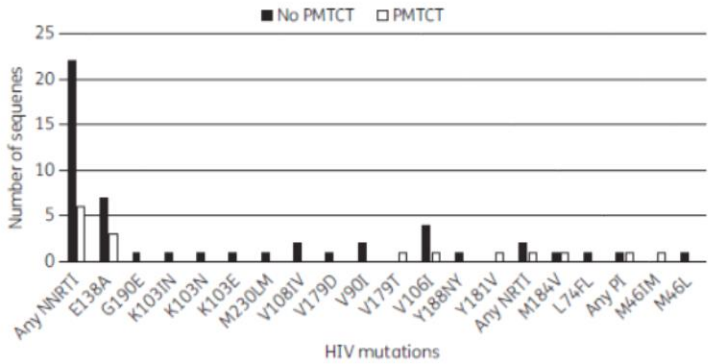

Figure 1. Profile of specific mutations (number) detected among newly diagnosed HIV-infected children, stratified by previous PMCTC exposure. The number of HIV sequences with the indicated mutations are shown in white bars for children exposed to PMTCT interventions ($n = 33$) and in black bars for children not exposed to PMTCT interventions ($n = 84$).

Moreover, in central Brazil, BF1 unique recombinant forms have been the second most prevalent variant following 'pure' subtype B.[55] This is the first study to describe circulation of recombinant HIV-1 subtype DB and subtype KC in Amazonas. HIV-1 subtype D was identified as BF1D recombinant in Rio de Janeiro in 1999.[56] Our results indicate that the epidemic in Amazonas is complex and diverse as they show that 10.3% of the children presented inter-subtype recombinants. Subtype-associated variability has shown different impacts on disease progression[57] and on the rate of CD4 cell count decline.[58] HIV-1 subtype-specific variability can have implications for resistance development.[22,23]

This study has some limitations. The median age of children in our study was >2 years. This differs from the approach followed by WHO for HIV DRM surveys, recommending enrolment of children for whom the duration of HIV infection is <18 months.[34] Some TDR mutations could revert in the oldest prenatally ART-naive infected children,[59] which might result in underestimation of their prevalence. In fact, the prevalence of any DRM was similar in the overall sample ($n = 117$) compared with the sample of children <18 months of age ($n = 39$) (21.4% versus 20.5%, respectively). However, the prevalence of any TDR was lower in the overall sample than in children <18 months (16.2% versus 20.5%, respectively) Nevertheless, we did not observe a significant trend of the prevalence of TDR with increasing age (children <1 year old when tested = 18.6%, 1–5 years = 11.8% and ≥5 years = 17.7%; P value for trends = 0.98). Moreover, approx. 70% of children in our study were reportedly PMTCT unexposed, while this will be less likely to occur in more recent studies once adoption of option B+, implemented in Brazil in 2013,[43] is more widespread. The potential inclusion of children with unreported ART exposure due to PMTCT or HIV treatment remains and should be considered a possible limitation of the study. We lack information about DRM in the mothers, so a possible association between the type of medication the mother was taking and the resistance patterns of their children cannot be evaluated. However, this study shows a high prevalence of DRM in children born to PMTCT-experienced mothers. Given the age group and PMTCT exposure, caution is warranted when comparing with other studies.

HIV-1 subtype showed a complex epidemic profile with expansion of subtype C. The diverse patterns of inter-subtype HIV-1 recombinants observed indicate a wide circulation of mosaic viruses in Amazonas state and highlight the need for surveillance of HIV-1 diversity in the region. Brazil is working to reduce perinatal infections in children. However, our data show that an extensive proportion of newly diagnosed HIV-infected infants and young children carry resistant viruses, in particular to NNRTI. Resistance-associated mutations are also present in a considerable proportion of children with no reported or recorded antiretroviral drug exposures. Together, these data support the use of genotypic tests prior to ART initiation and, in line with WHO guidelines,[45] the updated HIV treatment guidelines regarding the use of PI as preferred antiretrovirals for first-line therapy (i.e. lopinavir/ritonavir-based first-line regimens) in HIV-infected infants and young children regardless of PMTCT history.

Brazil is currently considering the adoption of option B+ for children and adolescents. In this new context, all children newly diagnosed with HIV would start ART immediately after HIV diagnosis, regardless of age, presence of symptoms, CD4 and viral load level. As part of the recommended clinical monitoring, a baseline resistance test in all HIV-positive children is already performed. At the national level, these data have the potential to provide strategic information for the assessment of ART-programme effectiveness, which may inform public health actions with immediate impact on the quality of treatment and care of children living with HIV.

This study shows how surveillance of HIV drug resistance may be performed using programmatic data in settings where HIV genotyping is available, recommended and performed in HIV-infected children through vertical transmission. These existing data could be used for surveillance purposes and have the potential to be expanded at the national level. HIV drug-resistance surveillance in Brazil is supported by a network of 22 reference laboratories (RENAGENO) that have the capacity to perform high-quality HIV genotyping, in order to provide reliable information. In addition, its information system (SISGENO) has been operating since 2007.[26] The surveillance plan could be based on a census of all cases that would improve representativeness.

Considering that Brazil presents a prevalence of vertical transmission of HIV higher than that in other parts of Latin America,[3] surveillance of initial resistance in children aged <18 months should be considered a priority.

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Transparency declarations

None to declare.

Author contributions

S. D. d. A. coordinated the study and acquired data. A. T., S. D. d. A. and M. S. designed the study. M. S. conducted the data analyses and wrote the article. S. D. d. A., A. T., W. M. M. and A. S. B. contributed to the interpretation of data. All authors contributed to the discussion of the results and critical revision of the manuscript. All the authors read and approved the final manuscript.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online.

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6. Discussão

Neste estudo, apesar de 94% das gestantes ter referido alguma visita de pré-natal, houve diagnóstico tardio de infecção pelo HIV em 18,8%, com essa identificação tendo sido feita durante o parto ou no pós-parto. Por outro lado, das 168 mulheres que foram testadas somente no pós-parto, 79% não tinha nenhuma visita de pré-natal. Tal constatação, leva à reflexão de possível dificuldade de acesso ao serviço de pré-natal, nesta região e ainda da disponibilização de serviços como exames de testagem para HIV. Para as situações de diagnóstico ainda na maternidade, o Ministério da Saúde já disponibiliza terapias estendidas como uso de nevirapina para os bebês expostos, nessas condições. Essa conduta minimiza parcialmente o risco de transmissão vertical. No entanto, para diagnósticos maternos posteriores ao parto, a perda de oportunidade de profilaxia é irreparável.

A correta identificação em tempo hábil de gestante infectada pelo HIV, ainda não é uma realidade no estado do Amazonas e principalmente em municípios, mais longínquos o que compromete a profilaxia na gestação. Diagnósticos do tipo *Point of care* (POC), já vem sendo utilizados no estado nos últimos anos e podem ser uma estratégia apropriada para as populações ribeirinhas. Além de agilizar o diagnóstico de gestantes e oportunizar a profilaxia, técnicas moleculares da mesma forma, facilitariam o diagnóstico precoce de crianças infectadas, agilizando início de TARVc.

Nas gestantes identificadas com infecção pelo HIV, a oferta de ARV variou de 70,8% no período entre 1999 e 2000 para 79,4% em 2011, evidenciando uma melhoria nas condutas profiláticas da TV.

Para as crianças envolvidas no estudo, dois fatores protetores puderam ser observados: o uso de AZT na gestação e o tipo de parto por cesariana eletiva. Ambos demonstrados em estudos prévios e já adotados como conduta pelo Ministério de Saúde do Brasil, no Protocolo de Prevenção da Transmissão Vertical do HIV. Entretanto no Amazonas, 25% das gestantes não receberam, no período estudado, PTMI durante a gestação e 19,3% foram submetidas à cesariana após início de trabalho de parto. Tais situações refletem perda de oportunidade para prevenção da transmissão vertical do HIV.

Dentre os fatores de risco encontrados nesta casuística, destaca-se a amamentação, experimentada por 10% das crianças acompanhadas. O diagnóstico materno tardio, já mencionado, foi observado em 83% das mulheres que amamentaram, sendo este o motivo desta prática nestes casos. Para as demais, já cientes do diagnóstico, o estigma social de não amamentar pode ser lembrado como motivação de manter a amamentação apesar do risco para o RN. A estratégia de uso de TARVc em todas gestantes ininterruptamente, conforme preconizado pelo MS na Opção B+, deve ser encorajado. Promove a garantia de controle da carga viral materna e reduz risco de infecção para o bebê, incluindo futuras gestações.

As taxas de transmissão vertical variaram durante os anos com tendência à regressão nos últimos anos do estudo, representando evolução nas abordagens de PTMI no estado. A taxa global do período estudado (6,6%) ainda se mantém acima da meta esperada de 1%. Mais esforços são necessários para completar com sucesso a cascata de cuidados de crianças verticalmente expostas ao HIV.

O presente estudo teve algumas limitações. Foram usados dados pediátricos de prontuários médicos e alguns dados referidos pelos cuidadores, porém não foram usados dados maternos de carga viral e LT - CD4, duração de terapia ARV ou adesão materna. A correlação de dados infantis com maternos poderia ampliar variáveis para análise, não disponibilizadas na atual.

Na coorte de crianças infectadas, a prevalência encontrada de mutações de resistência foi alta, de 21,4% e acima de algumas já reportadas no país anteriormente, para população pediátrica, que variavam de 0 a 12,8%, porém um trabalho brasileiro da Bahia reportava taxas ainda maiores (27 a 33%). Da mesma forma, a prevalência de resistência transmitida foi alta, 16,2% o que pode comprometer tratamentos futuros sendo esta mais alta do que todos estudos brasileiros mencionados. Nessa coorte, 70% das crianças não havia sido submetida à profilaxia da transmissão materno infantil, o que tende a não mais ser observado em novos estudos, uma vez que a adoção de Opção B+ foi implementada no Brasil em 2013.

Baseado no estudo genotípico, pode ser observada um complexo padrão na epidemia no estado. Já era esperado, ter subtipo B como mais frequente, de acordo com o que ocorre no resto do país. O subtipo C, que prevalece na região sul do país, já havia sido identificado na região norte anteriormente, evidenciando um padrão sul

a norte de disseminação. Foi observada uma diversidade de HIV -1 circulantes e a co-circulação de subtipos puros como B, C e F e de formas únicas recombinantes (BD e KC) e novos mosaicos compostos de sequencias dos subtipos D e K.

O Brasil ainda tem taxas de transmissão vertical mais altas do que alguns dos outros países da América Latina. A vigilância de resistência transmitida em crianças menores de 18 meses, teria papel prioritário para tomada de decisões terapêuticas, neste contexto. Os dados gerados no estudo podem contribuir para abordagem clínica de crianças infectadas pelo HIV, na região.

7. Conclusões

As estratégias de prevenção apresentaram melhoria ao longo do tempo, porém ainda ocorre uma taxa alta de TV, tendo sido 6,6% no período estudado.

Entre as mães, 76,1% havia feito uso de terapia antirretroviral em algum momento da gestação. Ter sido amamentado esteve relacionado com risco de infecção na criança.

A cascata de cuidados necessita de atenção para efetivamente conseguir reduzir para níveis satisfatórios, a transmissão do HIV de mãe para filho, no estado do Amazonas.

Na coorte de crianças infectadas, muitas carreavam ao nascer, vírus resistentes em extensa proporção, em particular à classe de ITRNN. Tal fato, fortalece a recomendação de manter a coleta de genotipagem prévia à terapia ARV, em crianças infectadas pelo HIV, o que já é conduta no país desde 2010. Apenas 28% delas havia utilizado alguma forma de profilaxia de TV.

Destaca-se que as resistências encontradas, não seriam possivelmente oriundas do uso de profilaxia materno infantil. Outrossim, tais resistências eram provavelmente originárias de má adesão por parte das mães que ao transmitir o vírus, já o faziam com formas de resistência por ela adquiridas. Retratam ainda um cenário de diagnóstico tardio nas mães e perda de oportunidades de prevenção para seus filhos, resultando em altas de transmissão vertical aqui demonstradas.

Os dados demonstrados, nos permitem concluir que a escolha da terapia inicial para crianças infectadas deve ser individualizada, levando em consideração as informações de resistência, para evitar regimes insatisfatórios que tragam consequências a longo prazo. Esses dados alertam para a otimização na escolha de terapia antirretroviral, em casos de não coleta de genotipagem prévia à TARVc. Evidenciam ainda, que a classe de ITRNN, pelos altos índices de resistência transmitida aqui apresentados, deve ser evitada para início de TARVc nas crianças dessa região.

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9. Anexos

9.1 Anexo A: Aprovação do CEP

FUNDAÇÃO DE MEDICINA TROPICAL DR. HEITOR VIEIRA DOURADO ((FMT-HVD))

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Coorte pediátrica de crianças expostas ou vivendo com HIV/Aids do Amazonas
Pesquisador: SOLANGE DOURADO DE ANDRADE
Área Temática:
Versão: 3
CAAE: 36157214.3.0000.0005
Instituição Proponente:
Patrocinador Principal: Fundação de Medicina Tropical do Amazonas - FMT/IMT/AM

DADOS DO PARECER

Número do Parecer: 1.054.945
Data da Relatoria: 08/05/2015

Apresentação do Projeto:
Globalmente, 3.2 milhões de crianças e adolescentes de 15 anos viviam com HIV/AIDS em 2013, sendo 9.1% de todas as pessoas infectadas pelo HIV no mundo. No ano 2013, 240.000 crianças adquiriam HIV globalmente.(1) Em 2011 foi lançado o Plano Global da Eliminação de novas infeções por HIV em crianças com a meta de reduzir as novas infeções entre criança em 90% até 2015. O número de novas infecções em crianças diminuiu
de 350.000 em 2009 para 199.000 (170.000 – 230.000) em 2013.(1) A taxa da transmissão vertical também tem diminuído de forma global. Em 2013, 16% (13-18%) das crianças nascidas de mãe vivendo com HIV foram infectadas, comparado com 25.8% em 2009.(1) Na América Latina e no Caribe, o número total de crianças que adquiriram HIV tem diminuído aproximadamente em 24% entre 2009 e 2013.(2) A Estratégia de Eliminação Regional de sífilis congênita e de transmissão vertical do HIV promovida pela Organização Pan-americana da Saúde desde 2009, tem conseguido diminuir a taxa de transmissão vertical na América Latina e no Caribe. Em 2011 a taxa foi de 14.2% (5.8%–18.5%), inferior aos 18.6% (10.5%–22.9%) reportados em 2010.(2) A meta para América Latina e o Caribe é reduzir a transmissão vertical do HIV para de 1% até 2015. (2)No Brasil, os casos de transmissão vertical representam 95.1% de casos aids entre indivíduos menores de 14 anos. (3) Em 2012, a taxa de detecção de casos de aids em menores de 5 anos, que serve como indicador aproximado para monitorar a redução da transmissão mãe-filho, (4) foi de 3.4 por

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1000.000 habitantes, sendo a região norte a segunda mais afetada (4.4 por 100.000 habitantes). Nos últimos 10 anos tem se detectado uma diminuição do 35.8% na taxa de detecção de casos de aids em crianças menores de 5 anos. (3) O último estudo multicêntrico nacional foi realizado em 2001, e reportou uma taxa de 7.1%, com algumas diferencias regionais. (5) O Brasil tem apresentado melhoras importantes na cobertura do
teste de HIV durante a gestação, sendo do 79% em 2011. A provisão de ARV para mulheres gravidas para prevenir a transmissão mãe-filho também melhorou. No entanto, somente um 63.86% de mulheres receberam antirretroviral para reduzir o risco de transmissão vertical em 2013, e 50% das crianças expostas ao HIV realizaram detecção virologia nos primeiros dos meses após do nascimento. As estratégias de prevenção de transmissão mãe-filho, como ART durante a gestação, ART durante o parto, e profilaxia pós-natal, parto cesariano programado, evitar a amamentação, não são aplicadas de forma universal. Este estudo permitirá conhecer as tendências da taxa de transmissão mãe filho do HIV no estado de Amazonas e quais são os fatores com maior influência nela. Também descrever e examinar as tendências do perfil clínico, laboratorial e terapêutico, das crianças infectadas pelo HIV/Aids.

Objetivo da Pesquisa:

Objetivo Primário:

Estimar a taxa de transmissão de mãe para filho (transmissão vertical) do HIV, no estado do Amazonas, Brasil, nos anos de 2015 a 2025, em
criança expostas ao HIV em seguimento em um serviço de referência em infectologia pediatria na cidade de Manaus e identificar fatores maternos e
neonatais associados à transmissão vertical do HIV.

Objetivo Secundário:

Entre crianças expostas ao HIV em seguimento entre o ano 2000 e 2012 em um serviço de referência em infectologia pediatria na cidade de
Manaus, estado do Amazonas:•Examinar a tendência da taxa de transmissão vertical do HIV em relação as tendências de estratégias de prevenção
como uso de ART durante a gestação, parto, profilaxia neonatal, e evitar a amamentação. •Identificar fatores maternos e neonatais associados à
transmissão vertical do HIV. • Descrever a informação clínica, de laboratório, e de tratamento das crianças vivendo com HIV/Aids atendidas num
serviço de referência em Manaus. • Descrever o uso de serviços. • Determinar efetividade ao longo do tempo dos antirretrovirais.

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Avaliação dos Riscos e Benefícios:

A coleta de sangue respeita a criança já que apenas vai se solicitar uma amostra de 4 ml coletada na primeira visita, e 1 ml a cada 6 meses de seguimentos, sempre coincidindo com a coleta para outros exames para evitar desconforto. A quebra do sigilo não se considera um risco já que os dados vão ser coletados e analisados anonimamente.

Comentários e Considerações sobre a Pesquisa:

O projeto intitulado "Coorte pediátrica de crianças expostas ou vivendo com HIV/Aids do Amazonas", da pesquisadora Solange Dourado, trata-se de protocolo unicêntrico, com financiamento próprio, sem coleta de material biológico e com uso de fontes secundárias de dados, como prontuários. A informação será obtida de forma retrospectiva, para a qual se pede dispensa de TCLE nesta emenda, e de forma prospectiva, conforme já aprovado anteriormente. Os pesquisadores apresentam instrumento adequado para a coleta de dados. Pela importância do agravo e natureza metodológica da investigação, considera-se que os benefícios suplantam os riscos. A pesquisa tem a anuência da DAM da FMT-HVD, apresentando viabilidade de execução nas condições propostas, incluindo os aspectos orçamentários. Os pesquisadores explicitam no protocolo os mecanismos de preservação da identidade dos sujeitos da pesquisa. As modificações necessárias foram realizadas no projeto, em decorrência da emenda.

Considerações sobre os Termos de apresentação obrigatória:

O protocolo apresenta os seguintes documentos em anexo: 1. PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_369347.pdf, 2. PB_PARECER_RELATOR_830900.pdf, 3. FOLHA DE ROSTO_TV.pdf, 3. TCLE, 4. TALE, 5. Carta anuencia Marcus Guerra, 6. Carta de anuencia casa Vhida, 7. Carta anuencia Dra. Graça, 8. FORM_ PADRAO_ARMAZ_BIOLOGICO, 9. Folha de resposta TV assinada, 10. Termo de dispensa TCLE para a coorte retrospectiva, 11. Instrumento coleta de dados, 12. Projeto detalhado.

Recomendações:

Conclusões ou Pendências e Lista de Inadequações:

Conclui-se, pela análise da emenda ao protocolo, que não foram verificadas pendências ou inadequações que inviabilizem, do ponto de vista ético, a execução do protocolo. Este é o parecer.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

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FUNDAÇÃO DE MEDICINA TROPICAL DR. HEITOR VIEIRA DOURADO ((FMT-HVD))

Considerações Finais a critério do CEP:

A presente Emenda ao projeto está APROVADA e os interessados ficam informados de apresentar a este CEP os relatórios parciais e final do estudo, conforme prevê a Resolução CNS nº 466/2012, utilizando o formulário de Roteiro para Relatório Parcial/Final de estudos clínicos Unicêntricos e Multicêntricos, proposto pela CONEP em nossa home page.

MANAUS, 08 de Maio de 2015

Assinado por:
Marilaine Martins
(Coordenador)

Endereço: Av. Pedro Teixeira, 25
Bairro: D. Pedro I **CEP:** 69.040-000
UF: AM **Município:** MANAUS
Telefone: (92)2127-3572 **Fax:** (92)2127-3572 **E-mail:** cep@fmt.am.gov.br

9.2. Anexo B

ID estudo: .....Coorte pediátrica de crianças expostas ou vivendo com HIV/Aids do Amazonas

UNIVERSIDADE DO ESTADO DO AMAZONAS
FUNDAÇÃO DE MEDICINA TROPICAL DR. HEITOR VIEIRA DOURADO

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Coorte pediátrica de crianças expostas ou vivendo com HIV/Aids do Amazonas

A sua criança está sendo convidado/a a participar do projeto de pesquisa acima citado. O documento abaixo contém todas as informações necessárias sobre a pesquisa que estamos fazendo. Sua colaboração neste estudo será de muita importância, mas se desistir a qualquer momento, isso não causará nenhum prejuízo a você.

A participante da pesquisa fica ciente que:

I). Este documento refere-se a um projeto de pesquisa Dra. Solange Dourado, da Fundação de Medicina Tropical Dr. Heitor Vieira Dourado (FMT-HVD, com o objetivo de descrever a sua doença, tratamento e transmissão.

As crianças expostas ao HIV são visitadas até os 24 meses de vida. Si neste momento não tem HIV, a criança e dada de alta. A criança HIV positiva é seguida no serviço de infectologia pediátrica até os 21 anos de idade. Os atendimentos se realizam a cada mês. Durante as visitas se fazem perguntas sobre a saúde da criança, os tratamentos, e se tomam amostras de sangue. Neste estudo, se você aceitar participar, uma das amostras de sangue tomada nas visitas a cada 6 meses ira ser armazenada. Para não incomodar, só vamos tomar a amostra quando tivermos que coletar sangue para outros exames. O seu prontuário será revisto para coletar os dados da doença, medicamentos, e transmissão são perguntados durante a rotina normal.

II) A sua participação nesta pesquisa não causará a você nenhum gasto com relação aos procedimentos realizados no estudo;

III). Você tem a liberdade de desistir ou de interromper a colaboração neste estudo no momento em que desejar, sem penalização nenhuma e sem prejuízo sobre o seu atendimento neste hospital.

IV) não vai gastar tempo por participar por que todo os dados coletados e a amostra de sangue irão acontecer durante a sua visita habitual.

V) nenhum tipo de recompensa ou remuneração será fornecida a você nesta pesquisa, sendo sua participação voluntária;

VI) Benefícios: Participar deste estudo pode trazer benefícios diretos para você ou para seu filho pois pode auxiliar vocês e a equipe a entender melhor suas dificuldades com a doença, o tratamento, a transmissão. Você estará ajudando para melhorar o conhecimento sobre a crianças expostas ou vivendo com HIV/aids, podendo beneficiar futuramente outras pessoas.

VII) Riscos: Não há grandes riscos por que as informações são e a amostra de sangue irão se coletar durante a rotina da visita e não a perguntas ne furadas a mais. A única diferença é que uma amostra de sangue vai ser armazenada e que o seu prontuário vai ser revisto para obter informações sobre a sua doença e os medicamentos.

VIII). Os dados obtidos durante a pesquisa serão mantidos em sigilo pelo pesquisador, assegurando a sua privacidade quanto aos dados confidenciais envolvidos na pesquisa. Não colocaremos seu nome em dado algum do estudo; em vez disso, usaremos um número para rotular suas informações.

IX). Os resultados poderão ser divulgados em publicações científicas mantendo sigilo dos dados pessoais;

X). Este documento é emitido em duas vias que serão assinadas por você como responsável pela criança e pelo pesquisador, devendo constar as rubricas das duas em todas as páginas, ficando uma via com cada uma de nós.

XI). Caso você desejar, poderá pessoalmente, ou por meio de telefone, entrar em contato com o pesquisador responsável para informações adicionais ou tomar conhecimento dos resultados parciais e finais desta pesquisa, no endereço Av. Pedro Teixeira, nº 25, Bairro Dom Pedro, telefone (92) 9132 2432(e-mail: douradosol@yahoo.com.br), ou poderá entrar em contato diretamente com o Comitê de Ética em Pesquisa – CEP/FMT-HVD, no mesmo endereço, telefone (92) 2127-3572.

ID estudo: .....Coorte pediátrica de crianças expostas ou vivendo com HIV/Aids do Amazonas

Eu, _____, declaro que obtive todas as informações necessárias, bem como todos os eventuais esclarecimentos quanto às dúvidas por mim apresentadas.

Você concorda de livre e espontânea vontade para que a criança do estudo acima descrito?

(_) Sim ( ) Não

Nome da criança: .....

Identificador do estudo: .....

Data: _____ / _____/ _____

_____ _____

Assinatura do pai, mãe ou Tutor da criança

Assinatura da Pesquisador responsável

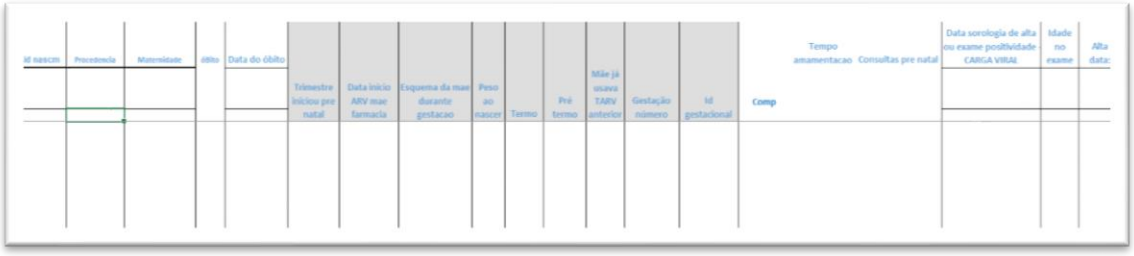
10.0. Apêndices

10.1 Equipe de Trabalho

.

	Nome (ordem alfabética)	Categoria	Formação	Vínculo
1	Adele Benzaken	Orientadora	Médica	FMT-HVD
2	Amilcar Tanuri	Co orientador	Médico	Pesquisador Visitante Senior FMT-HVD
3	Luiz Henrique Canellas Bastos	Pesquisador	Médico pediatra	FMT-HVD
4	Meritxell Sabidó	Co orientadora	Médica	Pesquisadora Visitante Senior FMT-HVD
5	Solange Dourado de Andrade	Doutoranda	Médica pediatra infectologista	FMT-HVD
6	Wuelton Marcelo Monteiro	Pesquisador	Farmacêutico -Bioquímico	FMT-HVD

10.2 Planilha de coleta de dados



10.3. Produção científica durante o Programa de Doutorado

10.3.1 Artigo 1:

Case Report

Revista da Sociedade Brasileira de Medicina Tropical 48(4):498-500, Jul-Aug, 2015
http://dx.doi.org/10.1590/0037-8682-0314-2014

Acute disseminated encephalomyelitis following inactivated influenza vaccination in the Brazilian Amazon: a case report

Solange Dourado de Andrade[1],[2], ***Maria Graciede Filha Santarém Andrade***[1],[2], ***Pablo José Santos***[1], ***Maria de Lourdes Galvão***[1], ***Mariana Martins de Barros***[1], ***Rajendranath Ramasawmy***[1],[3], ***Izabella Picinin Safe***[1], ***Wuelton Marcelo Monteiro***[1],[3], **Meritxell Sabidó**[1],[4] **and** ***Maria das Graças Costa Alecrim***[1],[3]

[1]. Fundação de Medicina Tropical Doutor Heitor Vieira Dourado, Manaus, Amazonas, Brasil. [2]. Centro de Referência para Imunobiológicos Especiais, Manaus, Amazonas, Brasil. [3]. Escola Superior de Ciências da Saúde, Universidade do Estado do Amazonas, Manaus, Amazonas, Brasil. [4]. TransLab. Departament of Medical Sciences. Faculty of Medicine. Universitat de Girona, Spain.

ABSTRACT

Here, we describe a case of acute disseminated encephalomyelitis (ADEM) that occurred during a plausible risk interval following inactivated influenza vaccination in a previously healthy 27-year-old man from Manaus, Brazil. He was treated with intravenous methylprednisolone and immunoglobulin. One-month follow-up revealed resolution of the brain lesions, but not of the spinal cord lesions. No recurrence or progression of the main neurological symptoms was observed. After two years of monitoring, the patient continues to experience weak lower limbs and urinary retention. Thus, we recommend that ADEM should be considered in a patient presenting with neurological symptoms after influenza vaccination.

Keywords: Acute disseminated encephalomyelitis. Inactivated influenza vaccination. Brazilian Amazon.

INTRODUCTION

Annual influenza vaccination is an important strategy for preventing seasonal influenza virus infections and their potentially severe complications, including death. Vaccination reduces the likelihood of acquiring and transmitting influenza infections[1]. The annual inactivated influenza vaccine (IIV) is safe to administer as an injection to both children and adults[2]. Minor side effects include self-limited reactions at the injection site such as pain, redness, and swelling[2]. Systemic events, such as mild and self-limited fever, malaise, and myalgia, can occur after the IIV has been administered. In some cases, fewer instances of ocular or respiratory symptoms, immediate hypersensitivity, increased risk for febrile seizures in young children, and temporally associated Guillain-Barré Syndrome have been reported[2].

Acute disseminated encephalomyelitis (ADEM) is an inflammatory demyelinating disease of the central nervous system[3]. Its onset is acute and often rapidly progressive[4]. It is traditionally monophasic, but some patients may have recurrences. ADEM typically presents with multifocal neurological signs, including motor, sensory, cranial nerve, and brainstem deficits, as well as nonspecific symptoms such as headache, malaise, and altered mental status (i.e., encephalopathy)[4]. The diagnosis is supported by the presence of one or more supratentorial or infratentorial demyelinating lesions on brain magnetic resonance imaging (MRI) and the absence of destructive *black hole* lesions on T1-weighted MRI[4]. Abnormal cerebrospinal fluid findings such as mild lymphocytic pleocytosis and slightly elevated cerebrospinal fluid (CSF) protein level are suggestive of ADEM, but not essential for diagnosis.

Prior viral infections (measles, smallpox, and chickenpox) are known risk factors, and ADEM has rarely been reported to be in temporal association with vaccinations. The rate of ADEM cases diagnosed following vaccination in a biologically plausible risk interval is as low as 0.1-0.2 per 100,000 vaccinated individuals of any age[5]. However, it is more common in children. ADEM has been reported following measles, mumps, rubella, smallpox, hepatitis B, and IIV[6]. Severe neurological complications including ADEM associated with influenza vaccination have been sporadically reported from countries with a good surveillance system[7-8]. Here, we report a case of ADEM following influenza vaccination that occurred in the western Brazilian Amazon.

Corresponding author: Dra. Solange Dourado de Andrade. Avenida Pedro Teixeira 25, Dom Pedro, 69040-000 Manaus, Amazonas, Brasil.
Phone: 55 92 2127-3473
e-mail: douradosol@yahoo.com.br
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CASE REPORT

We describe a case of neurological complications that occurred during a biologically plausible risk interval following IIV administration in a 27-year-old previously healthy man living in Manaus (State of Amazonas, Brazil). The vaccine was administered six days before symptoms onset as part of the city's immunization campaign in 2012. The patient worked as an administrative staff, had never smoked, and did not report any previous chronic or severe health conditions or infections. There was no travel history or contact with animals or rural areas before the onset of symptoms. He did not show any clear precipitating factors (e.g., infections), and was admitted with symptoms of fever, vomiting, headache, blurred vision, nuchal pain and fluctuating alertness and normal neurological examination. Under suspicion of bacterial meningitis, a broad-spectrum antibiotic (meropenem) was prescribed, but he showed no clinical improvement despite intravenous administration every 8 h for 14 days. He was hospitalized for 25 days from the day of symptoms onset.

The patient developed progressive weakness in all four limbs eight days post-vaccination. Neurological signs and symptoms progressed until he was unable to walk, and were associated with urinary retention, constipation, and paresthesia over the trunk and legs. The CSF was colorless with 85 white cells/μL, (normal range: 0-5 cells/μL), 100% lymphocytes, 149.82 mg/dL CSF protein (normal range: 15-60 mg/dL), and 63 mg/dL glucose (normal range: 50-80 mg/dL). His CSF was subjected to Gram staining, Ziehl-Neelsen staining for *Mycobacterium tuberculosis*, and nankin staining for fungi. The CSF was also subjected to polymerase chain reaction (PCR) to test for Flaviviruses and the results were negative for all (dengue, yellow fever, Ilheus, Rocio, and Saint Louis encephalitis viruses). Fluid-attenuated inversion-recovery (FLAIR) and T2-weighted MRI images revealed brain and spinal cord lesions (**Figure 1A** and **1B**). Thick blood smear was negative for *Plasmodium* spp. Serology tests were negative for human immunodeficiency virus 1 (HIV-1), viral hepatitis, rubella immunoglobulin M (IgM), and rubella immunoglobulin G (IgG). Respiratory viruses (adenovirus, influenza A1, influenza B, parainfluenza, and respiratory syncytial virus) were tested for using a nasopharyngeal swab and all were negative. No antinuclear antibodies, rheumatoid factor, and alpha fetoprotein were detected in the serum.

Cranial MRI showed multiple hyperintense lesions on T2 and FLAIR, and they involved the periventricular and subcortical white matter, as well as large areas of the medulla oblongata and pons and the splenium of the corpus callosum, bilaterally and asymmetrically. Cervical and dorsal spine MRI revealed focal areas of abnormal signal density with contrast enhancement, and these were randomly distributed on the sides of the cervical-dorsal cord that runs from the brainstem-spinal transition to the cone. The joint analysis of the MRI of the brain and spinal cord indicated ADEM. Based on the temporal association, exclusion of alternative etiologies, neurological findings, and MRI displaying diffuse white matter lesions, we diagnosed our patient's condition to be ADEM following influenza vaccination.

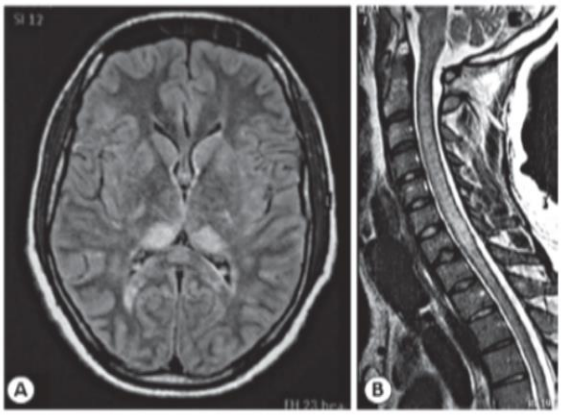

FIGURE 1 - A: FLAIR MRI showing *bilateral hyperintensity* of the *pulvinar nuclei of the thalamus*. B: T2-weighted MRI showing diffuse hyperintensity throughout the spinal cord. FLAIR: fluid-attenuated inversion-recovery; **MRI:** magnetic resonance imaging.

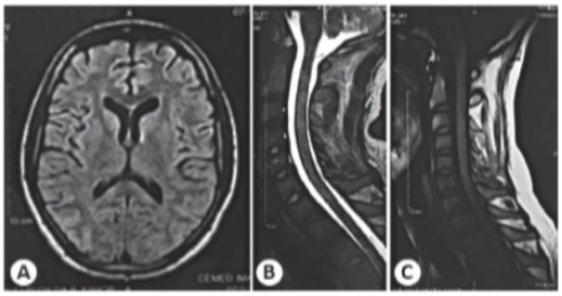

FIGURE 2 - Follow-up using T2-weighted MRI. One month after symptoms initiation, A: FLAIR MRI of the brain showing no alterations. B: Spinal cord MRI showing myelopathy. C: T2-weighted MRI four months after treatment initiation showing resolution of the spinal cord lesions. MRI: magnetic resonance imaging; **FLAIR:** fluid-attenuated inversion-recovery.

In the hospital, the patient was treated with intravenous methylprednisolone (500 mg daily for five consecutive days), intravenous immunoglobulin (2,500 mg/kg daily for five consecutive days), and vitamins B and C. After being discharged, he received a second cycle of intravenous methylprednisolone at the same dose and physiotherapy at home (2h/day for 2 months and 1h daily thereafter). The one-month follow-up MRI revealed resolution of the brain lesions (**Figure 2A**), but not of the spinal cord, which were suggestive of inflammation (**Figure 2B**). The last spinal MRI performed four months after symptoms onset showed resolution of the lesions (**Figure 2C**). After two years of monitoring, neurological examination showed the persistence of paraparesis and paresthesia in the lower limbs, and the patient continued to experience weakness in his lower limbs and urinary retention. No recurrence or progression of symptoms was seen, suggesting a monophasic episode that ruled out multiple sclerosis.

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DISCUSSION

Acute disseminated encephalomyelitis has no pathognomonic clinical features. However, a combination of neurological symptoms in particular temporally associated with an infection or vaccination should alert the clinician of a possible diagnosis of ADEM[5]. Its clinical presentation includes fever, altered consciousness, and multifocal neurological findings. Symptom onset is usually rapid with progression over hours to a peak in days. Focal presentation in ADEM is rather heterogeneous and depends upon the location and degree of demyelination within the central nervous system. MRI is essential for ADEM diagnosis; diffuse or multifocal white matter lesions on FLAIR or T2-weighted sequences are characteristic of ADEM[4]. Lumbar puncture may reveal lymphocytic pleocytosis or elevated protein level. Abnormal CSF is suggestive of ADEM, but not a requisite for diagnosis.

Based on the MRI findings and on excluding acute infective condition, we hypothesized that the IIV might have been related to ADEM in our patient. We then applied the causality criteria to this case, an essential step before diagnosis. According to the World Health Organization (WHO) causality assessment criteria, the association of any event with vaccination is classified as very likely, probable, possible, unlikely, unrelated, or unclassifiable. In the present case, the relationship between influenza vaccination and ADEM would be classified as possible[9]. Although the WHO criteria are generally well accepted and widely used, it is important to note that there are significant limitations when applying them to vaccine-related neurological adverse events[10]. A neurological event with a reasonable time relationship to IIV administration is automatically classified as possible, despite the fact that this event could be explained by an underlying disease, pathogens, or other drugs. Following clinical evaluation, the relationship between the same event and vaccination would be classified as unlikely because an alternative condition would explain its occurrence[10].

In the most recent review of vaccine-related adverse events conducted by the Institute of Medicine, it was concluded that there is insufficient or lack of epidemiological evidence to either accept or reject a causal association between ADEM and most vaccines, and that the mechanistic evidence based on natural infection is weak[11]. This distinction between temporal coincidence and causality is difficult, thus certain causality criteria have been developed. According to the Brighton Collaboration definition, the event observed in the present case was ADEM with level 1 (achieved) certainty [12].

Once ADEM is diagnosed, treatment should begin as soon as possible. Most patients with ADEM improve with treatment, but complete recovery occurs in only 10-46% patients, with motor deficits and/or cognitive impairment often persisting in the remainder. In fulminant cases, death may occur in 4-12% patients. Corticosteroids are accepted as first-line therapy[3]. For steroid-unresponsive patients, plasma exchange or intravenous immunoglobulin may be used. Poor outcome of treatment is associated with older age, female sex, degree of functional impairment at clinical onset, CSF protein level, spinal cord involvement, peripheral nervous system damage, and poor response to corticosteroids[5]. A few patients may either have recurrences that respond to glucocorticoid therapy or are at risk for development of multiple sclerosis.

Our findings underscore the need to consider ADEM when a patient presents with neurological symptoms after influenza vaccination, which is usually administered annually to children and adults in many countries. In our patient, the symptoms and signs compatible with ADEM presented with a reasonable time relationship after IIV administration.

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10.3.2 Artigo 2:

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Article

Children and Adolescents with Perinatal HIV-1 Infection: Factors Associated with Adherence to Treatment in the Brazilian Context

Maria Letícia Santos Cruz [1,*], Claudete A. Araújo Cardoso [2], Mariana Q. Darmont [1], Paulo Dickstein [1], Francisco I. Bastos [3], Edvaldo Souza [4], Solange D. Andrade [5], Marcia D'All Fabbro [6], Rosana Fonseca [7] and Simone Monteiro [8]

1 Infectious Diseases Department, Hospital Federal dos Servidores do Estado, Rua Sacadura Cabral 178, Rio de JaneiroRJ 20221-161, Brazil; marianadarmont@gmail.com (M.Q.D.); paulo.dickstein@gmail.com (P.D.)
2 Department of Maternal and Child Care, School of Medicine, Fluminense Federal University, Rua Marquês de Paraná, 303, Niterói RJ 24033-900, Brazil; claudetecardoso@id.uff.br
3 Health Information, Fundação Oswaldo Cruz, Biblioteca de Manguinhos suite 229, Av. Brasil 4365, Rio de Janeiro RJ 21045-900, Brazil; francisco.inacio.bastos@hotmail.com
4 Department of Pediatrics, Instituto de Medicina Integral Prof. Fernando Figueira, Rua dos Coelhos 300, Recife PE 50070-550, Brazil; edvaldo.es@gmail.com
5 Department of Pediatrics, Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Av. Pedro Teixeira 25, D Pedro I, Manaus AM 69040-000, Brazil; douradosol@yahoo.com.br
6 Centro de Doenças Infecciosas e Parasitárias de Campo Grande, Rua Bahia 280, Jardim dos Estados, Campo Grande MS 79002-380, Brazil; fabbro@uol.com.br
7 Department of Pediatrics, Hospital Fêmina, Grupo Hospitalar Conceição, Rua Mostardeiro 17, Moinhos de Vento, Porto Alegre RS 90430-001, Brazil; rosana040@yahoo.com.br
8 Laboratory of Environmental and Health Education, Instituto Oswaldo Cruz, Fiocruz. Av. Brasil 4365, Rio de Janeiro RJ 21045-900, Brazil; msimone@ioc.fiocruz.br
* Correspondence: mleticia@diphse.com.br; Tel.: +55-21-2233-0018

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Abstract: Challenges to the adherence to combination antiretroviral therapy among the pediatric population should be understood in the context of the trajectories of families, their interaction with healthcare services, and their access to material and symbolic goods. The present study analyzed individual, institutional and social factors that might be associated with the caregivers' role in the treatment adherence of children and adolescents living with HIV (CALHIV). Based on semi-structured interviews and questionnaires applied to 69 caregivers seen at pediatric AIDS services of five Brazilian macro-regions, we observed that adherent caregivers had better acceptance of diagnosis and treatment, were less likely to face discrimination and social isolation secondary to AIDS-related stigma and tended to believe in the efficacy of treatment, and to be more optimistic about life perspectives of CALHIV. Interventions aiming to improve adherence and to promote the health of CALHIV should take in consideration the interplay of such different factors.

Keywords: patient adherence; HIV infection; children; adolescents; vulnerability; caregivers

1. Introduction

The key aim of HIV infection treatment is to suppress viral replication; this aim requires optimal adherence to combination antiretroviral therapy (cART) and should be monitored over time by periodical assessment of serum HIV viral load (VL). Treatment failure usually requires the prompt return to optimal adherence and/or the adoption of new therapeutic regimens in order to avert progressive immune deficiency, clinical deterioration and, eventually, death. Regarding both

individual-level infection dynamics and the health of the public, non-adherence to treatment favors the appearance viral resistance and eventual transmission of drug-resistant HIV strains [1].

Studies on cART adherence among children and adolescents living with HIV (CALHIV) point to some barriers that involve several domains: intrapsychic, family and social relationships, service characteristics, as well as factors intrinsic to the medications themselves. These barriers include the following: difficulties of communication between parents and children, non-disclosure of diagnosis, premature attribution of the responsibility for the treatment to children themselves, cognitive deficits, high levels of stress, poor quality of life [2–4], adverse effects of medication, fear of discrimination and/or effective stigma, having the biological parents as caregivers, having lost one parent, and long treatment duration [4–7].

However, the results of studies on treatment adherence exhibit some inconsistencies, particularly within the pediatric population [5,8]: factors such as age, gender and education and some caregivers' sociodemographic characteristics were found to be associated with both adherence and non-adherence [9]. These inconsistencies suggest that adherence to treatment involves nonlinear interactions among the factors under analysis and that some underlying structures of interdependence among them are not systematically considered in an adequate manner [10]. Emphasis on the interactive, dynamic nature of these factors and their interplay is key for the analysis of interventions aimed at optimizing adherence to treatment. Several studies indicate the need for multifaceted, long-lasting and flexible actions, addressing both social (stigma) and situational (service location and functioning) factors, as well as highlighting the characteristics and untoward effects associated with the different therapeutic regimens [11].

The hypothesis informing the present study was that the challenges faced by CALHIV to adhere to cART should be understood within the context of the trajectories and living conditions of the caregivers who are responsible for administering medications and their interaction with healthcare services.

Based on the aforementioned considerations, the present study sought to identify and analyze the individual/family, institutional and social factors that contribute in an integrated manner to foster a better (or worse) adherence to cART among the pediatric population. For this purpose, we first classified CALHIV seen at pediatric AIDS services as adherent or non-adherent to treatment based on biological criteria (VL testing history). Next, we compared the following characteristics of the caregivers who were responsible for administering treatment to CALHIV: history of HIV diagnosis and diagnosis communication, their socioeconomic and psychosocial profile, as well as their views and practices relative to cART, their interaction with healthcare services and concerns about HIV-related stigma.

2. Materials and Methods

The present study is part of a larger multicity project investigating the adherence to cART of CALHIV seen at five pediatric AIDS services in Brazil, located in five cities (Rio de Janeiro, Recife, Manaus, Campo Grande and Porto Alegre). The project was approved by the IRBs (CAAE 15843613.7.1001.5240) of all five participating institutions and was supported by the Department of Sexually Transmitted Diseases/AIDS and Viral Hepatitis, Brazilian Health Ministry [12]. The study used different methodological procedures described on Figure 1 and included 250 caregivers. The number of participants from each center was proportional to the total number of CALHIV seen at these facilities.

As a first step, all 250 caregivers answered structured questionnaires that included socioeconomic profile, their quality of life evaluated with the short version of World Health Organization questionnaire (WHOQoL-bref) [13], their anxiety and depression scores evaluated with the Hospital Scale for Anxiety and Depression [14], and their alcohol and drug using habits evaluated with the Alcohol, Smoking and Substance Involvement Screening Test [15]. The study abstracted clinical, laboratory and pharmacy data, including all available results of VL exams during cART, for each child. We also reviewed the situation where the diagnosis of CALHIV occurred and have stablished two categories: diagnosis

due to symptomatic condition versus diagnosis as follow-up of HIV-exposed child (those born to women living with HIV). Caregivers were also submitted to semi-structured interviews aiming to better understand the participants' life perspectives and the meaning of living with AIDS and to be under treatment. Healthcare professionals from the multi-professional staff at the five participating institutions were trained to apply the questionnaires and to perform semi-structured interviews with the 250 caregivers. The principal investigators visited each participating center to provide specific training to the staff members who were in charge of data collection with the purpose of standardizing procedures used in the survey and qualitative components. The methods and findings from the survey, including clinical profile, caregivers' characteristics and use of cART were summarized in a previous publication [16].

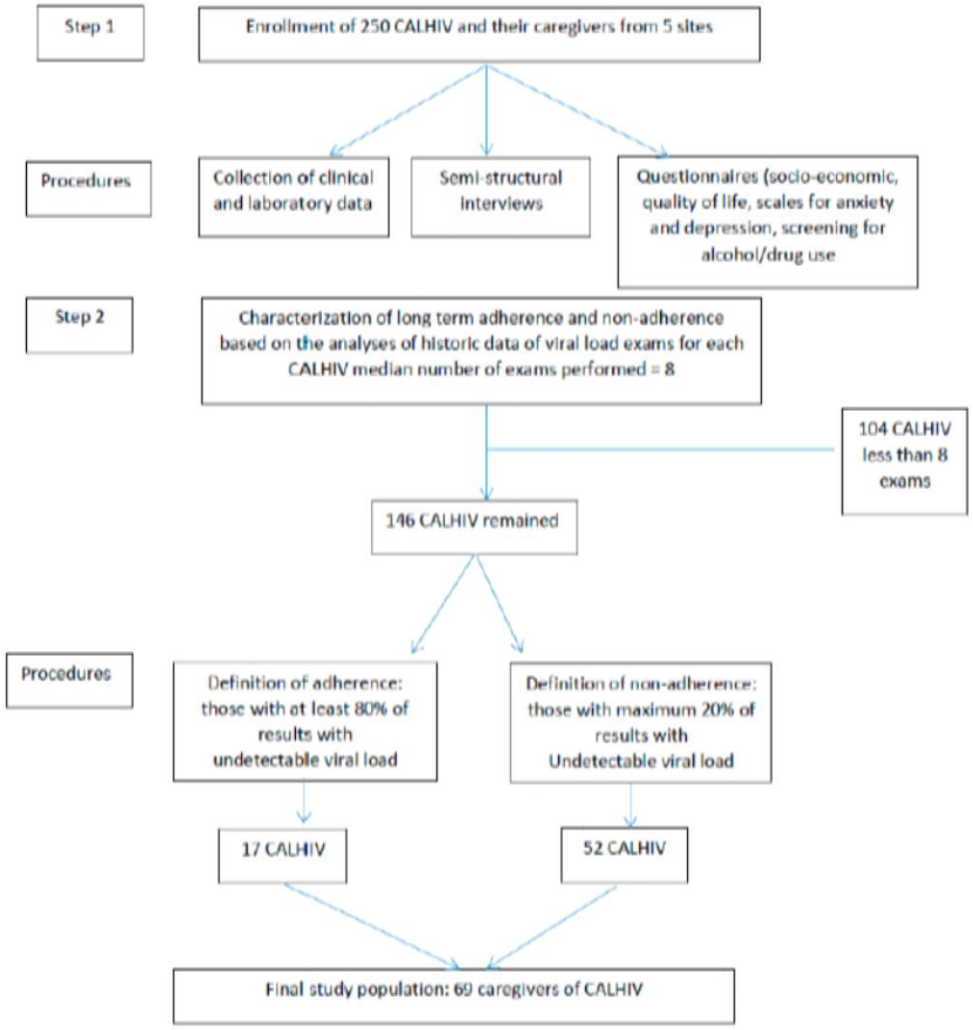

Figure 1. Study design and population.

Given that cART demands long-term commitment, at the second step of the study we characterized the CALHIV as "adherent" or "non-adherent" based on VL tests performed over time, since the beginning of treatment. The clinical coordination of the study defined patients with 80% or more VL serial results below the limit of detection as "adherent" and those with less than 20% of such results over time as "non-adherent". Since exams had been collected for routine care, at this point we

observed a wide variability in the number of exams performed for each participant CALHIV. Analysis of data showed that the median number of exams performed per CALHIV was 8 and we opted to exclude 104 CALHIV who had less than eight available VL results. Among the remaining 146 CALHIV, 17 met the criteria to be considered adherent and 52 non-adherent, corresponding to 69 caregivers. It was interesting to notice that there were 77 CALHIV who did not meet criteria to be considered either adherent or non-adherent. These CALHIV showed variable patterns of viral suppression, alternating VL with results below the level of detection with results showing viral replication in blood.

Most of the adherent CALHIV were from Recife, 12/17 (70%); among the non-adherent patients, 20/52 (38%) were from Recife, 15/52 (29%) were from Rio de Janeiro, 9/52 (17%) were from Manaus, 6/52 (12%) were from Campo Grande, and 2/52 (4%) were from Porto Alegre.

The present paper comprises analysis of the interviews, sociodemographical and laboratory data of CALHIV of these 69 caregivers, based on the contribution of social science approach in the comprehension of the nexus between cultural systems, social hierarchies and access to material and symbolic goods within the context of social behaviors of individuals and social groups. Our aim was to investigate intra- and inter-group similarities and differences between the 17 caregivers of the adherent CALHIV and the 52 caregivers of non-adherent CALHIV that were likely to reveal the dynamics of the factors associated with adherence to cART in this population.

After the transcription of the 69 interviews, the data analysis was organized and interpreted following four steps. The first one was exhaustive reading of the material to identify emerging themes and categories derived from the objectives of the study. The second step was the development of analytical categories based on the theoretical perspective of the research. The third step was the codification of the empirical material in order to identify the analytical categories. Lastly was the interpretation of the coded material in accordance with the study objectives, literature review and theoretical concern [17].

3. Results

The questionnaire data showed that the socioecomonic characteristics and the proportion of biological mothers were similar among the caregivers of the 17 adherent and 52 non-adherent CALHIV; in addition, 69% of the caregivers of adherent CALHIV and 71% of the caregivers of non-adherent CALHIV did not report to have abused alcohol or other substances. The groups differed in terms of high scores for anxiety according to standard scales (19% of caregivers of adherent CALHIV *vs.* 44% of caregivers of non-adherent CALHIV), high scores for depression according to standard scales (6% of caregivers of adherent CALHIV *vs.* 25% of caregivers of non-adherent CALHIV), and in the fact children's diagnosis was based on their symptoms (38% of caregivers of adherent CALHIV *vs.* 48% of caregivers of non-adherent CALHIV), as opposed to those tested as follow-up of HIV-exposed children. Table 1 shows clinical characteristics of adherent and non-adherent CALHIV.

Table 1. Clinical characteristics of adherent and non-adherent CALHIV receiving antiretroviral treatment in five centers in different geographic regions of Brazil.

Characteristics	Adherent (n = 17)	Non-Adherent (n = 52)
Male gender (%)	59%	58%
Mean age at study participation (y)	11.6	11.2
In use of first ART regimen (%)	53%	33%
Had AIDS defining condition at treatment beginning (%)	53%	39%

The interviews' analysis revealed that caregivers of adherent CALHIV differ from non-adherent caregivers, regarding the following categories: acceptance of own and/or child's diagnosis, valorization of and availability for care delivery, belief in the efficacy of treatment and survival perspectives, connection between users and healthcare team and support from family and community networks. As shown in Table 2, these categories were classified in three adherence-related dimensions

as follows: (1) individual: the context of testing for HIV and the communication of diagnosis, psychological/behavioral factors and caregivers views and practices related to the benefits of cART and the life perspectives of CALHIV; (2) institutional: the characterization of CALHIV's access to cART and of the interactions between caregivers and professionals at the pediatric AIDS services; (3) social: the nature of the (social-, family- and community-based) support received by the caregivers and putative experiences of social isolation secondary to AIDS-related stigma.

Table 2. Categories that differentiate caregivers of adherent and non-adherent CALHIV based on interviews with caregivers of CALHIV receiving antiretroviral treatment in five centers in different geographic regions of Brazil.

	Individual	Institutional	Social
Caregivers of adherents ($N = 17$)	• Greater acceptance of own and/or child's diagnosis • Valorization of and availability for care delivery • Belief in the efficacy of treatment and survival perspectives; invests in the child's future (e.g., education) • Acknowledgment of the relevance of disclosing the diagnosis to the child/adolescent • Commitment to drug administration	• Greater connection (exchanges) and dialogue between users and healthcare professionals resulting in the commitment of both • Interaction between healthcare teams and therapeutic resources contributes to redefine the fatality of AIDS and enlarge the life perspectives of people living with HIV/AIDS (PLHIV)	• Receives support from family and community networks • Does not experience social isolation due to AIDS-related stigma
Caregivers of non-adherents ($N = 52$)	• Difficulty to accept own and/or the child's diagnosis • Feelings of guilt for having transmitted HIV to the child • Limited belief on ART effects and life and future perspectives • Difficulty to disclose the diagnosis to the child/adolescent • Difficulties to tolerate own treatment	• Less connection (exchange) between users and healthcare professionals • Limited interaction between professionals and users does not favor redefining the fatality of AIDS and contributes to the lack of life perspectives of PLHIV	• Fragility of the potential family and community network support • Experiences of social isolation due to AIDS-related stigma

The vast majority of the 52 caregivers of non-adherent CALHIVrefer to experiences of discrimination and social isolation, which are secondary to AIDS-related stigma. These experiences contribute to the difficulty in accepting the diagnosis of HIV infection. More than a half of caregivers in this group report non-acceptance of diagnosis and the experience of suffering among family or community members, as illustrated bellow.

> *"(...) it's quite difficult because of the prejudice . . . I'd even accept (it). Just me. What I can't accept it's because my son has it and he's a child and I could have helped if I'd known when I was pregnant. That's the only thing that tortures me."* (Boy's mother; Rio de Janeiro)

> *"His mother also suffered a lot. But we didn't know the cause of her suffering (. . .) She killed herself, she didn't let the disease manifest in her."*

In turn, most of the 17 caregivers of adherent CALHIV believe that the children need and deserve respect, affection and special care. This perspective is partly due to their view of the affected children as innocent victims and their understanding of the negative effects of AIDS-related stigma. Adherence to treatment thus becomes an opportunity to repair (or reduce) the damages associated with the infection.

> *"The person who lives (with HIV) is like this, he/she has to be well understood, be well-cared for, with affection (. . .) So I said, I want to keep her. And she said: see, Mrs. X, are you aware of the*

girl's problem? I said: indeed, that's why I want (to take care) (. . .) I want it very much, more than anything else. And now I won't give her to anyone else. It has to be me; I will take responsibility for everything." (Girl's grandmother; Recife)

The value attributed to CALHIV care, which seems to be translated into efforts to ensure their adherence to treatment, as well as family support were associated with the acceptance of diagnosis. AIDS, which is still believed to be a fatal and morally condemnable disease, has gained a new meaning for the interviewees due to the possibilities of living with HIV that are presented by healthcare professionals.

"... when I learned about that stuff he has, I refused to accept, I cried a lot. Dr. Y gave me a lot of advice, that I should have to (. . .) that I should accept him the way he is, she explained me how to take care of him, but it was very difficult; it's still difficult." (Boy's grandmother 2; Recife)

The caregivers' belief in the quality of care delivered at the service are common to all caregivers of adherent CALHIV. Their belief in the efficacy of regular treatment to expand life perspectives of CALHIV and to preserve their rights to health and education are evident in their investment in education and their plans for the future (to have a family, to work); this attitude is illustrated by the following excerpt of a caregiver of an adherent patient:

"So I think about him growing up, graduating (. . .) working, getting married (. . .) I wonder, will X get married? Will he have children? How will X's daily life be? (. . .) I'm very insistent that he studies, to have a good job, work somewhere like a bank, something good for him." (Boy's aunt; Recife)

In our study, commitment to treatment has been frequently accompanied by taking responsibility for related actions, such as showing up for consultations, refilling prescriptions and administering medications. In some cases, such responsibility was explicitly manifested by expressions such as "not to fail, not to miss" or sentences such as *"If I fail, I'll kill myself"*.

"I always come, on the right date. I've never missed a single day. She's been seeing Dr. W for 10 years now, and has only missed one appointment. (. . .) When the day comes, I've got to come and get the medication, I come, I take it at the right time. I never miss a date, never miss the time." (Mother; Recife)

Interviews were conducted by healthcare professionals affiliated with the clinical services and their participation in the study was seen as an opportunity by the caregivers to obtain further information, to discuss the issue and to receive orientations about treatments, valued chiefly by caregivers of adherent CAVHIV.

"This is what it can be done (...) help with words, with words, with strength. Words of affection, of strength, positive words. To guide about something that I don't understand, about something that I don't have, that I can't do, that I can't be (. . .) Thank you. You bringing me here was a pleasure (. . .) It was good, I even let it off my chest (. . .) I was all locked up here. It was good for me, I was a help (. . .) a good help indeed, a huge help." (Girl's grandmother; Recife)

On the opposite side, most caregivers of non-adherent CALHIV have negative thoughts about the treatment, although they rarely admit difficulties in following the prescriptions. Biological mothers frequently had troublesome experiences in taking their own medication due to the physical and mental side effects of the drugs. This is illustrated by the following excerpt:

"You see, to take this medication (. . .) is awful, you know? Because it's so many medicines (. . .) Sometimes I say: when I die and the doctors open me, there'll be only pills inside me, because I take so many medicines. And then, when I take them, I feel ill (. . .) And when I don't take them I don't

feel anything (. . .) do I really have this (disease)? Because I take it and I feel ill, an agony. I want to throw up, I throw up all day long, I feel dizzy, that kind of stuff, you know? And when I don't take (the medication) I feel nothing (. . .) they said I have HIV, but I didn't see the test (results), I didn't see anything. What the doctors tell me to do, I do, but not all, you know? Because at times I give myself a rest." (Girl's mother; Recife)

As illustrated by the excerpt presented above, the medications used came to represent the disease itself because the interviewee was asymptomatic before she started taking the medications; in other words, from her subjective perspective, the symptoms came to acquire concrete existence and to characterize her condition as "ill" (although imprecisely) due to the use of medication. Discomfort is also related to the perception that treatment is a continuous and endless battle because the medication will not solve the problem (cure AIDS). In this context, the treatment of children tends to be more difficult than the treatment of adults due to the scarcity of therapeutic options, which are often available in poorly tolerated forms (e.g., having an unpleasant taste).

Around 40% of caregivers of non-adherent CALHIV refer at least one reason for disbelief about the positive effects of treatment on extending life expectancy and on maintaining the quality of life. The narratives of these caregivers suggest CALHIV lack future life perspectives. Such disbeliefs seem to be reflected in the caregivers' lack of valorization of long-term investments or projects, such as education, as shown by the following excerpt of a child's grandmother interview:

"She's doing well at school, but I think that she won't have a future (. . .) She's thinking of going to college, work, getting a better job, something good for her (. . .) she tells me: Grandma, I'll graduate, I'll help you. But I don't think it will happen (. . .) (no matter) all she learns, all she devotes herself to, all I do for her, that everybody here does . . . But I don't think she has a long life, a future. It's just a (...) like something (she does as) entertainment so as not to just pass through life. But I know that it has no cure. The person has no future (. . .) I think it's a pity. You see, you look at her so healthy in appearance, so full of life. But it's a life (. . .) so short, because if she stops taking the medication, she has a short life." (Girl's grandmother; Manaus)

Among caregivers of non-adherent CALHIV it was common to observe difficulties with disclosure of the diagnosis to CALHIV and fear that the use of medication might lead to disclosure of the diagnosis to partners, friends and family members.

"... my son will soon be a man and will want to have a family of his own, and what will happen? It will be a huge problem for him (. . .) I keep thinking what I'll say when he's 15, 16 and finds a girlfriend (. . .) because it's difficult to me (. . .) sometimes he asks me: mom, why do we take so many medicines? I never knew how to sit down with him and explain. These things hurt me; they're accumulating inside me (. . .)" (Boy's mother; Rio de Janeiro)

"To me it means much sadness, too much prejudice (. . .) it breaks my heart, I get sad (. . .) the harmful thing is not the HIV, but all the prejudice (. . .) it ties me down. I don't have strength (. . .) Sometimes I take the medicine, sometimes I don't, because sometimes there's someone at home who cannot see it (. . .) I remove the labels from the medication, but then, they ask me why I remove the labels from the medication." (Girl's mother; Rio de Janeiro)

4. Discussion

The study has a multi-step design developed to unable identification of caregivers' and CALHIV's commitment to treatment, based on biological criteria (VL testing history). We recognize that the rigor of selection process that excluded those CALHIV who had less than 8 available VL results during follow-up may have created a bias, since those with less exams are more likely to be non-adherent. Nevertheless, we have tried to compensate the possible exclusion of the most non-adherent patients defining that among the remaining CALHIV, those with less than 20% of exams showing viral suppression should be considered as non-adherent.

Although the socioeconomic profile and access to pediatric AIDS services were similar among the 69 caregivers of CALHIV under analysis, in all five investigated cities, a comparative analysis between the 17 caregivers of the adherent and the 52 caregivers of non-adherent CALHIV (defined after biological criteria, viral load results) showed how individual, institutional and social factors interactively influence cART adherence.

Twelve of the 17 caregivers of adherent CALHIV were from the same center in Recife. The Recife center, like those in Manaus and Campo Grande, serves the metropolitan population and is a reference center for the treatment of CALHIV from neighboring towns and rural areas. Among the five participating centers, the center in Recife stood out as providing care to the largest number of CALHIV and consequently, for recruiting the largest proportion of participants in this study. This good result might be associated with the wide experience of the staff in the management and care of pediatric patients and their families. However, this hypothesis needs to be further investigated in future studies to achieve a better understanding of the role that healthcare services play in CALHIV treatment adherence.

In regard to the history of HIV diagnosis, the adherent CALHIV were, in general, diagnosed during the process of investigating the medical and family histories of seropositive parents; whereas the non-adherent CALHIV were tested due to the presence of symptoms suggestive of HIV infection. HIV diagnosis delay was found to be a major influence on treatment adherence, reinforcing the relevance of prenatal care and programs that aim to prevent HIV vertical transmission as a first, key intervention to improve treatment adherence of infected children. These families might have greater opportunities to develop relationships with healthcare staff and to commit themselves to the necessary care. However, even in cases where this first opportunity is lost, later interaction between CALHIV/relatives and healthcare staff might contribute to redefine their fatalistic perspective of AIDS, with a consequent broadening of the life perspectives of people living with HIV and their families.

A study conducted at an AIDS service in Rio de Janeiro found that children diagnosed due to disease manifestation in the first months or years of life might be undervalued and discriminated against by their families. The study also made evident that some families are afraid of disclosing the diagnosis to CALHIV and secrecy may become an additional source of discrimination. Some CALHIV present a slow evolution of the infection, and diagnosis might be delayed for many years, which might slow the assumption of their new identity. Last but not least, the study also documented CALHIV who grow up in institutions may face difficulties to adapt to their families when they return home [18].

Fear of AIDS-related stigma might influence the acceptance and disclosure of HIV diagnosis among the population under study. Excerpts from caregivers' narratives suggest that among caregivers of non-adherent CALHIV failure to cope with AIDS diagnosis might be secondary to the internalization of a deteriorated social identity due to the associated stigma [19]. Thus, a perverse circle may emerge, whereby the fear of being rejected is fed by experiences of social isolation and a lack of social and family support, which in turn contribute to the non-acceptance and non-disclosure of the diagnosis. A meta-analysis of studies carried out in 2002–2012 on the impact of HIV on the quality of life of adults (3348 participants, assessed based on WHOQoL-BREF or WHOQoL-HIV-BREF) found that the lowest scores were related to the "social relationships" domain. This finding was attributed to the interference of stigma and discrimination within relationships with family members, friends and coworkers [20].

The communication of an HIV diagnosis to children and adolescents may represent a challenge, especially in low-income countries. A review of studies published from 1997 to 2008 showed that social, economic and racial disadvantages are associated with a greater difficulty in disclosing such a diagnosis due to a greater concern about stigma and discrimination [21]. Although caregivers are aware of the potential benefits of communicating the diagnosis, this communication is often delayed based on the idea that children are immature or are not interested in the matter [8]. In addition, some caregivers express the fear that the children might tell other people about their condition, and they have a sense of guilt for having transmitted the infection to their children.

Among the caregivers of adherent patients, the predominant attitude was one of acceptance of the status of living with HIV and a firm belief in the patient's high survival chances and future perspectives, once optimal treatment adherence is achieved. These findings reiterate that disclosure of the HIV diagnosis to children should be a component of care provided to this population of patients because it is associated with positive outcomes regarding treatment adherence and health status. A systematic review of studies on cART adherence showed that high esteem and the acceptance of a seropositive status facilitate treatment adherence [22]. Additionally, a qualitative study with 17 caregivers of CALHIV aged zero to 18 years old that was conducted in Belgium found that caregivers who accept the diagnosis of disease tend to better incorporate useful information and are more motivated to fight for the child's health [6].

The establishment of good interactions with the healthcare staff and their trust regarding the benefits of proper treatment adherence has been identified as a key element of optimal adherence. In contrast, families of non-adherent patients had limited relationships with healthcare professionals and usually mistrust the potential benefits of treatments for CALHIV's survival, and consequently did not redefine their fatalistic worldviews. These caveats usually originated from experiences of social isolation and lack of social, family and community support, which are associated with AIDS-related stigma. These findings show that in addition to providing access to diagnosis and treatment, it is also necessary to address the social exclusion of people living with HIV/AIDS and their relatives (which is caused by AIDS-related stigma as well as precarious living conditions) by means of sound partnerships between institutions, patients and their families [23].

Finally, attempts at achieving satisfactory adherence to treatment among CALHIV should consider the dynamic nature of the process and the life stories of the families living with HIV. The main strength of our study is the combination of direct (biological effects) and indirect (questionnaires, interviews) methods of assessment treatment adherence [24]. In addition to distinguishing itself from the cross-sectional approach that measures treatment adherence in an exclusively dichotomous manner (adherent *vs.* non-adherent), which is predominant in the treatment adherence literature, the approach used in the present study enabled the consistent identification of groups of caregivers of adherent or non-adherent CALHIV and to analyze their characteristics and mutual differences.

This comparison has self-evident limitations. The considerable difference in the size of the two groups (adherent and non-adherent) did not allow the analysis of characteristics with relatively low frequencies. Nevertheless, the characterization of the individual, institutional and social factors that determine the role that caregivers play in the adherence of CALHIV to cART showed how these factors act in a complementary manner within a complex articulation regarding the dynamics of adherence or non-adherence to treatment. Thus, despite the similarities in their socioeconomic profile and access to reference services, we detected differences among the families that proved to be associated with CALHIV's adherence/non-adherence to treatment. By contributing to a more thorough understanding of the dynamics involving the factors involved in cART adherence in the pediatric population, this study provides useful information that can guide a revision of the practices performed at pediatric AIDS services.

5. Conclusions

Pediatric AIDS healthcare teams should anticipate possible hindrances to treatment adherence and seek to minimize them. Our findings call attention to dynamic situations in which individual, institutional and social factors should be worked out together with the involved families based on dialogue, active listening and facilitating caregivers, children and adolescents to overcome the conditions that keep them vulnerable to non-adherence to treatment [25].

In addition to improving the interaction between families and healthcare services, the outcomes of the treatment of CALHIV might benefit from interventions that target cultural and structural aspects. Participation of the community in dealing with AIDS-related stigma is an integral part of strategies that seek to overcome obstacles and promote human rights and effective changes in the legislation and

discriminatory practices [26]. Within this context, the process of the primary socialization of CALHIV must be a focus of attention to prevent the next generation from being affected by HIV-related stigma.

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Abbreviations

The following abbreviations are used in this manuscript:

cART	combined antiretroviral therapy
VL	viral load
HIV	Human immunodeficiency virus
CALHIV	children and adolescents living with HIV
AIDS	Acquired Immunodeficiency Syndrome
IRB	Institutional Review Board
WHOQoL-HIV-BREF	World Health Organization instrument for evaluation of quality of life among people living with HIV (short version)
PLHIV	person living with HIV

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Neonatal infections: Case definition and guidelines for data collection, analysis, and presentation of immunisation safety data☆

Stefania Vergnano[a], Jim Buttery[b], Ben Cailes[a], Ravichandran Chandrasekaran[c], Elena Chiappini[d], Ebiere Clark[e], Clare Cutland[f], Solange Dourado de Andrade[g], Alejandra Esteves-Jaramillo[h], Javier Ruiz Guinazu[i], Chrissie Jones[a], Beate Kampmann[j,k], Jay King[h], Sonali Kochhar[l], Noni Macdonald[m], Alexandra Mangili[n], Reinaldo de Menezes Martins[o], César Velasco Muñoz[p], Michael Padula[q], Flor M. Muñoz[r], James Oleske[s], Melvin Sanicas[t], Elizabeth Schlaudecker[u], Hans Spiegel[v], Maja Subelj[w], Lakshmi Sukumaran[x], Beckie N. Tagbo[y], Karina A. Top[m], Dat Tran[z], Paul T. Heath[a,*], The Brighton Collaboration Neonatal Infections Working Group[1]

[a] Paediatric Infectious Diseases Research Group, Institute for Infection and Immunity, St George's University of London, UK
[b] Monash Medical Centre, Clayton, Victoria 3168, Australia
[c] Madras Medical College, India
[d] Meyer Children's Hospital, Florence University, Italy
[e] Enhanced Vigilance Systems, UK
[f] University of the Witwatersrand, Wits Health Consortium, DST/NRF Respiratory and Meningeal Pathogens Research Unit (RMPRU), South Africa
[g] Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Brazil
[h] Global Pharmacovigilance, Sanofi Pasteur, USA
[i] GSK Vaccines, Wavre, Belgium
[j] Imperial College, London, UK
[k] MRC Unit, The Gambia
[l] Global Healthcare Consulting, India
[m] Departments of Paediatrics, Dalhousie University, Halifax, NS, Canada
[n] Novartis Vaccine/GSK Vaccines, USA
[o] Bio Manguinhos/Fiocruz, Brazil
[p] ISGlobal, Barcelona Ctr. Int. Health Res. (CRESIB), Universitad de Barcelona, Spain
[q] Division of Neonatology, The Children's Hospital of Philadelphia and University of Pennsylvania, USA
[r] Baylor College of Medicine, Houston, TX, USA
[s] Department of Pediatrics, Rutgers – New Jersey Medical School, Newark, NJ, USA
[t] Bill & Melinda Gates Foundation, Seattle, WA, USA
[u] Division of Infectious Diseases, Global Health Center, Cincinnati Children's Hospital Medical Center, USA
[v] Henry Jackson Foundation, Bethesda, MD, USA
[w] National Institute of Public Health (NIJZ), Slovenia
[x] Centers for Disease Control and Prevention, Division of Pediatric Infectious Diseases – Emory University School of Medicine, USA
[y] Institute of Child Health, University of Nigeria Teaching Hospital, Nigeria
[z] Division of Infectious Diseases, Department of Paediatrics, The Hospital for Sick Children, University of Toronto, Canada

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* Corresponding author.
E-mail addresses: contact@brightoncollaboration.org, secretariat@brightoncollaboration.org (P.T. Heath).

[1] Brighton Collaboration homepage: http://www.brightoncollaboration.org.

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ABSTRACT

Maternal vaccination is an important area of research and requires appropriate and internationally comparable definitions and safety standards. The GAIA group, part of the Brighton Collaboration was created with the mandate of proposing standardised definitions applicable to maternal vaccine research. This study proposes international definitions for neonatal infections.

The neonatal infections GAIA working group performed a literature review using Medline, EMBASE and the Cochrane collaboration and collected definitions in use in neonatal and public health networks. The common criteria derived from the extensive search formed the basis for a consensus process that resulted in three separate definitions for neonatal blood stream infections (BSI), meningitis and lower respiratory tract infections (LRTI). For each definition three levels of evidence are proposed to ensure the applicability of the definitions to different settings.

Recommendations about data collection, analysis and presentation are presented and harmonized with the Brighton Collaboration and GAIA format and other existing international standards for study reporting.

1. Preamble

1.1. Need for developing case definitions and guidelines for data collection, analysis, and presentation for neonatal infections as an adverse event following immunisation

Considering the enormous public health benefit that can potentially be derived by vaccinating women in pregnancy to protect their newborns against specific infections, it is now imperative to establish safety and efficacy standards in this area. This includes the need to develop definitions for neonatal infections. Such definitions need to be flexible enough to reflect changes in the pattern of infections that may occur following vaccination and to include infections as possible adverse events [1,2]. Considering that vaccination may delay the onset of infections from the neonatal period to later in infancy, the definitions also need to be applicable to the young infant.

Providing standardised definitions of neonatal infections is equally relevant for global efforts to address child mortality since the majority of deaths in children less than five years now occur in the neonatal period and neonatal infections are the third most common cause of death in newborns [3]. The majority of deaths occur in low and middle-income countries (LMIC) and therefore standardised definitions for global use must specifically reflect the needs of LMICs. Global deaths from neonatal sepsis and other infections were estimated to be 328,000 and 342,000 in 1990 and 2013, respectively (age-standardised death rates 4.7 and 4.9 per 100,000, respectively) [4]. The other most common types of fatal neonatal infections in 2013 were lower respiratory infections (196,500 deaths), diarrhoeal diseases (44,800), tetanus (26,000), meningitis (20,600), and malaria (16,800) [4].

A variety of definitions for neonatal infections have been proposed and applied in both community and hospital studies (for example from the Young Infant Clinical Study Group (YICSG)) [5], or as part of verbal autopsy studies [6].

In high-income countries, neonatal intensive care has advanced dramatically over the last decades. Neonatal infections cause a significant burden of morbidity and mortality in the extremely preterm population in these settings. As a result, neonatal networks around the world have produced many case definitions for infections, especially focusing on preterm infants. The better known case definitions are from the National Institute of Child Health and Human Development Neonatal Research Network (NICHD) [7], Australian and New Zealand Neonatal Network (ANZNN) (https://npesu.unsw.edu.au/data-collection/australian-new-zealand-neonatal-network-anznn), European Neonatal Network (ENN) [8], the Vermont-Oxford-Network (VON) (https://public.vtoxford.org) and the neonatal infection network (neonIN: www.neonin.org.uk). Some infectious disease networks have focused specifically on healthcare-associated infections, such as neoKISS [9]. With a similar drive to monitoring hospital associated infections, other organisations such as the Centers for Diseases Control (CDC) [10], the European Centre for Disease Control (ECDC) (http://ecdc.europa.eu/en/healthtopics/Healthcare-associated_infections/point-prevalence-survey/Pages/Point-prevalence-survey.aspx) and the European Medicine Agency (EMA) (http://www.ema.europa.eu/docs/en_GB/document_library/Report/2010/12/WC500100199.pdf) have proposed yet more neonatal infection definitions.

In the neonatal period, the immaturity of the immune system, particularly in premature infants, confers distinctive clinical, physical and outcome characteristics to infections compared with other age groups: neonates are more vulnerable to a broad range of pathogens, including those of generally low virulence such as *Listeria*, paraechoviruses or *Candida*. Different pathogens such as bacteria, viruses, fungi or parasites often present in a clinically indistinguishable pattern in neonates, and localised infections may present with systemic signs making the clinical diagnosis difficult and often impossible without imaging confirmation and/or laboratory support. Moreover, a number of non-infectious syndromes, such as respiratory distress syndrome in the premature infant, inborn errors of metabolism and congenital malformations such as serious cardiac anomalies, have initial clinical presentations similar to severe infections [11].

Even when laboratory tests are available, diagnostic tools to guide clinicians are limited. Traditional blood culture methods lack sensitivity, particularly in neonates where only small samples can be obtained. This leads to a high number of negative results, leaving a large percentage of bacterial infections microbiologically unconfirmed [12]. Whilst the diagnosis of some entities such as HIV and CMV has benefited from the use of novel PCR-based molecular diagnostic tools, this has not happened for all neonatal infections. Interpretation of molecular results from non-sterile samples, such as nasopharyngeal aspirates, can be problematic [13].

The lack of a standardised clinical or laboratory diagnosis for neonatal infections explains the heterogeneity in the neonatal infection definitions in current use, particularly for probable bloodstream infections [14].

There is currently no uniformly accepted definition of neonatal infections following immunizations. However, the development of standardised definitions is now essential in order to facilitate comparability of data and outcomes across clinical trials and epidemiological surveillance studies in which women have received vaccines in pregnancy as well as other clinical trials and interventions aimed at reducing neonatal morbidity and mortality.

1.2. Methods for the development of the case definition and guidelines for data collection, analysis, and presentation for neonatal infections as an adverse event following immunisation

Following the process described in the overview paper [15], the Brighton Collaboration – GAIA: *Neonatal Infections Working Group* was formed in 2015 and included members with clinical, academic, public health, and vaccine industry background. The composition of the working and reference group as well as results of the web-based survey completed by the reference group with subsequent discussions in the Working Group can be viewed at: http://www.brightoncollaboration.org/internet/en/index/working_groups.html.

To guide the decision-making for the case definition and guidelines, a literature search was performed using Medline, Embase and the Cochrane Libraries, the search terms are available in Appendix 1.

The search resulted in the identification of 4422 references. Only references with full abstracts (in English language) were included. All abstracts were screened for possible reports of neonatal infections. 1205 articles with potentially relevant material were reviewed in more detail. This review resulted in a detailed summary of 432 articles, including information on the diagnostic criteria or case definitions used. Case reports, editorials and letters were excluded. Where relevant a description of the vaccine used, the time interval since immunisation, and any other symptoms were extracted. Multiple key references were hand searched and definitions from existing neonatal networks, infection surveillance networks and websites of public health organisations such as the Centers for Disease Control (CDC), the European Centre for Disease Control (ECDC) and the European Medicine Agency (EMA) were also searched for neonatal and perinatal infection definitions.

Across the different manuscripts selected, a large number of definitions were found with a variable number and type of clinical, laboratory and microbiological criteria. The quality of the manuscripts was heterogeneous but this review did not grade the evidence as it was not considered to be relevant for the task of extracting the definitions used.

The definitions from the manuscripts were extracted and entered into spreadsheets listing clinical, laboratory and radiological criteria by 14 members of the group independently and then reviewed for consistency by the coordinator (SV). The data were separated according to the syndrome described: sepsis, meningitis and respiratory tract infections and congenital infections. Percentages of the clinical and laboratory indicators were calculated. The syndromes were not separated according to single pathogens or class of pathogens.

The data extracted from the published literature were collected recognizing the limitation that each study reported different data and definitions for the clinical or laboratory signs and these were not always specified nor clearly described. The studies from neonatal units in high-income countries were reporting both clinical and laboratory confirmed infections while community studies from middle- or low-income countries used mostly clinical definitions. This heterogeneity made data extraction a somewhat subjective exercise. Proposed definitions for specific congenital infections were also discussed, but were eventually excluded from this guideline and recommended for consideration as a specific group of definitions for a future Brighton collaboration Working Group.

The results of this work were presented to the Working Group together with the standard definitions currently in use from the aforementioned networks and the group discussed the definitions in a series of teleconferences until consensus was obtained.

1.3. Rationale for selected decisions about the case definition of neonatal infections as an adverse event following immunisation

For the purpose of this guideline the term "infection" includes neonatal bacteraemia and sepsis (of early or late onset), meningitis, pneumonia and other respiratory infections such as bronchiolitis, caused by bacteria, parasites, viruses or fungi. Localised eye and ear infections were excluded from these guidelines as were encephalitis, urinary tract infections and intestinal infections. The term "neonatal" includes infants from birth (day 0) up to and including 28 postnatal days.

The term "neonatal infection" was chosen to include different infection syndromes during the neonatal period (proven blood stream infections, probable blood stream infections, meningitis and respiratory tract infections).

Ultimately, the group reached agreement on 3 separate definitions for neonatal infections, each with 3 or more diagnostic levels. It is important to emphasise that within the definition context, however, the diagnostic levels must not be misunderstood as reflecting different grades of clinical severity. They instead reflect diagnostic certainty. The case definition has been formulated such that the Level One definition is highly specific for the condition. As maximum specificity normally implies a loss of sensitivity, one or two additional diagnostic levels have been included in the definition, offering a stepwise increase of sensitivity from level one to level three, while retaining an acceptable level of specificity at all levels. In this way it is hoped that all possible cases of neonatal infections can be captured, regardless of the setting or population in which they are being assessed. This is of particular relevance in LMICs where the resources available to assess events, e.g. laboratory facilities, may be more limited.

1.3.1. Rationale for individual criteria or decisions made related to the case definition

1.3.1.1. Neonatal invasive blood stream infections. The GAIA neonatal infections Working Group included in level 1 the microbiological confirmation of infection as this is the recognised diagnostic gold standard. It was decided to use the term "validated" method of identification because it was recognised that this is a rapidly changing field, especially with regard to molecular tests. It is hoped that this will allow the definition to be as inclusive as possible as these methods continue to advance.

The group opted to include a list of organisms commonly considered non-pathogenic (often called "skin commensals"), but still capable of causing opportunistic infections in certain situations, for example, in the presence of central lines, as well as a list of recognised pathogens in order to reduce uncertainty and differences in reporting.

The number of clinical criteria was chosen by reviewing the available definitions in the literature and by consensus. It was decided to include a level 3 definition based solely on clinical signs and taken from a systematic review of studies that reported clinical signs predictive of severe illnesses or mortality in young infants aged 0–59 days, endorsed by the World Health Organization (WHO) [16]. The limited set of clinical signs for which extensive evidence supporting their value exists was reported to have high sensitivity and reasonable specificity. This ensures that the case definition has relevance in all populations and settings.

With regard to the criterion of abnormal white cell count (WCC), it is recognised that ethnic variations exist, for example many African Americans have a WCC that is persistently below the normal range for people of European descent, a condition called "benign ethnic neutropenia" [17]. This should be considered when evaluating a case.

1.3.1.2. Neonatal meningitis. As above, the GAIA neonatal infections Working Group included in level 1 the microbiological confirmation of infection as this is the recognised diagnostic gold standard.

In recognition that delays in undertaking a lumbar puncture may mean that antibiotics have already been given before CSF is obtained, which may make microbiological confirmation less likely, the group included a definition based on the presence of CSF pleocytosis. CSF pleocytosis was defined as ≥20 cells/mm^3 for ≤28 day-olds and ≥10 cells/mm^3 for 29–89 day-olds based on data from large studies [18,19] with no adjustment made for traumatic taps [20].

1.3.1.3. Respiratory tract infections (RTI). The GAIA neonatal infections Working Group provided a single definition for RTI which aimed to include bacterial, fungal and viral pathogens to allow ease of use. The different pathophysiology of viral and bacterial or fungal infections is reflected in the use of diagnostic imaging. Radiographic features (e.g. lobar infiltrate) were accepted without microbiological confirmation for bacterial and fungal infections, but viral low respiratory tract infections required laboratory confirmation, even in the presence of X-ray findings consistent with a viral diagnosis.

The number of clinical criteria chosen arose from the consensus of the group after careful review of available evidence and current definitions in use.

The Working Group were aware of the proposed WHO candidate case definitions for RSV vaccine efficacy trials and believe that both sets of guidelines are consistent.

1.3.2. Influence of treatment on fulfilment of case definitions [21]

In the context of infection a response to antimicrobial treatment might be considered towards fulfilment of the neonatal infections case definition. However, the Working Group decided against this. A treatment response or its failure is not in itself diagnostic and may depend on variables such as clinical status, time to initiation of treatment, other clinical parameters and for many infections, particularly viral, no treatment is currently available.

Inflammatory markers were included although it was recognised that viral infections often are not accompanied by an inflammatory response and newborns often do not present a strong inflammatory response, particularly extremely preterm infants.

1.3.3. Timing post immunisation

Specific time frames for onset of symptoms following immunisation are not included because there are many factors that may influence the impact of vaccination in pregnancy on events in the newborn period. Such factors include the vaccine given, the length of gestation at vaccination of the mother and at birth, the presence of pre-existing immunity and concomitant illnesses in the newborn.

We postulate that a definition designed to be a suitable tool for testing causal relationships requires ascertainment of the outcome independent from the exposure (e.g. immunisations). Therefore, to avoid selection bias, a restrictive time interval from immunisation to the onset of neonatal infections should not be an integral part of such a definition. Instead, where feasible, details of this interval should be assessed and reported as described in the data collection guidelines.

Further, events often occur outside the controlled setting of a clinical trial or hospital. In some settings it may be impossible to obtain a clear timeline of the event, particularly in less developed or rural settings. In order to avoid selecting against such cases, the case definition avoids setting arbitrary time frames.

Table 1
Neonatal invasive blood stream infections: bacterial/fungal/viral.

LEVEL 1	LEVEL 2	LEVEL 3 [22]
Recognised pathogen[a] identified using a validated method and from a normally sterile site[b] If an organism normally considered non-pathogenic is isolated from blood cultures[a]: Level 1 requires its identification from at least 2 blood cultures taken from two different sites, or at 2 different times, PLUS 1 of the criteria as per level 2 of evidence	Not meeting Level 1 of evidence **AND** 3 or more criteria: • Temperature ≥37.5 °C or <35.5 °C[f] • Tachycardia[d] or new or more frequent episodes of bradycardia[d] • New or more frequent episodes of apnea[d] or increased oxygen requirement or increased requirement for ventilatory support • Lethargy or moving only when stimulated or hypotonia or irritability • Difficulty in feeding or abdominal distention • Pallor or poor perfusion[d] or hypotension[d] • Abnormal White Cell Count[d] or I/T ratio >0.2 • Abnormal platelet count [d] • Increased[e] inflammatory markers (CRP, procalcitonin) • Metabolic acidosis as defined by a base excess (BE)[d]	Not meeting Level 1 or 2 of evidence **AND** 2 or more of the following criteria: • Temperature ≥37.5 °C or <35.5 °C[f] • Tachypnea[d] or severe chest indrawing or grunting or cyanosis • Change in level of activity • History of feeding difficulty • History of convulsions

[a] See list of pathogens and non-pathogens in Appendix 1.
[b] Sterile site: blood, sterile urine (catheter urine or supra-pubic aspirate), pleural fluid, ascitic fluid, broncho-alveolar lavage, bone biopsy, synovial fluid.
[d] *Definitions*: Apnea: pause in breathing >20 s; CRP or calcitonin levels above the local normal standards; Tachypnea/fast breathing: respiratory rate >60 breaths per minute; Tachycardia: heart rate >180 beats per minute; Bradycardia: heart rate <100 beats per minute; c Poor perfusion: CRT > 2. d 4000 or >20,000 × 10^9 cells/L; Low Platelets/Thrombocytopenia: <100,000 × 10^9/L; Metabolic acidosis: <−10 mmol/L (−10 mEq/L)
[e] Increased according to locally defined and validated reference ranges.
[f] Also refer to Brighton collaboration case definition for fever [23].

1.3.4. Differentiation from other (similar/associated) disorders

Using the level 2 or 3 of evidence there is risk that the above definitions will include other neonatal pathologies such as congenital heart diseases or inborn errors of metabolism within the blood stream infections (BSI) and meningitis definitions or even respiratory distress syndrome and transient tachypnea of the newborn in the most premature neonates within the RTI definition. Congenital malformations and inborn error of metabolism are relatively rare events however, and distinction based on clinical response to treatment, laboratory investigations and imaging may be possible in most settings.

1.4. Guidelines for data collection, analysis and presentation

The case definition is accompanied by guidelines, which are structured according to the steps of conducting a clinical trial, i.e. data collection, analysis and presentation. Neither case definition nor guidelines are intended to guide or establish criteria for management of ill infants, children, or adults. Both were developed to improve data comparability.

Table 2
Bacterial/fungal/viral meningitis.

LEVEL 1	LEVEL 2	LEVEL 3a	LEVEL 3b
Recognised pathogen[a] identified using a validated method from cerebrospinal fluid (CSF) If an organism normally considered non-pathogenic is identified from the CSF, LEVEL 1 of evidence additionally requires all LEVEL 2 criteria: i.e. CSF pleocytosis AND temperature criteria AND 1 or more clinical criteria	CSF pleocytosis[d] OR positive IgM antibodies to a specific pathogen in the CSF **AND** Recognised pathogen[a] identified using a validated method from a normally sterile site[b] (other than CSF) **AND** Temperature ≥37.5 °C or <35.5 °C[c] **AND** 1 or more criteria: • History of convulsions • Lethargy or irritability • Coma • Apnea[d] • Bulging fontanel • Neck stiffness	CSF pleocytosis[d] **AND** NO d pathogen[a] identified using a validated method from a normally sterile site[b] **AND** Temperature ≥37.5 °C or <35.5 °C[c] **AND** 3 or more criteria: • History of convulsions • Lethargy or irritability • Coma • Apnea[d] • Bulging fontanel • Neck stiffness	No lumbar puncture done or no sample available **AND** Temperature ≥37.5 °C or <35.5 °C[c] **AND** 4 or more criteria: • History of convulsions • Lethargy or irritability • Coma • Apnea[d] • Bulging fontanel • Neck stiffness

[a] See list of pathogens and non-pathogens in Appendix 1.
[b] Sterile site: blood, sterile urine (catheter urine or supra-pubic aspirate), pleural fluid, ascitic fluid, broncho-alveolar lavage, bone biopsy, synovial fluid.
[c] Also refer to Brighton collaboration case definition for fever [23].
[d] CSF pleocytosis: ≥20 cells/mm³ for <28 day-olds and ≥10 cells/mm³ for 29–89 day-olds. # i–89 day-olds.

1.5. Periodic review

Similar to all Brighton Collaboration case definitions and guidelines, review of the definition with its guidelines is planned on a regular basis (i.e. every three to five years) or more often if needed.

2. Case definitions of neonatal infections

2.1. Neonatal invasive blood stream infections: bacterial/fungal/viral

Table 1 presents the case definition for neonatal invasive blood Stream Infections: bacterial/fungal/viral.

2.2. Bacterial/fungal/viral meningitis

Table 2 presents the case definition for bacterial/fungal/viral meningitis.

2.3. Respiratory bacterial/fungal/viral infection

Table 3 presents the case definition for respiratory bacterial/fungal/viral infection.

3. Guidelines for data collection, analysis and presentation of neonatal infections

It was the consensus of the Brighton Collaboration GAIA *Neonatal Infections Working Group* to recommend the following guidelines to enable standardised data collection, analysis, and presentation of information regarding neonatal infections in the context of pregnancy vaccination. The availability of information may vary depending upon resources, geographical region, and whether the source of information is a prospective clinical trial, a post-marketing surveillance or epidemiological study, or an individual report of a neonatal infection.

Guidelines for the collection, analysis and presentation of safety data in clinical trials of vaccines in pregnant women are also available and should be referred to for more generic guidance.

3.1. Data collection

3.1.1. Source of information/reporter

For all cases and/or all study participants, as appropriate, the following information should be recorded:

(1) Date of report.
(2) Name and contact information of person reporting[2] and/or diagnosing the event as specified by country-specific data protection law.
(3) Name and contact information of the investigator responsible for the subject, as applicable.
(4) Relation to the patient (e.g. immuniser [clinician, nurse], family member [indicate relationship], other).

3.1.2. Vaccinee/control

For all cases and/or all study participants, as appropriate, the following information should be recorded:

3.1.2.1. Demographics.

(5) Case/study participant identifiers (e.g. first name initial followed by last name initial) or code (or in accordance with country-specific data protection laws).
(6) Date of birth, age, and sex. With neonatal data disaggregated from older infants.
(7) Gestational age, birth weight and methods used for their assessment.

[2] If the reporting centre is different from the vaccinating centre, appropriate and timely communication of the adverse event should occur.

Table 3
Respiratory bacterial/fungal/viral infection.

LEVEL 1	LEVEL 2	LEVEL 3 [24,25]
New or progressive or persistent infiltrate or shadowing or fluid in the intrapleural cavity or interlobar fissure on chest X-ray	New or progressive or persistent infiltrate or shadowing or fluid in the intrapleural cavity or interlobar fissure on chest X-ray	**2 or more criteria:** Difficulty in breathing/Tachypnea[c]
AND	**AND 4 or more criteria:**	• Severe chest indrawing
Recognised virus[c] identified using a validated assay from an upper respiratory sample	• Temperature ≥37.5 °C or <35.5 °C[e]	• Nasal flaring
OR	• Tachypnea[c] or Nasal flaring or Chest indrawing or Grunting	• Grunting
Recognised pathogen[a] identified using a validated method and from a normally sterile site[b]	• Desaturations or increased oxygen requirements or increased ventilator requirements or oxygen saturation <95%	• Wheezing
AND 3 or more criteria:	• Apneas[c]	• Stridor
• Temperature ≥37.5 °C or <35.5 °C[e]	• Increased respiratory secretions or Increased suctioning requirements	• Fever
• Tachypnea[c] or Nasal flaring or Chest indrawing or Grunting	• Cough or wheeze or crepitations	
• Desaturations or increased oxygen requirements or increased ventilator requirements or oxygen saturation <95%	• Increased CRP or procalcitonin[d]	
• Apneas[c]		
• Increased respiratory secretions or Increased suctioning requirements		
• Cough or wheeze or crepitations		
• Increased CRP or procalcitonin[d]		

[a] See list of pathogens and non-pathogens in Appendix 1.
[b] Sterile site: blood, sterile urine (catheter urine or supra-pubic aspirate), pleural fluid, ascitic fluid, broncho-alveolar lavage, bone biopsy, synovial fluid.
[c] See list of definitions in Table 1.
[d] Increased according to locally defined and validated reference ranges.
[e] Also refer to Brighton collaboration case definition for fever [23].

3.1.2.2. Clinical and immunisation history. For all cases and/or all study participants, as appropriate, the following information should be recorded:

(8) Mother: Maternal history of infections or risk factors for infections (e.g. GBS colonisation, peripartum fever), indication whether any antimicrobials were used in pregnancy or in labour, type and route of administration; underlying diseases/disorders, type of delivery and indicate whether the delivery occurred in a facility or at home, describe obstetric care available in terms of basic or comprehensive; immunisation received in pregnancy with dates, type, batch and reaction for all infections, available serology as applicable, any other medications use during pregnancy including non prescription medications.
(9) Newborn: report whether the newborn was admitted to hospital and the type of facility (e.g. emergency department, ward, neonatal unit) or was in the community. Indicate the level of neonatal care available (e.g. ventilator support) and give the type of neonatal care staff available and their level of training. Indicate the presence of central lines, whether the newborn received surgical interventions and their type.
(10) Newborn: Report the medication history (other than treatment for the event described) including prescription and non-prescription medication as well as medication, topical treatments, parenteral nutrition or treatment with long half-life or long-term effect (e.g. immunoglobulins, blood transfusion and immunosuppressants).
(11) Facility: indicate whether microbiology laboratory investigations are available and describe the methods used for bacterial identification or the molecular techniques used to identify organisms viral, fungal, parasitic or bacterial. Give an indication of the quality control in place. Indicate whether biochemistry, haematology and radiology facilities are available.
(12) Immunisation history (i.e. previous immunizations and any adverse event following immunisation (AEFI)), in particular occurrence of neonatal infection after a previous immunisation.

3.1.3. Details of the immunisation

For all study participants, as appropriate, the following information about pregnancy vaccination should be recorded:

(13) Date and time of immunisation(s), gestational age at the time of immunisation. Context of immunisation (routine clinic, outbreak situation, clinical trial, etc.)
(14) Description of vaccine(s) (name of vaccine, manufacturer, lot number, dose (e.g. 0.25 mL, 0.5 mL, etc.) and number of dose if part of a series of immunizations against the same disease).
(15) The anatomical sites (including left or right side) of all immunizations (e.g. vaccine A in proximal left lateral thigh, vaccine B in left deltoid).
(16) Route and method of administration (e.g. intramuscular, intradermal, subcutaneous, and needle-free (including type and size), oral, intranasal, other injection devices).
(17) Needle length and gauge.

3.1.4. The adverse event

(18) For all cases at any level of diagnostic certainty and for reported events with insufficient evidence, the criteria fulfilled to meet the case definition should be recorded.
Specifically document:
(19) Clinical description of signs of neonatal infection and if there was confirmation of the infection (i.e. positive identification using validated method).
(20) Date/time of onset,[3] first observation[4] and diagnosis,[5] end of episode[6] and final outcome.[7]
(21) Concurrent signs and diseases.
(22) Measurement/testing
- Values and units of routinely measured parameters (e.g. temperature, blood pressure) – in particular those indicating the severity of the event;
- Method of measurement (e.g. type of thermometer, oral or other route, duration of measurement, etc.);
- Results of laboratory examinations, surgical and/or pathological findings and diagnoses if present.

(23) Treatment given for neonatal infection, especially antimicrobials, including which antimicrobials (e.g. antibiotics, antivirals, immunoglobulins), dosing and duration of treatment.
(24) Outcome[6] at last observation.
(25) Objective clinical evidence supporting classification of the event as "serious"[8]
(26) Exposures from 24 h before and after immunisation (e.g. food, environmental) considered potentially relevant to the reported event.

3.1.5. Miscellaneous/general

(27) The duration of surveillance for neonatal infection should be predefined based on
- Biologic characteristics of the vaccine e.g. live attenuated versus inactivated component vaccines;
- Biologic characteristics of the vaccine-targeted disease;
- Biologic characteristics of neonatal infection including patterns identified in previous trials (e.g. early-phase trials); and
- Biologic characteristics of the vaccinee (e.g. nutritional status, underlying disease like immunosuppressing illness).

(28) The duration of follow-up reported during the surveillance period should be predefined likewise. It should aim to continue to resolution of the event.
(29) Methods of data collection should be consistent within and between study groups, if applicable.
(30) Follow-up of cases should attempt to verify and complete the information collected as outlined in data collection guidelines.
(31) Investigators of patients with neonatal infection should provide guidance to reporters to optimise the quality and completeness of information provided.
(32) Reports of neonatal infection should be collected throughout the study period regardless of the time elapsed between immunisation and the adverse event. If this is not feasible due to the study design, the study periods during which safety data are being collected should be clearly defined.

3.2. Data analysis

The following guidelines represent a desirable standard for analysis of data on neonatal infections to allow for comparability of data, and are recommended in addition to the data analysed for the specific study question and setting.

Reported events should be classified in one of the following five categories including the three levels of diagnostic certainty. Events that meet the case definition should be classified according to the levels of diagnostic certainty as specified in the case definition. Events that do not meet the case definition should be classified in the additional categories for analysis.

Event classification in 5 categories[9]

- Event meets case definition
  (1) Level 1: Criteria as specified in the neonatal infections case definition (separately for BSI, meningitis and RTI)
  (2) Level 2: Criteria as specified in the neonatal infection case definition (separately for BSI, meningitis and RTI)
  (3) Level 3: Criteria as specified in the neonatal infections case definition (separately for BSI, meningitis and RTI)
- Event does not meet case definition

Additional categories for analysis

[3] The date and/or time of onset is defined as the time post immunisation, when the first sign or symptom indicative for neonatal infection occurred. This may only be possible to determine in retrospect.
[4] The date and/or time of first observation of the first sign or symptom indicative for neonatal infection can be used if date/time of onset is not known.
[5] The date of diagnosis of an episode is the day post immunisation when the event met the case definition at any level.
[6] The end of an episode is defined as the time the event no longer meets the case definition at the lowest level of the definition.
[7] E.g. recovery to pre-immunisation health status, spontaneous resolution, therapeutic intervention, persistence of the event, sequelae, death.
[8] An AEFI is defined as serious by international standards if it meets one or more of the following criteria: (1) it results in death, (2) is life-threatening, (3) it requires inpatient hospitalisation or results in prolongation of existing hospitalisation, (4) results in persistent or significant disability/incapacity, (5) is a congenital anomaly/birth defect, (6) is a medically important event or reaction.

[9] To determine the appropriate category, the user should first establish, whether a reported event meets the criteria for the lowest applicable level of diagnostic certainty, e.g. Level three. If the lowest applicable level of diagnostic certainty of the definition is met, and there is evidence that the criteria of the next higher level of diagnostic certainty are met, the event should be classified in the next category. This approach should be continued until the highest level of diagnostic certainty for a given event could be determined. Major criteria can be used to satisfy the requirement of minor criteria. If the lowest level of the case definition is not met, it should be ruled out that any of the higher levels of diagnostic certainty are met and the event should be classified in additional categories four or five.

(4) Reported neonatal infection (separately for BSI, meningitis and RTI) with insufficient evidence to meet the case definition[10]
(5) Not a case of neonatal infection[11] neither BSI, meningitis or RTI

The interval between maternal immunisation and reported neonatal infection could be defined as the interval from the date/time of immunisation to the date/time of onset[2] of the first signs consistent with the definition. The timing of onset of a neonatal infection may be defined by the age of the infant at the time of onset using specific periods of infancy as follows:

Periods of infancy for age of clinical recognition of a neonatal infection

Time period	Days
Prenatal	<Day 1 of life
Neonatal[a]	1–27[b]
Early neonatal[a]	1–6[b]
Late neonatal[a]	7–27
Post neonatal	28–364

[a] Use either Neonatal or divide into early neonatal and late neonatal.
[b] Day 1 = first 24 h of life.

The duration of a possible neonatal infection could be analysed as the interval between the date/time of onset[2] of the first signs consistent with the definition and the end of episode[5] and/or final outcome[6]. Whatever start and ending are used, they should be used consistently within and across study groups.

If more than one measurement of a particular criterion is taken and recorded, the value corresponding to the greatest magnitude of the adverse experience could be used as the basis for analysis. Analysis may also include other characteristics like qualitative patterns of criteria defining the event.

The distribution of data (as numerator and denominator data) could be analysed in predefined increments (e.g. measured values, times), where applicable. Increments specified above should be used. When only a small number of cases is presented, the respective values or time course can be presented individually.

Data on neonatal infections obtained from neonates born to women vaccinated during pregnancy should be compared with those obtained from an appropriately selected and documented control group(s) or known background rates of neonatal infections in comparable populations, and should be analysed by study arm and dose where possible, e.g. in prospective clinical trials.

3.3. Data presentation

These guidelines represent a desirable standard for the presentation and publication of data on neonatal infections following maternal immunisation to allow for comparability of data, and are recommended as an addition to data presented for the specific study question and setting. Additionally, it is recommended to refer to existing general guidelines for the presentation and publication of randomised controlled trials, systematic reviews, and meta-analyses of observational studies in epidemiology (e.g. statements of Consolidated Standards of Reporting Trials (CONSORT), of Improving the quality of reports of meta-analyses of randomised controlled trials (QUORUM), and of Meta-analysis Of Observational Studies in Epidemiology (MOOSE), respectively) and the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Strengthening the Reporting of Observational Studies in Epidemiology for Newborn Infection (STROBE-NI) guidelines (Fitchett, in press) (http://www.equator-network.org).

All reported events of neonatal infections should be presented according to the categories listed above.

Data on possible neonatal infections events should be presented in accordance with data collection guidelines and data analysis guidelines.

Terms to describe neonatal infection such as "low-grade", "mild", "moderate", "severe" or "significant" are highly subjective, prone to wide interpretation, and should be avoided, unless clearly defined.

Data should be presented with numerator and denominator (n/N) (and not only in percentages), if available. It should be clear if the denominator represents a population denominator (live births) or neonates admitted to a facility. The source of the denominator data should be reported and calculations of estimate described (e.g. manufacturer data like total doses distributed, reporting through Ministry of Health, coverage/population based data, etc.).

The incidence of cases in the study population should be presented and clearly identified as such in the text.

If the distribution of data is skewed, median and interquartile range are usually the more appropriate statistical descriptors than the mean. However, the mean and standard deviation should also be provided.

Any publication of data on neonatal infection should include a detailed description of the methods used for data collection and analysis as possible. It is essential to specify:

- The study design;
- The method, frequency and duration of monitoring for neonatal infection;
- The trial profile, indicating participant flow during a study including drop-outs and withdrawals to indicate the size and nature of the respective groups under investigation;
- The type of surveillance (e.g. passive or active surveillance);
- The characteristics of the surveillance system (e.g. population served, mode of report solicitation);
- The search strategy in surveillance databases;
- Comparison group(s), if used for analysis;
- The instrument of data collection (e.g. standardised questionnaire, diary card, report form);
- Whether the day of immunisation was considered "day one" or "day zero" in the analysis;
- Whether the date of onset[2] and/or the date of first observation[3] and/or the date of diagnosis[4] was used for analysis; and
- Use of this case definition, in the abstract or methods section of a publication.[12]

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[10] If the evidence available for an event is insufficient because information is missing, such an event should be categorised as "Reported neonatal infection with insufficient evidence to meet the case definition".
[11] An event does not meet the case definition if investigation reveals a negative finding of a necessary criterion (necessary condition) for diagnosis. Such an event should be rejected and classified as "Not a case of neonatal infection".
[12] Use of this document should preferably be referenced by referring to the respective link on the Brighton Collaboration website (http://www.brightoncollaboration.org).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2016.03.046.

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