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FERNANDA DE OLIVEIRA DEMITTO TAMOGAMI

DESFECHOS DE TRATAMENTO E REGIMES TERAPÊUTICOS USADOS PARA TB-HIV

Rio de Janeiro
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Tese apresentada ao Programa de Pós-graduação em Pesquisa Clínica em Doenças Infecciosas, do Instituto Nacional de Infectologia Evandro Chagas para obtenção do grau de doutora em Pesquisa Clínica em Doenças Infecciosas

Orientadora Profa. Dra. Valeria Cavalcanti Rolla

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Aos que insistem em dedicar parte de suas vidas à luta contra tuberculose e aids

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Não há fatos eternos, como não há verdades absolutas.

(*Friedrich Nietzsche*)

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RESUMO

Tuberculose (TB) é a maior causa de morte entre pessoas que vivem com HIV/AIDS (PVHA). O tratamento antirretroviral (TARV) em pacientes co-infectados representa um desafio devido às interações da rifampicina com os antirretrovirais (ARV). Pacientes com TB ativa podem apresentar níveis reduzidos de hemoglobina (Hb), o que pode afetar a morbidade associada à TB. Objetivos: analisar a efetividade dos regimes terapêuticos; caracterizar a dinâmica dos níveis de Hb; examinar as relações entre anemia e distúrbio inflamatório sistêmico, associação entre anemia persistente e desfechos desfavoráveis, e avaliar sobrevida em pacientes TB-HIV durante terapia anti-tuberculose (ATT). Tratou-se de um estudo de coorte retrospectivo longitudinal de pacientes com TB-HIV entre 2008 e 2016 tratados no Laboratório de Pesquisa Clínica em Micobacterioses do Instituto Nacional de Infectologia Evandro Chagas, Fiocruz- Rio de Janeiro. Foi utilizado modelo de riscos proporcionais de Cox para avaliar a efetividade do TARV. Os pacientes foram subdivididos em virgens de ARV (VT) e previamente expostos a ARV (PE). Para sobrevida foi utilizado o método de Kaplan Meier. Parâmetros bioquímicos e contagens de células sanguíneas foram utilizados para traçar o perfil de inflamação sistêmica antes do ATT. As análises foram realizadas no software estatístico R. O primeiro artigo mostrou que para os VT, o diagnóstico de IRIS foi um fator independente significativo para mortalidade precoce. Em relação a efetividade do TARV, TB prévia e abuso de álcool foram associadas ao insucesso no grupo VT, para os PE foi CV elevada antes do início do TARV e tratamento baseados em inibidor de protease. O segundo artigo evidenciou que a maioria dos pacientes com anemia antes do início da ATT persistiu com tal condição até o dia 180. Esses indivíduos exibiram elevado grau de perturbação inflamatória (DIP), que foi inversamente correlacionado com níveis de Hb. Níveis basais de Hb mais baixos foi determinante dos desfechos desfavoráveis. Conclui-se então que os fatores de risco para mortalidade e falha do ARV foram diferentes entre VT e PE, sendo o último grupo alvo para ensaios com drogas compatíveis com rifampicina para melhorar os desfechos do tratamento desfavoráveis. A anemia persistente em PVHA durante o curso de ATT está intimamente relacionada com DIP crônica.

Palavras-chave: tuberculose, HIV, efetividade, sobrevida, anemia, inflamação.

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ABSTRACT

Tuberculosis (TB) is the leading cause of death among people living with HIV/AIDS (PLWH). Antiretroviral treatment (ART) in patients with TB-HIV represents a challenge due to the interactions of rifampicin with ART. Patients with TB may have reduced levels of hemoglobin (Hb), which can directly affect the morbidity associated with TB. The objectives were: to analyze the effectiveness of the different therapeutic regimens; characterize the dynamics of Hb levels; examine the relationship between anemia and systemic inflammatory disorder, the association between persistent anemia and poor outcomes, and to evaluate the survival in TB-HIV during the antitubercular therapy (ATT). This was a longitudinal retrospective cohort study, with TB-HIV between 2008 to 2016, evaluated and treated at Clinical Research Laboratory for Mycobacterioses at national Institute of Infectology Evandro Chagas, Fiocruz in Rio de Janeiro. To assess the effectiveness of ART Cox's proportional hazards model was used, patients were subdivided into ARV-naïve (VT) and previously exposed to ARV (PE). The Kaplan Meier method was used for survival analyzes. Biochemical parameters and blood cell counts were used to trace the profile of systemic inflammation in patients stratified by anemia before to ATT. The analyzes were performed using the statistical software R. The first article showed that for VT patients, IRIS was a risk factor for early mortality. Regarding the analyses of effectiveness of ART, previous TB and alcohol abuse were associated with failure in the VT group, and for PE were high baseline VL and treatment with protease inhibitor based regimens. The second article showed that the majority of patients with anemia before TB treatment (pre-ATT) persisted with this condition until day 180. These individuals exhibited a high degree of inflammatory disorder (DIP), which in turn was inversely correlated with Hb levels. Multivariate regression analysis revealed that the lowest baseline Hb levels were the main determinant of the unfavorable outcomes. It is concluded that the risk factors for ARV mortality and failure were different for VT patients from those PE, being the last target group for trials with less toxic drugs and compatible drugs with rifampicin to improve unfavorable treatment outcomes. Persistent anemia in PLWH during ATT is closely related to chronic DIP.

Keywords: tuberculosis, HIV, TARV, effectiveness, survival, anemia, inflammation.

LISTA DE ABREVIATURAS E SIGLAS

3TC	Lamivudina
aOR	*Adjusted odds ratio*
ABC	Abacavir
ATT	Tratamento antituberculostático/ *Antitubercular therapy*
ARV	Antirretroviral
ATZ	Atazanavir
AUC	Área sob a curva
AZT	Zidovudina
CBC	*Complet Blood Count*
CDC	Center for Disease Control and Prevention
CIs	*Confidence Intervals*
CV/*VL*	Carga Viral/ *Viral Load*
CYP	Enzimas do citocromo
D0	*Baseline (day0)*
D4T	Estavudina
D60	*Day 60*
D180	*Day 180*
DDI	Didanosina
DIP	*Degree of inflammatory perturbation*
DRV	Darunavir
EFZ	Efavirenz
FIOCRUZ	Fundação Oswaldo Cruz
FPV	Fosamprenavir
HR	*Hazard ratio*
Hb	Hemoglobina/ *Hemoglobin*
HIV	Vírus da imunodeficiência humana
II	Inibidor de integrasse
INI	Instituto Nacional de Infectologia
IP/*PI*	Inibidor de protease/*Protease inhibitor*
IQR	*Interquartile range*

IRIS	Síndrome inflamatória da reconstituição imune/ *Immune reconstitution inflammatoru syndrome*
ITRN	Inibidor de Transcriptase Reversa Análogo do Nucleosídeo
ITRNN	Inibidor de Transcriptase Reversa não Análogo do Nucleosídeo
KM	Kaplan-Meier
LAPCLINTB	Laboratório de Pesquisa Clínica em Micobacterioses
LPV	Lopinavir
MDP	*Molecular degree of perturbation*
Mtb	*Mycobacterium tuberculosis*
NVP	Nevirapina
OR	*Odds Ratio*
PE	Previamente exposto a terapia antirretroviral
PCR	Polymerase Chain Reaction - Reação em Cadeia da Polimerase
PVHA/ PLWA	Pessoas vivendo com HIV/AIDS/ *People living with HIV/AIDS*
SD	*Standard deviation*
RAL	Raltegravir
RTV	Ritonavir
SQV	Saquinavir
TANN	Testes de amplificação de ácidos nucleicos
TARV	Terapia antirretroviral
TCLE	Termo de consentimento livre e esclarecido
TDF	Tenofovir
TB	Tuberculose/ *Tuberculosis*
VT	Virgem de tratamento antirretroviral
WHO	World Health Organization

SUMÁRIO

1 INTRODUÇÃO

1.1- Epidemiologia

A tuberculose (TB) é uma doença infectocontagiosa conhecida há séculos, causada por *Mycobacterium tuberculosis* (Mtb). A TB é um problema de saúde pública, e está ligada à pobreza, aglomerações e à má distribuição de renda (MINISTÉRIO DA SAÚDE, 2012). Seu desenvolvimento e evolução são dependentes de fatores como os aglomerados humanos, desnutrição e a baixa resistência imunológica, estando este último fator intimamente relacionado com casos de infecção provocada pelo vírus da imunodeficiência humana (HIV). A TB é a doença mais comum entre as pessoas vivendo com HIV/AIDS (PVHA). Fatal se não detectada ou tratada, a TB é a principal causa de morte entre PVHA, responsável por quase uma em cada três mortes associadas ao HIV (WORLD HEALTH ORGANIZATION, 2020).

Estima-se que em 2018 cerca de 10 milhões de pessoas em todo o mundo tenham desenvolvido TB, sendo uma das dez principais causas de morte, e a principal causa por um único agente infeccioso. O Brasil pertence a um grupo de 20 países com o maior número absoluto de casos de TB. Em 2018, a incidência estimada foi de 95.000 casos, com uma tendência ascendente partir de 2016 (WORLD HEALTH ORGANIZATION, 2019).

Sendo a TB responsável por 1,6 milhões de mortes anualmente e com o surgimento do HIV, a partir do início dos anos 80, houve uma mudança no perfil clínico e epidemiológico de TB. A TB associada ao HIV resultou no aumento da incidência, da prevalência e da mortalidade por TB (CHAISSON et al., 1987). A associação destas duas doenças é sinérgica, interativa e recíproca, na qual ocorre um aumento da viremia plasmática de HIV nas pessoas com TB-HIV e um aprofundamento da imunossupressão (queda na contagem de células CD4+) levando ao aumento da mortalidade (HAVLIR; BARNES, 1999).

O risco de um indivíduo não infectado por HIV desenvolver TB ao longo da vida é de 5% a 10%, mas alcança 50% entre PVHA. PVHA possuem 26 vezes mais chances de desenvolver TB em relação às pessoas não infectadas que vivem no mesmo país (WORLD HEALTH ORGANIZATION, 2015). Em pacientes soropositivos para HIV, ocorre um aumento no risco de desenvolver TB dependendo do estágio da imunossupressão (HAVLIR; BARNES, 1999) e um aumento do risco de reinfecção

exógena (RUFFINO-NETTO, 2007). A associação do HIV com o Mtb tem impactado simultaneamente a epidemiologia, a história natural e a evolução clínica de ambas as doenças, representando uma combinação letal, uma doença acelerando o progresso da outra, sendo assim um desafio para a saúde pública (MAHER; SEKAJUGO, 2010).

1.2- Diagnóstico de TB

Os exames utilizados para o diagnóstico de TB incluem a baciloscopia do espécime coletado, exames de imagem, a cultura da amostra clínica em meios apropriados e o uso de testes moleculares rápidos, como o Xpert-MTB-RIF (KAUFMANN; PARIDA, 2007). O diagnóstico definitivo de TB é feito pela cultura ou através de métodos moleculares (WORLD HEALTH ORGANIZATION, 2015).

Durante décadas, os países com recursos limitados têm contado com baciloscopia como o principal método para diagnosticar TB. Porém a microscopia do escarro não é um teste sensível (particularmente em crianças e PVHA) e não fornece nenhuma informação sobre o perfil de resistência dos bacilos. Além disso, microscopia não é capaz de distinguir entre Mtb e micobactérias não-tuberculosas (WORLD HEALTH ORGANIZATION, 2015) sendo necessário realizar a cultura e identificação, ou o uso de testes moleculares para um diagnóstico completo.

A cultura, considerada o padrão ouro para o diagnóstico de TB, permite a identificação do Mtb e a realização do teste de sensibilidade aos antimicrobianos, além de aumentar o rendimento diagnóstico da baciloscopia em 20-40%. Os meios sólidos mais recomendados são o Löwenstein-Jaensen e o Ogawa-Kudoh (KUDOH; KUDOH, 1974), mas suas desvantagens são que os resultados podem levar semanas para serem concluídos, além de requerer um laboratório bem equipado, profissionais altamente treinados e um eficiente sistema de transporte para assegurar a viabilidade das amostras (WORLD HEALTH ORGANIZATION, 2015). Já os meios líquidos, Middlebrook 7H9 e 7H12, são utilizados nos métodos automatizados disponíveis no Brasil, entre eles MGIT®, no qual o tempo de resultado varia entre 5 a 12 dias, quando positivo, e 42 dias, quando negativo. Os meios líquidos permitem o crescimento das micobactérias em menor tempo, o que contribui para um diagnóstico mais rápido, (ANVISA, 2004), porém sofrem contaminação mais frequentemente que os meios sólidos.

Os testes de amplificação de ácidos nucleicos (TAAN) são métodos rápidos com alta especificidade e sensibilidade. Entre as metodologias mais empregadas nos TAAN, está a Reação em Cadeia da Polimerase (PCR, do inglês Polymerase Chain Reaction), que pode ser aplicada diretamente na amostra biológica, como no escarro ou na colônia suspeita (DROBNIEWSKI et al., 2013). Uma metodologia TAAN, recomendada pela WHO e pela ANVISA é o ensaio comercial Xpert MTB/RIF^{TM}. Esse ensaio pode confirmar a presença de Mtb e identificar mutações que conferem resistência da micobactéria à rifampicina em até 2 horas (IOANNIDIS et al., 2011).

1.3- Tratamento anti-TB

O regime de tratamento de primeira linha para TB no Brasil para todos os pacientes com TB sensível desde 2010 é a combinação de rifampicina 600mg, isoniazida 300mg, pirazinamida 1600mg e etambutol 1100mg durante 2 meses na fase inicial ou chamada “fase intensiva” do tratamento, seguido de rifampicina e isoniazida durante 4 meses ou "fase de manutenção", totalizando 6 meses de tratamento. Há situações com indicação para a ampliação do tempo de tratamento da segunda fase, tais como: formas meningoencefálicas e osteoarticulares, pacientes com baciloscopias de acompanhamento negativas, com evolução clínica e/ou radiológica insatisfatórias, pacientes com baciloscopia positiva no quinto ou sexto mês de tratamento (PNCT, 2019).

Um grande problema na utilização destes fármacos é a duração do tratamento, o número de doses e as reações adversas associadas (PROGRAMA NACIONAL DE CONTROLE DA TUBERCULOSE; MINISTÉRIO DA SAÚDE, 2019). Em PVHA observa-se com frequência uma maior incidência de reações adversas (BLUMBERG, 2003).

A rifampicina é uma medicação de extrema importância no tratamento da TB. Estudos mostraram que a taxa de recaída da TB e o risco de falha do tratamento é maior em pacientes que foram tratados sem esta droga no esquema (ATOMIYA; UIP; LEITE, 2002; JINDANI; NUNN; ENARSON, 2004; OKWERA, A et al., 2006). Um estudo conduzido no INI-Fiocruz mostrou uma tendência à maior mortalidade em pacientes tratados para TB sem uma rifamicina no esquema, sugerindo que, mesmo no contexto de disponibilidade de terapia antirretroviral (TARV), o uso de esquemas

de TB menos eficazes pode influenciar para um pior prognóstico de pacientes soropositivos para HIV (SCHMALTZ et al., 2012a). Atualmente, a necessidade de novos esquemas terapêuticos alternativos sem o uso de rifampicina, mas com eficácia semelhante, tem aumentando, especialmente em PVHA, para compatibilizar a TARV com um melhor tratamento para TB.

A rifampicina é um potente indutor do citocromo P450 e da glicoproteína P (uma bomba de efluxo que joga para o espaço extracelular muitos medicamentos utilizados no tratamento de TB e de HIV); por esse motivo, reduz dramaticamente as concentrações plasmáticas dos inibidores de protease (IP) e inibidores de transcriptase reversa não nucleosídeos (ITRNN), uma vez que estes fármacos utilizam a mesma via de metabolização (CDC, 2000). No ano 2000, o Center for Diseases Control and Prevention (CDC) publicou uma nota autorizando o uso de rifampicina apenas com a associação de saquinavir e ritonavir em doses de 400mg e 400mg (CDC, 2000) porque o ritonavir tem um efeito inibidor do CYP3A4 e poderia compensar o efeito indutor do CYP causado pela rifampicina. O uso de efavirenz e nevirapina também foi autorizado devido a pequena redução plasmática causada pela rifampicina na classe de análogos não nucleosídeos da trascriptase reversa [(para efavirenz a redução é de 30% da área sob a curva (AUC)],. Apesar de autorizado pelo CDC dos EUA e pelo Ministério da Saúde do Brasil, a associação de rifampicina e IP (saquinavir e ritonavir) levou a reações adversas graves e descontinuação do tratamento em pacientes virgens de terapia antirretroviral (ROLLA et al., 2006a).

Rifabutina é um fármaco derivado da rifamicina e possui largo espectro de atividade antimicrobiana, incluindo as micobactérias (BRUNA et al., 1983). Rifabutina está disponível no Brasil desde 2012, tomada em doses de 300mg uma vez ao dia e têm eficácia similar a rifampicina (DAVIES; CERRI; RICHELDI, 2007; DEPARTAMENTO DE DST /AIDS E HEPATITES VIRAIS; MINISTÉRIO DA SAÚDE, 2012; GONZALEZ-MONTANER et al., 1994; MCGREGOR et al., 1996; SCHWANDER et al., 1995). Comparada à rifampicina, a Rifabutina tem um efeito significativamente menor nos fármacos metabolizados pelas enzimas do citocromo P450 (BURMAN; GALLICANO; PELOQUIN, 2001). O uso de Rifabutina foi recomendado com IPs porque a Rifabutina é um indutor do citocromo menos potente do que a rifampicina. Contudo, ela é metabolizada por isoformas do citocromo P450 3A4, dos quais ritonavir é um inibidor potente. A fim de evitar a toxicidade da Rifabutina, recomenda-se a

redução da dose deste medicamento quando administrado concomitantemente com IPs. O regime recomendado de forma geral é 150mg de Rifabutina/dia ou 300mg em dias alternados. Porém, para que a dose de Rifabutina seja efetiva, é preciso uma boa adesão à TARV (CDC, 2000).

Em 2012, o Ministério da Saúde emitiu uma Nota Técnica a respeito da TARV, na qual disponibilizava a Rifabutina para que pudesse ser mantida a TARV de resgate com IPs em pacientes falhados, oferecendo opções terapêuticas com rifamicinas às PVHA, sendo recomendada nestas situações na dose de 150mg/dia, tanto na fase intensiva quanto na fase de manutenção (DEPARTAMENTO DE DST /AIDS E HEPATITES VIRAIS, 2012).

1.4- Tratamento Antirretroviral

O uso da TARV mudou a história natural da infecção por HIV ao promover redução significativa da carga viral (CV) e, consequentemente, de doenças oportunistas, melhorar a qualidade de vida e sobrevida dos pacientes (QUINN, 2008; REIS et al., 2011). Inicialmente, em 1987 os antirretrovirais (ARV) ofereciam apenas ganhos temporários, quando usados em monoterapia ou terapia dupla, devido à baixa eficiência na atividade antirretroviral e recuperação limitada da competência imunológica do paciente. Em 1987 foi disponibilizado o AZT (Zidovudina), um inibidor da transcriptase reversa análogo do nucleosídeo (ITRN), tornando-se o primeiro medicamento para a AIDS. Em seguida outros fármacos foram sintetizados: didanosina (DDI) e lamivudina (3TC). Inibidores da transcriptase reversa análogos do nucleosídeo (ITRNNs) foram desenvolvidos, tais como o efavirenz e nevirapina sendo incluídos no esquema terapêutico recomendado. Em 1996, novas drogas ARVs foram introduzidas, representadas pelos IPs, aumentando as opções de tratamento (HOARE, 2003). A combinação dessas novas classes de drogas (usualmente 2 ITRNs + ITRNN ou 2 ITRNs + IP) passou a ser chamada Terapia Antirretroviral Altamente Ativa, aqui denominado TARV.

A partir de meados dos anos 1990 a utilização de novas classes de drogas ARVs (ITRNNs e IPs) provara sua eficácia em PVHA mesmo nos casos de imunossupressão avançada. Uma significativa queda na morbidade e mortalidade por AIDS foram relatadas (DELPIERRE et al., 2008; PALELLA; LOVELESS; HOLMBERG, 1998). O uso da TARV no tratamento de PVHA e a profilaxia de infecções oportunistas

reduziram as mortes relacionadas à AIDS em vários países, incluindo o Brasil (MATIDA et al., 2005; SCHMALTZ et al., 2012a).

O efavirenz é o antirretroviral mais utilizado atualmente no tratamento de PVHA, principalmente em pacientes com TB. Existem poucas alternativas de tratamento neste cenário, o que complica o tratamento da infecção por HIV em caso de intolerância ou resistência ao efavirenz. Ainda em meados de 1990 uma nova classe, os Inibidores de Integrase (IIs), como raltegravir e posteriormente o dolutegravir se mostraram alternativas para o uso de IP. Um estudo, Reflat TB I, de 2014 mostrou que raltegravir 400mg duas vezes ao dia era uma alternativa ao efavirenz para o tratamento de pacientes com TB-HIV (GRINSZTEJN et al., 2014a). Este mesmo grupo, no estudo de Reflat TB II, concluiu que o raltegravir era inferior ao efavirenz em termos de eficácia (NATHALIE DE CASTRO, 2019).

Apesar de ser uma droga muito bem tolerada, o raltegravir possui baixa barreira genética (isso é, apenas uma única mutação já confere resistência ao fármaco) e não deve ser utilizado em doentes com resistência ao efavirenz (BEALE; ROBINSON, 2000; REINKE; STEFFEN; ROBINSON, 2001). Já o dolutegravir, um II de segunda geração, possui elevada barreira genética, sendo necessárias várias mutações para que haja resistência virológica a esse fármaco. Dolutegravir demonstrou grande potência contra HIV e um perfil de segurança extremamente atraente, que permite o uso uma vez ao dia, com poucos eventos adversos em indivíduos que iniciam TARV (GARVEY et al., 2008; MIN et al., 2010).

Recentemente um estudo foi publicado mostrando que entre PVHA que recebem tratamento para TB com rifampicina, o uso de dolutegravir duas vezes ao dia foi considerado eficaz e bem tolerado pelos pacientes (DOOLEY et al., 2019). Sendo assim, em 2019, o uso do dolutegravir foi ampliado para utilização no Sistema Único de Saúde (CONITEC, 2019) e recomendado como segunda escolha para tratamento da TB em PVHA. O efavirenz foi mantido como primeira opção devido a facilidade posológica (dose fixa combinada uma vez ao dia), o que aumenta a adesão. Entretanto, para se usar efavirenz é necessário uma genotipagem previa, devido à grande prevalência de resistência primária. Caso não seja possível fazê-la a tempo para a introdução precoce do TARV, o dolutegravir está indicado.

Pacientes que apresentavam resistência a ITRNNs, antes do advento do dolutegravir, precisavam usar IPs. Aqueles que precisavam tratar tuberculose

concomitantemente tinham como opção não usar rifamicina no esquema, usar rifampicina caso o IP usado fosse o lopinavir com booster de ritonavir (nesse caso dobrando a dose dos IPs) ou usar rifabutina (150 mg/dia). Atualmente, pacientes que já usaram diversos esquemas antirretrovirais, que acumularam muitas mutações, muitas vezes não podem deixar de usar IP para tratamento do HIV. Tendo em vista a descontinuação do lopinavir pelo Ministério da Saúde, a única rifamicina que pode ser usada no tratamento da tuberculose nesses pacientes é a rifabutina.

A adesão dos pacientes à TARV é um fator essencial para o sucesso do tratamento e aumento da sobrevida. Entretanto, muitos fatores contribuem para uma baixa adesão tais como: longa duração do tratamento, o número de pílulas e as reações adversas associadas às drogas em uso, as condições de vida da pessoa e o acesso ao tratamento nos serviços de saúde (MELCHIOR et al., 2007). Ainda assim, a introdução da TARV levou ao aumento da sobrevida, melhora da qualidade de vida e mudança da percepção a respeito desse agravo (PALELLA; LOVELESS; HOLMBERG, 1998; SEPKOWITZ, 2001).

1.5- Sobrevida

Entre os pacientes com TB-HIV a taxa de mortalidade é mais alta nos primeiros três meses após o início do tratamento anti-TB (ELLIOTT et al., 1995; NUNN et al., 1992; WORODRIA et al., 2011; ZACHARIAH et al., 2007).

No Brasil, o tempo médio de sobrevida de pacientes após o diagnóstico de AIDS tem aumentado significativamente. Nas décadas de 80 e 90, a mediana de sobrevida desses pacientes era de cinco meses (CHEQUER et al., 1992), em 1995 e 1996 esta mediana aumentou para 18 e para 58 meses respectivamente (MARINS et al., 2003). Um estudo publicado em 2002 descreveu uma mediana de sobrevida de 7 anos em indivíduos infectados por HIV, a partir do diagnóstico de AIDS, atendidos no INI- Fiocruz entre os anos de 1997 e 1999 (GADELHA et al., 2002).

Em um estudo no qual o tempo de sobrevivência de PVHA foi investigado, os autores concluíram que a TB foi um dos fatores mais importantes para a progressão para AIDS e a TARV foi considerada como uma medida eficaz para suprimir a replicação de HIV e melhorar a sobrevida de PVHA (MIRZAEI et al., 2013).

As taxas de letalidade são notoriamente altas em pacientes co-infectados por TB/HIV (22,4% a 67%) (DURO et al., 2017; ERBES, 2006; SILVA et al., 2010). Nestes

pacientes a mortalidade está relacionada com alguns preditores como, apresentação tardia aos serviços de saúde, pacientes com TB e AIDS em estágios avançados, diagnóstico tardio da TB, contagem baixa de CD4 no início do tratamento, ocorrência de TB multidroga resistente, episódios simultâneos de outras complicações relacionadas ao HIV e falta de acesso a TARV (AKKSILP et al., 2007; GADKOWSKI et al., 2009; MANOSUTHI et al., 2006a; MOORE et al., 2007; SCHMALTZ et al., 2012a). Em estudo conduzido no INI-Fiocruz, foi observado que há diferentes preditores de mortalidade para pacientes com TB/HIV virgens de TARV e aqueles previamente experimentados. Uma das conclusões deste estudo foi que a demora em diagnosticar TB em pacientes que faziam uso prévio da TARV era significativamente associada a maior mortalidade (SCHMALTZ et al., 2012a).

Apesar da considerável redução da incidência de TB entre PVHA tratadas com TARV, a TB continua sendo a principal causa de morte entre as PVHA (WORLD HEALTH ORGANIZATION, 2019) e a adesão dos pacientes à TARV tornou-se um fator essencial para o sucesso do tratamento e aumento da sobrevida (MELCHIOR et al., 2007).

Diversos estudos mostraram que o uso de TARV durante o tratamento da tuberculose diminuiu a mortalidade de pacientes com TB/HIV (CARVALHO et al., 2002; GIRARDI et al., 2001; OLALLA, PG et al., 2002; SCHMALTZ et al., 2012a). Além do uso concomitante, o início precoce da TARV (duas semanas após início do tratamento de TB), melhora significativamente a sobrevivência das PVHA com CD4 muito baixo (ABDOOL KARIM et al., 2010; BLANC et al., 2011a).

1.6- Anemia

A anemia afeta cerca de um quarto da população mundial, afetando a saúde dos pacientes e comprometendo o desenvolvimento social e econômico da população. As causas multifatoriais podem contribuir, individualmente ou em grupo, para a anemia, entre essas causas estão as doenças infecciosas como a TB (BALARAJAN et al., 2011).

Anemia pode ser definida como níveis de hemoglobina (Hb) abaixo de 12,0 g/dL para mulheres e 13,5 g/dL para homens (WORLD HEALTH ORGANIZATION, 2017).

A anemia pode ter diversas causas, incluindo deficiência de ferro, carências vitamínicas e inflamação crônica. A anemia é comum no momento do diagnóstico da TB, com estimativas de prevalência variando entre 32% a 96%, dependendo da localização, desenho do estudo e população estudada (BARZEGARI et al., 2019). Comumente pacientes com TB ativa apresentam níveis baixos de Hb, o que pode afetar diretamente a morbidade associada à TB (ISANAKA et al., 2012; LEE et al., 2006; SAHIRATMADJA et al., 2007). Os níveis de Hb tendem a diminuir à medida que a positividade da baciloscopia aumenta (SAATHOFF et al., 2011) e a presença de anemia no diagnóstico da TB foi associada à negatividade tardia da baciloscopia (AGRAWAL, 2017; NAGU et al., 2014). Também já foi associada a um maior risco de letalidade em pacientes com TB (ISANAKA et al., 2012; KWON et al., 2014), sua prevalência foi significativamente maior em pacientes TB-HIV em comparação com aqueles soronegativos para HIV (ABAY et al., 2018; BACELO et al., 2015; HELLA et al., 2018; ISANAKA et al., 2012; SAATHOFF et al., 2011; VAN LETTOW et al., 2005)

Em pacientes com TB, a anemia também foi atribuída a inflamação crônica (GIL-SANTANA et al., 2019). Anemia também está relacionada à progressão acelerada da doença em PVHA (MOCROFT et al., 1999).

Mais recentemente, uma investigação em PVHA virgens de TARV relatou que a anemia e inflamação sistêmica foi associada a maior risco de falha da TARV (SHIVAKOTI et al., 2015), uma possível explicação para a associação entre anemia e desfechos de tratamento desfavoráveis é que as baixas concentrações de Hb refletem um avanço da doença de base. Em outro estudo recente realizado pelo grupo do LAPCLINTB (dados não publicados) a anemia foi associada a gravidade de TB: histórico de perda de peso maior que 10%, hospitalizações e coinfecção com HIV. Sugerindo assim, que a anemia poderia ser usada como um preditor de gravidade da TB, uma vez que o exame de hemograma é um teste simples, barato e disponível no mundo todo (VALERIA CAVALCANTI ROLLA, 2019)

Porém ainda não foi investigada a relação entre anemia e inflamação sistêmica no contexto do ATT em PVHA e se a recuperação da anemia durante a ATT está relacionada à melhora do prognóstico.

1.7- Objetivos

1.7.1. Objetivos Artigo 1

Analisar a efetividade dos diferentes regimes terapêuticos usados para pacientes com TB-HIV.

Comparar a efetividade da TARV em pacientes VT e nos PE ao TARV.

Avaliar a mortalidade precoce (100 dias) após início do tratamento de TB de acordo com os regimes ARVs utilizados.

1.7.2. Objetivos Artigo 2

Avaliar a trajetória e caracterizar a dinâmica dos níveis de Hb em PVHA com TB ao longo do tratamento.

Examinar as relações entre anemia e distúrbio inflamatório sistêmico em PVHA co-infectadas com TB.

Analisar a associação entre anemia persistente e desfechos clínicos desfavoráveis do tratamento de pacientes com TB-HIV.

2 DESENVOLVIMENTO

2.1. Artigo 1

Fernanda de Oliveira Demitto Tamogami

PREDICTORS OF EARLY MORTALITY AND EFFECTIVENESS OF ANTIRETROVIRAL THERAPY IN TB-HIV PATIENTS FROM BRAZIL

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Predictors of Early Mortality and Effectiveness of Antiretroviral Therapy in TB-HIV patients from Brazil

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Short title: Prediction of ART effectiveness in TB-HIV patients from Brazil.

2.1.1- Abstract

The implementation of antiretroviral (ARV) therapy caused a significant decrease in HIV-associated mortality worldwide. Nevertheless, mortality is still high among people living with HIV/AIDS and tuberculosis (TB). In countries where ARVs are in current use, some TB-HIV patients are already failing the first line efavirenz-based regimen and seem to have different response to second line therapy and predictors of mortality. We performed a retrospective cohort study including 273 patients diagnosed with TB-HIV and treated at a referral center in Rio de Janeiro, Brazil, between 2008 and 2016. Multivariate analysis and Cox regression models were used to evaluate the effectiveness of ARV therapy regimens (VL<80 copies from the 4^{th} to 10^{th} months after TB therapy introduction) and to identify predictors of early mortality (100 days after TB therapy initiation) considering ARV-naïve and ARV-experienced patients adjusting for sociodemographic, clinical and therapeutic covariates. Survival analysis included 273 patients, out of whom 154 (56.4%) were ARV-naïve and 119 (43.6%) were ARV-experienced. Seven deaths occurred within 6 months of anti-TB treatment, 4 in ARV-naïve and 3 in ARV-experienced patients. Multivariate analysis revealed that in ARV-naïve patients, the chance of death was substantially higher in patients who developed immune reconstitution inflammatory syndrome during the study follow up (HR=40.6, p<0.01). For ARV-experienced patients, similar analyses failed to identify factors significantly associated with mortality. Variables independently associated with treatment failure for the ARV-naïve group were previous TB (adjusted OR [aOR]=6.1 p=0.03) and alcohol abuse (aOR=3.7 p=0.01). For ARV-experienced patients, a ritonavir boosted. Protease Inhibitor-based regimen resulted in a 2.6 times higher risk of treatment failure compared to the use of efavirenz based ARV regimens (p=0.03) and High baseline HIV VL (p=0.03) were predictors of treatment failure. Risk factors for mortality and ARV failure were different for ARV-naïve and ARV-experienced patients. The latter patient group should be targeted for trials with less toxic and rifampicin-compatible drugs to improve TB-HIV treatment outcomes and prevent death.

2.1.2- Introduction

Antiretroviral (ARV) therapy was one of the greatest achievements in medicine of the last decade due to the significant decrease in HIV-associated mortality, most significantly observed in low and high-developed countries [1,2]. Since 1986, the Brazilian Ministry of Health offers antiretrovirals free of charge, as well as assessment of CD4+ lymphocyte counts, HIV viral load (VL) and more recently genotyping, to all patients in the public health system [3]. However, although this policy has been implemented in Brazil and other high burden of tuberculosis (TB) countries, mortality is still high among people living with HIV/AIDS and TB. One of the reported reasons for this scenario is the loss of follow up of patients after diagnosis of HIV infection, with patients being reluctant to initiate ARV due to misinformation and/or awareness about the benefits of this therapy [4] in addition to toxicity of TB-HIV concomitant therapy [5].

In 2009, Sant'Anna et al. conducted a study in TB-HIV patients to evaluate the HIV VL control after ARV therapy implementation in ARV-naïve (those persons who have never received ARV before) and ARV-experienced patients (those who have used ARV regimens previously) from Rio de Janeiro, Brazil [6]. The authors found that, for ARV-naïve patients, the best results were achieved with efavirenz-based regimens. However, for ARV-experienced patients, the effectiveness was lower than that found in naïve patients, and efavirenz based regimens were not effective, which was attributed to probable acquired drug resistance [6]. Additional studies in this population revealed that ARV regimens containing a Protease Inhibitor (PI) boosted with ritonavir were associated with better virologic control but also linked to increased incidence of severe adverse reactions [7]. Recently, Rifabutin incorporation in the Brazilian HIV program [8] brought some progress in TB-HIV treatment, increasing the possibilities of concomitant ARV regimens. ARV-experienced patients have a few options of effective ARV drugs that usually cannot be used with rifampicin. Therefore, treatment outcomes in ARV-experienced patients receiving therapy for TB and AIDS could be improved with Rifabutin [8]. Moreover, new ARVs were incorporated in the Brazilian HIV guidelines, such as the PI Darunavir and a new class of Integrase Inhibitors (II) (Raltegravir), which although not yet broadly used, could also positively influence ARV effectiveness [9].

Another study conducted by our group [10] has previously shown that the predictors of early mortality in ARV-naïve or ARV-experienced patients with TB

diagnosis appear to be different. Among ARV-naïve patients, mortality was influenced by TB severity and no ARV use during TB treatment, possibly because of a late presentation for medical assistance. For ARVexperienced patients, delays in TB diagnosis for more than 120 days as well as poor control of viral replication were associated with higher mortality rates [10].

Although several previous studies have explored different aspects of clinical and pharmacological management of TB-HIV patients, scarce data is available on effectiveness of ARV therapy in patients undergoing TB treatment, and mainly in ARV-experienced patients, especially in Brazil. Since more ARVs are becoming available in most of the TB high burden countries, the number of patients with TB who will be already on ARV therapy might increase. Therefore, it is critical to evaluate the effectiveness of ARV regimens used during TB treatment and risk factors associated with early mortality in TB-HIV patients, not only in ARV-naïve but also considering previous use of ARVs. Although no differences in mortality rate has been shown so far between ARV-naïve and ARV-experienced patients, different predictors of mortality may drive implementation of distinct approaches in clinical management of these groups. In the present study, we explored this matter in a population from a high burden area of TB-HIV in Brazil.

2.1.3- Methods

Ethics Statement

The study was approved by the Institutional Review Board of the Instituto Nacional de Infectologia Evandro Chagas (CAAE: 71191417.8.0000.5262). Written informed consent was obtained from all participants, and all clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

Population and Design

A retrospective cohort study was conducted at the Clinical Research Laboratory on Mycobacteria (LAPCLIN-TB) of the Instituto Nacional de Infectologia (INI) Evandro Chagas, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil, between 2008 and 2016. Patients with 18 years and older, HIV-seropositive, with clinical signs and symptoms of TB with or without a positive culture of any site (pulmonary or extrapulmonary) were included. For those who had a negative culture, a positive therapeutic test with TB

drugs was considered, after excluding other opportunistic diseases for differential diagnosis. Patients that initiated TB treatment and were diagnosed later with Non-tuberculous Mycobacteria as well as those who showed rifampicin and isoniazid resistance (multidrug resistance) were excluded.

Definitions

TB was classified as pleuropulmonary (when restricted to the lungs and/or pleura), extrapulmonary (when just one extra-pulmonary site was identified) or disseminated (involving spleen, liver, bone marrow, or at least 2 noncontiguous sites) [11].

Comorbidity is when existence two or more diseases simultaneously in the same person, such as diabetes, chronic obstructive pulmonary disease, hepatitis B and C, hypertension, amongst others.

TB-associated immune reconstitution inflammatory syndrome (IRIS) was defined as a documented worsening of TB signs or symptoms during antituberculous treatment and following the initiation of ARV, not explained by any other diseases or by an adverse effect of drug therapy, as described previously [12,13]. Suggestive histopathologic findings of a lymph node biopsy and/or an improvement of CD4+ cell counts after appearance of the clinical signs of IRIS corroborated the diagnosis in some patients. If a biopsy was not available, lymph node enlargement with inflammatory signs temporally related with ARV introduction was considered IRIS.

The cause of death was determined after thorough review of relevant clinical, microbiological and pathological data of each deceased patient. Only deaths due to TB were analyzed. Early deaths were defined as deaths in the first 100 days of TB therapy. ARV effectiveness was defined as HIV VL ≤ 80 copies/mL between months 4 to 10 after TB therapy initiation.

TB diagnosis and follow up visits

Visits included in this study were done at baseline (TB diagnosis and treatment initiation), 15 days and 30, 60, 90, 120 e 180 days after TB therapy initiation. ARV were initiated after TB treatment according to decision from each physician and following the Brazilian TB treatment Guidelines [14].

Information collected at the baseline visit included sociodemographic data as well as previous TB and ARV treatments, clinical presentation of TB, comorbidities like

diabetes, hypertension, hepatitis (B and C), opportunistic diseases as well as CD4 counts and HIV VL.

Antituberculous and Antiretroviral Therapy

ARV therapy was offered according to contemporary Brazilian National Guidelines that were periodically updated [14].

The first line antituberculous regimen was the combination of rifampicin, isoniazid and pyrazinamide during the two initial months, followed by rifampicin and isoniazid during four months, except when the continuation phase needed to be extended to seven months such as in cases with central nervous system TB. From July 2009 on, ethambutol was added to the intensive phase regimen following a new recommendation of the National TB program of the Brazilian Ministry of Health [15]. TB treatment was adjusted in cases of severe adverse reactions, drug resistance and ARV regimens that precluded the use of rifampicin.

Statistical Analysis

Descriptive statistics was used to present data, which was presented as proportions, mean ± standard deviation (SD), or median and interquartile range (IQR), depending on Gaussian distribution assessed by the Kolmogorov-Smirvov test. The Fisher's and Chi-square tests were used to compare categorical variables between study groups. Continuous variables were compared using the Mann-Whitney *U* test. Bivariate analysis was used to describe the association with socio-demographic and clinical variables from baseline exposure to ARV. The first outcome was assessed based on the following status: patients who did not die from TB had their follow-up censored on the 100^{th} day after the start of antituberculous therapy. Overall survival was assessed using the Kaplan-Meier (KM) method. We used Cox proportional hazards regression models to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). A multivariate regression model was used to evaluate the effectiveness of ARV considering ARV-naïve and ARV-experienced. Variables with univariate p-value ≤ 0.2 were selected to the multivariate model to assess the odds ratios (OR). The analyzes were performed using SPSS version 24.0 software package. All analyses were pre-specified. Differences with two-tailed p-values <0.05 were considered statistically significant.

2.1.4- Results

Cohort description

During the period from 2008 to 2016, 285 patients were screened, 11 patients were excluded because they were diagnosed with atypical Mycobacteria and one with multidrug resistant TB.

Survival analysis included 273 patients, out of whom 154 (56.4%) were ARV-naïve and 119 (43.6%) were ARV-experienced. Seven early deaths were observed, 4 in ARV-naïve patients and 3 in ARV-experienced. There were 12 cases of TB-associated IRIS, among which 4 (33.34%) were pulmonary, 1 (8.33%) was extrapulmonary and 7 (58.33%) were disseminated TB. Eight (5.2%) ARV-naïve and 4 (3.4%) ARV-experienced patients developed IRIS (Table 1). Eleven IRIS cases occurred in patients using nucleoside reverse transcriptase inhibitors (NRTI) + non-nucleoside reverse transcriptase inhibitors (NNRTI) and 2 cases in those who used NRTI +PI during TB treatment. None of the IRIS cases had resistance to first line TB drugs. For the effectiveness analysis, 186 patients with HIV VL results available at the end of TB treatment were included, 116 (62.4%) were ARV-naïve and 70 (37.6%) were ARV-experienced. By the end of follow up, 127 (68.3%) patients had VL ≤ 80 copies/mL, with 87 (68.5%) in the ARV-naïve group and 40 (31.5%) in the ARV-experienced group. In addition, 59 (31.7%) had VL > 80 copies/mL, 29 (49.2%) in the ARV-naïve group and 30 (50.8%) in the ARV-experienced. Thus, effectiveness of ARV therapy was significantly higher in the group of ARV-naïve individuals (odds ratio [OR] 2.1, 95% CI: 1.1–4.0, p = 0.02).

Baseline characteristics of both groups are shown in Table 1. Comorbidities and previous TB were more frequent in the ARV-experienced group, while loss of more than 10% of body weight, HIV VL ≥5 log and disseminated clinical forms of TB were more frequent in the ARV-naïve group. Positive blood cultures were more frequent in the ARV-naïve group while ARV-experienced individuals exhibited a trend to have more monoresistance to TB drugs.

In ARV-naïve patients, the univariate model (Table 2) showed that the risk of dying was higher in patients who developed IRIS after commencement of ARV therapy (hazard ratio [HR] = 6.8, 95% CI: 1.3–35.1, p = 0.005). The median time between onset of ARV and time at which IRIS occurred was 84 days (IQR 27–152). The median time

between ARV onset and HIV VL drop before IRIS onset was 50.5 days (IQR 16-5-249). In addition, increases of 1 log in baseline HIV VL values had a tendency to exhibit augmented risk of early death (≤ 100 days after therapy initiation) as well, however, without reaching statistical significance (Table 2). A multivariate regression analysis model confirmed association between occurrence or IRIS and increased risk of early death (Table 2). For ARV-experienced patients, univariate and multivariate analyses failed to reveal risk factors significantly associated with early death (Table 3).

ARV effectiveness

The response to ARV were analyzed considering if patients were ARV-naïve or ARV-experienced. In univariate Cox regression model (Table 4) alcohol abuse ($p<0.01$), viral hepatitis ($p=0.02$) and previous TB ($p=0.02$) were factors associated with ARV failure for the ARV-naïve group. In the multivariate model, variables associated with a worst effectiveness were previous TB ($p=0.03$), alcohol abuse ($p=0.01$) and a borderline significance for viral hepatitis ($p=0.06$).

The risks factors associated with treatment failure for ARV-experienced patients in the univariate analysis were baseline HIV VL (log10) and ARV treatment with PI-based regimens. In the multivariate analysis, patients treated with a ritonavir boosted PI had 2.6 times higher risk of treatment failure compared to the use of other regimens and the other factor associated with treatment failure was baseline VL (log10). No other risk factors were identified in both groups (Table 5).

2.1.4- Tables

Table 1. Baseline characteristics of the study participants stratified according to exposure to antiretroviral therapy at enrollment.

Characteristic	Study population (N = 273)		p-value
	ARV-naïve	ARV-experienced	
	n = 154 (56.4%)	n = 119 (43.6%)	
Male	114 (74.0)	81 (68.1)	0.3
Race: white	69 (44.8)	36 (30.3)	0.1
Age z 40 years	67 (43.5)	52 (43.7)	1.0
Educational level			1.0
≥ 5 years of schooling	93 (60.4)	72 (60.5)	
< 5 years of schooling	60 (39.6)	47 (39.5)	
Family income			0.7
> 2 Minimal wage	26 (18.2)	17 (16.0)	
≤ 2 Minimal wage	117 (81.8)	89 (84.0)	
Not married status	105 (68.2)	81 (68.1)	1.0
Homo/bisexual	54 (35.5)	36 (30.3)	0.4
Smoker	80 (52.3)	62 (53.4)	0.9
Alcohol abuse	48 (31.6)	41 (34.5)	0.7
Use of illicit drugs	40 (26.1)	37 (31.4)	0.3
Comorbidity	44 (28.6)	50 (42.0)	0.02
Viral hepatitis (B or C)	12 (8.4)	14 (12.0)	0.4
Weight loss (>10%)	130 (85.0)	75 (63.0)	<0.01
Previous tuberculosis	11 (7.1)	54 (45.4)	<0.01
Positive hemoculture for *M. tuberculosis*	14 (15.6)	5 (5.8)	0.05
Resistant to any TB drug	19 (22.1)	7 (10.9)	0.08
Baseline VL ≥ 5 log	95 (70.4)	28 (29.2)	<0.01
Clinical form of tuberculosis			0.006
Pleuropulmonary	79 (51.3)	76 (63.9)	
Extrapulmonary	15 (9.7)	18 (15.1)	
Disseminated	60 (39.0)	25 (21.0)	
Baseline $CD4^+$ cell count median (IQR)	169 (129–195)	205 (134–278)	0.17
Paradoxical Reaction (IRIS)			0.56
Yes	8 (5.2)	4 (3.4)	
No	145 (94.8)	114 (96.6)	
ARV use before TB			-
NRTI+NNRTI	NA	39(32.8)	
NRTI+PI	NA	35 (29.4)	
NRTI+II	NA	2 (1.7)	
NRTI +PI+II	NA	1 (0.8)	
NRTI	NA	1 (0.8)	
ARV use after TB			<0.01
NRTI+NNRTI	125 (94.0)	47 (42.0)	
NRTI+PI	7 (5.3)	57 (50.9)	
NRTI+II	1 (0.8)	4 (3.6)	
NRTI +PI+II	0 (0.0)	3 (2.7)	
NRTI	0 (0.0)	1 (0.9)	

p-value based on Chi-squared test; NA, nonapplicable; ARV = antiretroviral
NRTI = nucleoside reverse transcriptase inhibitors; NNRTI = non-nucleoside reverse transcriptase inhibitors; PI = protease inhibitors; II = integrase inhibitors

Table 2. Cox analysis for early mortality (100 days) for ARV-naïve patients.

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	HR adjusted	95% CI	p-value
IRIS	6.8	(1.3–35.1)	0.005	40.6	(5.1–320.8)	<0.01
Baseline HIV VL (log10)	3.6	(0.8–16.8)	0.11	5.4	(0.9–33.0)	0.07

Only IRIS and baseline HIV VL had univariate p-values ≤ 0.2 and thus were selected to be used in the multivariate model. HR: Hazard ratio; CI: confidence interval;
IRIS = Paradoxical reaction

Table 3. Cox analysis for early mortality (100 days) for ARV-experienced patients.

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	HR adjusted	95% CI	p-value
Age (years)	1.1	(0.9–1.2)	0.08	1.1	(1.0–1.3)	0.09
Extrapulmonary TB	6.6	(0.9–47.2)	0.06	2.8	(0.2–35.3)	0.43
Baseline HIV VL ≥ 5 log	4.7	(0.4–51.6)	0.11	6.5	(0.5–86.3)	0.16

Only age, extrapulmonary TB and baseline HIV VL ≥ 5 log had univariate p-values < 0.2 and thus were selected to be used in the multivariate model. HR: Hazard ratio; CI: confidence interval

Table 4. Factors associated with antiretroviral therapy failure for ARV-naïve patients.

Variable	Univariate analysis			Multivariate analysis		
	OR	95% CI	p-value	OR adjusted	95% CI	p-value
Alcohol abuse	5.2	(2.1–12.8)	<0.01	3.7	(1.3–10.0)	0.01
Viral hepatitis (B or C)	6.2	(1.4–28.0)	0.02	5.0	(1.0–25.9)	0.06
Previous tuberculosis	5.8	(1.3–26.2)	0.02	6.1	(1.2–31.3)	0.03

OR: Odds ratio; CIs: confidence intervals

Table 5. Factors associated with ARV failure for ARV-experienced patients.

Variable	Univariate analysis			Multivariate analysis		
	OR	95% CI	p-value	OR adjusted	95% CI	p-value
Baseline HIV VL (log10)	1.6	(1.1–2.2)	0.009	1.5	(1.0–2.1)	0.03
Protease Inhibitor based regimens	4.9	(1.7–13.8)	0.003	3.6	(1.1–11.1)	0.03

OR = Odds ratio; CI = confidence intervals; VL = viral load

2.1.5- Discussion

Tuberculosis in HIV-infected patients is still a challenge due to a significant number of deaths concentrated in the first months of TB treatment, toxicity and interactions of TB and ARV drugs in addition to IRIS, which has been described worldwide [16].

In our study, ARV-naïve and ARV-experienced patients were different with regard to several characteristics. ARV-naïve patients had more B or C hepatitis, higher HIV VL at baseline, more disseminated TB with positive blood cultures, significant weight loss and more TB drug resistance. ARV-experienced patients had more frequently previous TB and exhibited more comorbidities. Furthermore, additional analyses on early survival demonstrated that IRIS and high baseline HIV VL were risk factors for early mortality in ARV-naïve but not for ARV experienced patients. Similarly to our results, the Camelia study [17] reported that even with early implementation of ARV therapy, no deaths associated with IRIS were observed, including in ARV-naïve TB-HIV patients. In addition, lack of ARV therapy in TB-HIV patients was a risk factor

in previous study [10] that was not detected in our recent analysis, probably due to the extensive use of ARV in our current cohort.

In previous studies from our group, a delay of more than 120 days from presentation of symptoms to TB diagnosis was linked to increased early mortality of ARV-experienced patients [10]. Such delay occurred in circumstances where a patient had a negative screening for acid-fast bacilli in sputum smears and the assistant physician decided to wait for a positive mycobacterial culture to start TB treatment, for example [10]. In recent years, Xpert -MTB-RIF has become available and a rapid TB screening has been done more frequently [18]; this could have contributed to a faster TB diagnosis in patients living with HIV, decreasing the impact of late TB treatment start in these patients mortality although the median time to diagnosis have not changed.

Noteworthy, our study revealed that occurrence of IRIS was an important risk factor for mortality in ARV-naïve patients, although in previous years such relationship had not been found [10]. In Brazil, a low incidence of IRIS in TB-HIV patients has been reported [19]. We have been working to better understand this phenomenon but the incidence rate have not changed in recent years in our site [20], differently from other countries in the south hemisphere with rates ranging from 22 to 36% [21–23].

Herein, we also analyzed the risk factors for poor response to ARV after TB diagnosis and, again, they were distinct for the two groups of patients differentiated based on ARV exposure. It is possible that there was a higher risk of hepatotoxicity associated with anti-TB and ARV drugs in patients already with alcohol or viral-induced hepatic injury. For ARV-experienced patients, a higher risk of treatment failure was observed in those with higher baseline HIV VL values and those treated with PIs, in other words, those patients with resistance to first line ARV (efavirenz-based regimens). For that population, the options are use lopinavir boosted with ritonavir (800–200 mg or 400–400 mg) and keep the anti-TB drugs in a fixed dose [24,25] or deconstruction of TB fixed dose combination to use Rifabutin (for those who have it available in the public health units) concomitant to others PIs [8]. Our previous study also revealed that patients treated with PI (in both situations described above) had 3.08 times more risk of treatment failure compared with patients using efavirenz-based ARV regimens [6]. Ritonavir boosted PI regimens are toxic and when added to

antitubercular drugs may result in interruption of both therapies; no other ARV regimen compatible with rifampicin is recommended in Brazil for instance.

However, some patients develop TB using efavirenz with undetectable HIV VL, and if it turns out to be a case which is sensitive to this drug, one could keep the same regimen and have lower risk of treatment failure. A new class of ARV, the integrase inhibitors, is being used in some countries and was recently incorporated in Brazil [9]. Raltegravir has been recently studied in an Agence Nationale de Rechèrche sur le SIDA (ANRS) clinical trial, which demonstrated that a regimen including this drug is as effective as efavirenz based regimens to treat TB-HIV ARV-naïve patients [26]. Raltegravir is a very well tolerated drug but has a low genetic barrier (needs few mutations to acquire resistance) and should not be used in patients with resistance to efavirenz. Another integrase inhibitor recently introduced in Brazil was Dolutegravir [9], which has a good genetic barrier and could be the treatment of choice for TB-HIV patients with resistance to efavirenz. A recent trial reported promising results in naïve patients with very few adverse reactions [27], this drug was already recommended by the WHO, recently [16]. We urgently need better therapies for TB-HIV patients to improve treatment effectiveness and survival. ARV-experienced patients should be a focus of new studies as well as identification of risk factors for mortality and treatment failure to allow effective interventions.

Our study had some limitations, such as the low number of participants in each group and the proportion of patients for whom HIV VL results were available to achieve effectiveness of ARV. Moreover, adherence to treatment was self-reported, and we have not considered due to the low level of reliability. If patients had not taken their drugs correctly, effectiveness of ARVs could have been harmed and we would not have been able to annotate reliably. Another limitation was the low number of IRIS cases that was not enough to explore subgroup analyzes. Regardless, the present study adds to the current knowledge in the field TB-HIV therapies as it demonstrates that risk factors for mortality and ARV failure were different for ARV-naïve and ARV-experienced patients and latter group should be targeted for trials with less toxic rifampicin-compatible drugs to improve TB-HIV treatment outcomes in high burden TB and HIV countries.

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2.2- Artigo 2

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IMPACT OF PERSISTENT ANEMIA ON SYSTEMIC INFLAMATION AND TUBERCULOSIS OUTCOMES IN PERSONS LIVING WITH HIV

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Impact of Persistent Anemia on Systemic Inflammation and Tuberculosis Outcomes in Persons Living with HIV

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2.2.1- Abstract

Tuberculosis (TB) is associated with systemic inflammation and anemia, which are aggravated in persons living with HIV (PLWH). Here, we characterized the dynamics of hemoglobin levels in PLWH coinfected with TB undergoing antitubercular therapy (ATT). We also examined the relationships between anemia and systemic inflammatory disturbance as well as the association between persistent anemia and unfavorable clinical outcomes. Here, data on several blood biochemical parameters and on blood cell counts were retrospectively analyzed in a cohort of 256 TB/HIV patients from Brazil during 180 days of ATT. Multidimensional statistical analyses were employed to profile systemic inflammation of patients stratified by anemia status (hemoglobin levels <12 g/dL for female and <13.5 g/dL for male individuals) prior to treatment and prediction of unfavorable outcomes, such as treatment failure, loss to follow up and death. Most of patients with anemia at pre-ATT persisted with such condition until day 180. Such individuals exhibited heightened degree of inflammatory perturbation (DIP), which in turn was inversely correlated with hemoglobin levels. Recovery from anemia was associated with increased pre-ATT albumin levels whereas persistent anemia was related to higher total protein levels in serum. Multivariable regression analysis revealed that lower baseline hemoglobin levels was the major determinant of the unfavorable outcomes. Our findings demonstrate that persistent anemia in PLWH during the course of ATT is closely related with chronic inflammatory perturbation. Early intervention to promote recovery from anemia may improve ATT outcomes.

2.2.2- Introduction

Tuberculosis (TB) remains as a leading cause of death from infection by a single pathogen and also among people living with human immunodeficiency virus (HIV) (1). Persons living with HIV (PLWH) exhibit up to 19 times higher risk of developing active TB (2). In addition, TB is one of the most common opportunistic infections in PLWH. In fact, a total of 1.5 million people died from TB in 2018, including 251,000 PLWH (1). Understanding the determinants of clinical outcomes of PLWH coinfected with TB is critical to improve patient care.

Anemia is also a global public health problem and is diagnosed based on concentration of hemoglobin (Hb), specifically when it falls below established cut-off values; 12.0 g/dL for women and 13.5 g/dL for men (3). Low concentrations of Hb are a frequent complication of both TB and HIV infections, and its occurrence is associated with increased morbidity and mortality (4). Several causes of anemia are described, including iron deficiency and chronic inflammation (5–7). Prevalence of anemia in TB patients is reported to range between 32 and 96% (8), whereas in PLWH, this estimate varies from 1.3 to 95% (4). The extreme discrepancies in frequency of anemia associated with either TB and/or HIV infections published by several studies are thought to be influenced by factors that include study design, geographic location as well as clinical and epidemiological characteristics of patients.

Many studies have associated anemia with poor prognosis and increased mortality after TB diagnosis (6, 7, 9). In patients with TB, anemia has been attributed to be caused by chronic inflammation (10). It has also been shown that anemia is related to accelerated HIV/AIDS disease progression in PLWH (11). This latter study concluded that Hb levels is a robust biomarker to predict death independent of CD4+ T-cell count and HIV viral load values (11). More recently, a prospective investigation of antiretroviral therapy (HAART)-naïve PLWH reported that concurrent anemia and systemic inflammation were associated with higher risk of HAART failure (12). A potential explanation for the association between anemia and poor outcomes in HIV/AIDS and/or TB is that low Hb concentrations reflect more advanced disease staging. It is still to be defined the relationship between anemia and systemic inflammation in the context of antitubercular treatment (ATT) in PLWH and whether recovery from anemia during ATT in PLWH is related to improved prognosis.

In a study from Brazil, we have recently described that risk factors for mortality were distinct between HAART-naïve and HAART-experienced PLWH patients coinfected with TB. Indeed, in HAART-naïve patients, but not in those who were already undertaking antiretrovirals, the odds of death were substantially higher in patients who developed immune reconstitution inflammatory syndrome (IRIS) during the study follow up (13). This finding suggests that inflammation during the course of ATT in PLWH is related to unfavorable outcomes. In the present study, we expanded our analyses to investigate the relationship between the presence and severity of anemia and the cellular and biochemical profile of systemic inflammation in PLWH and TB in Brazil. We also tested whether low levels of Hb measured at pre-ATT could be used to predict unfavorable outcomes.

2.2.3- Methods

Ethics Statement

The study was approved by the Institutional Review Board of the Instituto Nacional de Infectologia Evandro Chagas (INI) (CAAE: 71191417.8.0000.5262). Written informed consent was obtained from all participants, and all clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

Population and Design

A prospective cohort has been followed at the Clinical Research Laboratory on Mycobacteria (LAPCLIN-TB) of the INI Evandro Chagas, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil, since 2000. The present study is a retrospective assessment performed between 2008 and 2016, with data obtained from this cohort. Data were collected from electronic medical records based on standardized information of a defined template used in each patient's visit for the whole cohort. PLWH 18 years and older, with clinical signs and symptoms of TB were included. The diagnosis of TB was made when Mycobacterium tuberculosis (Mtb) detection was positive in any sample collected (acid fast bacilli smear, Gene Xpert or culture from clinical specimens). In cases without bacteriological confirmation, the diagnosis was established by suggestive imaging analysis, histopathological examination, together with clinical and epidemiological findings consistent with TB. For those who had a negative culture, a positive therapeutic test with TB drugs was considered, after excluding other

opportunistic diseases for differential diagnosis. Patients that initiated TB treatment and were diagnosed later with non-tuberculous mycobacteria as well as those who showed rifampicin and isoniazid resistance (multidrug resistance) were excluded. Patients with bone, mammary, renal or ocular TB were excluded, since these clinical forms can have very subtle, asymptomatic presentations, making it difficult to be compared to the other forms.

Definitions

Anemia was defined according to World Health Organization (WHO) guideline criteria: Hb value < 13.5 g/dL for men and < 12.0 g/dL for women.

Tuberculosis was classified as pleuropulmonary (when restricted to the lungs and/or pleura), extra-pulmonary (when just one extra-pulmonary site was identified) or disseminated (involving spleen, liver, bone marrow, or at least 2 non-contiguous sites).

Discharge due to cure, with or without etiologic confirmation of the diagnosis of TB, was considered a favorable outcome. Patients were defined as cured through clinical and/or radiologic improvement. Unfavorable outcome was defined as death, loss to follow up and treatment failure following the WHO guidelines. The cause of death was determined after thorough review of relevant clinical, microbiological and pathological data of each deceased patient.

Antiretroviral and Antitubercular Therapies

Highly active antiretroviral therapy was offered according to contemporary Brazilian National Guidelines that were periodically updated (14). The first line ATT regimen was the combination of rifampicin, isoniazid and pyrazinamide during the two initial months, followed by rifampicin and isoniazid for 4 months, except when the continuation phase needed to be extended to 7 months such as in cases with central nervous system TB. From July 2009 on, ethambutol was added to the intensive phase regimen following a new recommendation of the National TB program of the Brazilian Ministry of Health (15). TB treatment scheme was adjusted in cases of severe adverse reactions, drug resistance and HAART regimens that precluded the use of rifampicin.

Follow Up Visits

Visits included in this study were done at baseline, 60 and 180 days after TB therapy initiation. HAART were initiated after TB treatment according to decision from each physician and following the Brazilian TB treatment Guidelines (14). Information collected at the baseline visit included socio demographic data as well as previous TB and HAART, clinical presentation of TB, comorbidities like diabetes, hypertension, hepatitis (B and C), opportunistic diseases as well as CD4+ T-cell count and HIV VL among other variables. At baseline and in the follow up timepoints, patients underwent blood tests according to the INI's clinical laboratory routine, with complete blood count and biochemical tests (creatinine, urea, total and direct bilirubin, albumin, alkaline phosphatase, uric acid, AST, GGT, ALT and total proteins).

Some patients (n = 06) who abandoned TB treatment (ATT loss to follow up) had recorded data on Complete Blood Count (CBC) and biochemical assessments in blood after the date of the outcome established by the present study (non-compliance), because those patients had been following up at INI by other specialties outside the TB outpatient clinic.

Statistical Data Analysis

Three timepoints were considered: baseline, day 60 (D60) and day 180 (D180) of ATT. To perform baseline analysis, were used data from 256 patients. Due to lack of data in the subsequent timepoints (6.6% were missing data at D60 and 25.4% at D180), only 191 (74.6%) patients with complete laboratory data at all timepoints were considered for longitudinal analysis. Descriptive statistics was used to present data, use the median values with interquartile ranges (IQR) as measures of central tendency and dispersion, respectively, for continuous variables. Categorical variables were described using frequency (no.) and proportions (%). The Pearson chi-square test was used to compare categorical variables between study groups. The Mann–Whitney U test (for two unmatched groups), the Wilcoxon matched pairs test (for two matched groups), the Kruskal–Wallis test (for more than 2 unmatched groups) or the Jonckheere-Terpstra permutation and asymptotic test (for time series) were used to compare continuous variables. The Spearman rank test was used to assess correlations between indicated markers, conditions and timepoints. A multivariable logistic regression analysis model was used to identify independent determinants of

persistent anemia and unfavorable treatment outcomes. The results were presented in the form of adjusted odds ratio (aOR) and 95% confidence intervals (CI).

The degree of inflammatory perturbation (DIP) is based molecular degree of perturbation (MDP) (16), an adaptation of the molecular distance to health previously described (17). In the present study, instead of using gene expression values, we inputted biochemical markers concentrations, HIV viral load and blood cells counts. Thus, herein, the average level and standard deviation of a baseline reference group (nonanemic at baseline) were calculated for each biomarker. The DIP score of each individual biomarker was defined by z-score normalization, where the differences in concentration levels from the average of the biomarker in reference group was divided by the reference standard deviation. The DIP score represents the differences by number of standard deviations from the control group.

Hierarchical cluster analysis (Ward's method) using values of z-score normalized data was employed to depict the overall expression profile of indicated markers in the study subgroups. In this analysis, the dendrograms represent the Euclidean distance (inferring degree of similarity).

All analyses were pre-specified. Differences with p-values below 0.05 after adjustment for multiple comparisons (HolmBonferroni) were considered statistically significant. The statistical analyses. were performed using mdp (version 1.8.0), rstatix (version 0.4.0), stats (version 3.6.2), and caret (version. 6.0.86) R packages.

2.2.4- Results

Characteristics of the Study Participants During the period from 2008 to 2016, 273 patients were screened, but 17 were excluded from all the analyses because of lack of data at baseline. Thus, the initial analysis included 256 patients, out of whom 219 (85.6%) were anemic and 37 (14.4%) were not anemic at baseline. The vast majority of study participants were male (71%), and the median age was 37 years old (IQR: 31–46). Individuals with anemia at baseline were similar to non-anemic participants with regard to, age, sex, overall frequency of comorbidities and life-habits (Table 1). Anemic patients more frequently self-reported weight loss (>10% of body weight) before initiating treatment and displayed lower CD4+ T-cell counts and higher HIV viral loads than those non-anemic at the study baseline (Table 1). Frequency of HAART

use before TB diagnosis was higher in non-anemic study participants (65% in non-anemic vs. 43% in anemic, p = 0.021; Table 1).

To perform the longitudinal analysis, 82 of these patients were excluded because due to lack of data at some time point of the TB treatment (as described in “Materials and Methods”). Thus, 191 patients were further considered, out of whom 161 (84.3%) were anemic and 30 (15.7%) were not anemic at baseline. The median TB treatment period was 189 days for both groups. At day 180 of treatment, CD4+ T-cell counts increased in both study groups, but values in the group of participants who were anemic at the study baseline persisted substantially lower than those measured in non-anemic patients (p = 0.018; Table 1). Nevertheless, both frequency of individuals with undetectable HIV viral loads and median values with detectable viral loads were indistinguishable between study participants stratified based on anemia at baseline. There was no difference in the type of antitubercular treatment regimen between the study groups.

Presence of Anemia Is Associated With Specific Cellular and Biochemical Profiles in Peripheral Blood of PWH

Coinfected with TB The overall differences in cell counts and values of biochemical parameters measured at pre-ATT for anemic and non-anemic TB patients are described in Supplementary Table 1. As expected, erythrocyte counts, and values of hematocrit and hemoglobin were lower in anemic compared to non-anemic study participants. In addition, anemic patients exhibited lower counts of several leukocytes including lymphocytes and eosinophils at the study baseline (Supplementary Table 1). Additional analyses of the CBC parameters using hierarchical clustering of z-score normalized data and computation of fold change were performed to evaluate the dynamicity of the values over time in each group (Figure 1A). We observed a distinct profile between the groups, with three clusters defined in the heatmap, where the latter cluster (hemoglobin, hematocrit and erythrocyte) was the most consistent in both groups, with few changes mainly in the group of patients without anemia before treatment (baseline). Furthermore, it was possible to observe that, in the anemic participants, there was a significant difference in all parameters over time, mainly when comparing the baseline with the end of TB treatment (D180).

In regard to biochemical parameters, statistically significant differences were found in levels of ALT, AST and GGT, which were all higher in anemic patients at baseline, whereas the levels of albumin were lower (Supplementary Table 1 and Figure 1B). Additional hierarchical cluster and fold change analysis performed with biochemical parameters revealed a distinct profile between the groups (Figure 1B). Again, small changes over time in the group without anemia at baseline were observed, with increased levels of uric acid and decreased levels of creatinine at D60 and with increase in albumin levels at D180, comparing with baseline. In the group that presented anemia at baseline, the differences in levels of biomarkers were more pronounced. We found that, at D60, a decrease in urea levels and increase in uric acid and albumin levels were detected compared to baseline. At D180, there were significantly higher values of albumin and lower values of direct bilirubin, alkaline phosphatase, AST, GGT and ALT, than those measured at the study baseline.

Correlation Between Cells and Biochemical Parameters With Hemoglobin

The results presented above indicate that anemia is associated with a distinct profile of cell counts and biochemical parameters in peripheral blood of patients with HIV-TB coinfection prior to initiation of ATT. We next examined the correlations between Hb levels and cell counts or values of the biochemical parameters (Figure 2). We observed that gradual increases in Hb values were related with decreases in percentage of neutrophils ($r = -0.27$; $p < 0.001$) and levels of ALT ($r = -0.26$; $p < 0.001$), AST ($r = -0.25$; $p < 0.001$), GGT ($r = -0.35$; $p < 0.001$), Alkaline Phosphatase ($r = -0.23$; $p < 0.001$), and Urea ($r = -0.13$; $p = 0.045$). Furthermore, frequency of lymphocytes ($r = 0.35$; $p < 0.001$) and monocytes ($r = 0.14$; $p = 0.021$), as well as levels of albumin ($r = 0.61$; $p < 0.001$) were increased proportionally to elevations in Hb levels (Figure 2). These findings reinforce the idea that degree of anemia is associated with changes in cellular and biochemical disturbances in peripheral blood.

Dynamic Change of Hemoglobin Levels Upon Initiation of Anti-TB Treatment

In order to better understand the impact of ATT commencement in the anemia, we prospectively investigated Hb levels at different time points of therapy (Figure 3). This approach revealed a differential dynamic of changes in Hb levels depending on the anemia status at the study baseline (Figure 3A). Indeed, a gradual increase in Hb

levels over time on treatment was observed in the group of anemic participants (linear trend p-value: < 0.001), whereas such levels did not substantially change in those who were not anemic at baseline. Curiously, 11 (36.6%) patients who were non-anemic at baseline developed anemia at day 60, from whom 8 (26.6% of the non-anemic group) were also anemic at day 180 of ATT (Figure 3B). Among the initially anemic patients, 83.85% were still anemic at day 60 and 63.35% persisted with anemia at day 180 of therapy (Figure 3B). A Sankey diagram was used to illustrate the dynamic change of anemia status over time on ATT (Figure 3C).

Hence, we observed that the vast majority of the participants who were anemic at the study baseline persisted with anemia until at least day 180 of therapy, whereas 19 (11.8%) individuals recovered from anemia at day 60 (early recovery), 36 (22.36%) recovered only by day 180 (late recovery), and 5 (3.1%) recovered at day 60 but were once again anemic at day 180 (transient recovery). The characteristics of these subpopulations are shown in the Supplementary Table 2. The dynamicity of hemoglobin levels in the different subgroups of anemic patients identified in the Sankey diagram is described in Figure 3D. Among the patients who had anemia at the baseline, with the exception of the transient recovery group, all exhibited a significant increase in hemoglobin levels over time of ATT (p-values < 0.05) (Figure 3D).

Persistent Anemia Is Associated With Augmented Degree of Inflammatory Perturbation

Given that the majority of anemic patients persisted with anemia during the time of ATT regardless of the gradual increase in hemoglobin levels, we tested whether such condition was related to a chronic and unresolved inflammatory disturbance. To do so, we employed a mathematical maneuver named Molecular Degree of Perturbation (MDP), which has been used by our group and others to estimate the overall degree of inflammation and/or immune activation (18–20). In the present study, we included cells (from CBC), viral load, CD4 counts and biochemical parameters (creatinine, urea, total and direct bilirubin, albumin, alkaline phosphatase, uric acid, AST, GGT, ALT and total proteins) to create a score henceforth named Degree of Inflammatory Perturbation (DIP) (Figure 4A). We found that in general, anemia was associated with increased DIP values measured at both baseline (Figure 4B) and at day 180 of ATT (Figure 4C), with the highest levels being detected in the group of persistent anemia.

Strikingly, the DIP score values exhibited strong inverse correlations with hemoglobin levels both at baseline ($r = -0.74$; $p < 0.001$) and at day 180 ($r = -61$; $p < 0.001$), highlighting that the degree of anemia and activation of inflammation are concurrent processes.

Additional analyses demonstrated that, as expected, patients who had an early recovery from anemia exhibited significantly higher baseline values for erythrocytes, Hb, hematocrit, neutrophils (Supplementary Figure 1) and albumin (Supplementary Figure 2) than those who did not recover. Patients who had a late recovery displayed significantly higher baseline values of Hb and hematocrit compared to those who persisted anemic (Supplementary Figures 1, 2). The prospective comparisons have also identified discrepancies in cell counts and concentrations of biochemical parameters between the subgroups of patients based on recovery from anemia, which are summarized in Supplementary Figures 1, 2.

The findings described above led us to hypothesize that the distinct profile of cell counts, and levels of biochemical parameters, measured at pre-ATT, is associated with persistent anemia. Thus, a stepwise binary multivariate logistic regression analysis was performed to test if biochemical parameters measured at pre-ATT (baseline) are able to predict recovery from anemia. Results demonstrated that increases in concentrations of albumin were directly associated with recovery from anemia (aOR: 2.67, 95% CI:1.05–6.75, $p = 0.04$) whereas increases in total proteins were directly associated with persistent anemia (aOR: 0.44, 95% CI: 0.24–0.78, $p = 0.01$) (Figure 3E). Similar trends in associations were observed when the major group of participants who recovered from anemia were further stratified in early and late recovery.

Lower Concentrations of Hemoglobin at Pre-ATT Are Associated With Increased Risk of Unfavorable Treatment Outcome

In the longitudinal study cohort, 18 patients (9.4%) developed unfavorable outcomes (death attributed to TB: n = 3; death attributed to HIV: n = 2; ATT failure: n = 1; ATT loss to follow up abandonment: n = 12). The majority of the cases of unfavorable outcomes was composed by individuals who experienced persistent anemia (14 out of 18 participants, 77.8%) (Figure 5A). In fact, the median values of Hb levels gradually increased upon initiation of ATT in patients who were successfully treated (linear trend

p < 0.001) but did substantially change in those who has unfavorable outcomes (Figure 5B). A hierarchical cluster analysis inputting average values of CBC (Figure 5C) and biochemical parameters (Figure 5D) demonstrated that there were differential trends in values between the study timepoints and the subgroups of favorable vs. unfavorable outcomes.

At study baseline, individuals who further developed unfavorable outcomes exhibited lower levels of Hb (p = 0.052), albumin (p = 0.035), uric acid (p = 0.001), urea (p = 0.006), and creatinine (p = 0.008) than those who were further successfully treated (Supplementary Table 3). A binomial logistic regression analysis was performed to test independent associations between the parameters analyzed and treatment outcome (Figure 5E). We found that increases in hemoglobin at pre-ATT were protective against unfavorable outcomes (aOR: 0.80, 95% CI: 0.64–0.99, p = 0.04) independent of the other factors (Figure 5E). These results highlight the importance of Hb as a prognostic marker in PLWH coinfected with TB.

2.2.5- Tables and figures

Table 1

Characteristic	All (*n* = 256)	Anemic at baseline (*n* = 219)	Non-anemic at baseline (*n* = 37)	*p*-value
Age (years), median (IQR)	37 (31–46)	37 (30.7–46)	37 (33–46)	0.504
Sex, no. (% male)	182 (71)	157 (71.7)	25 (67.6)	0.996
Weight loss (>10%), no. (%)	190 (74.2)	174 (79.5)	16 (43.2)	<0.01
Smoking, no. (%)	131 (51.2)	114 (52)	17 (45.9)	0.948
Use of illicit drugs, no. (%)	74 (28.9)	60 (27.3)	14 (37.8)	0.185
Alcohol abuse[1], no. (%)	88 (34.3)	80 (36.5)	8 (21.6)	0.133
Baseline CD4 count (cells/mm^3), median (IQR)	170.5 (52–321.2)	153 (42.5–304.5)	294 (158–560)	<0.01
Baseline Viral Load log10 (copies/mL), median (IQR) (*n* = 165)	4.3 (1.69–0.5.23)	4.41 (2.5–5.31)	1.79 (1.69–4.22)	<0.01
D180 CD4 count (cells/mm^3), median (IQR)	292.5 (165–432)	258 (157.5–403)	423 (266–603.5)	0.018
D180 Detectable Viral Load log10 (copies/mL), median (IQR) (*n* = 76)	3.21 (1.80–4.67)	3.17 (1.79–4.68)	3.42 (2.03–4.03)	0.766
D180 Undetectable VL, no. (%)	133 (52)	105 (65.2)	25 (38.5)	0.320
Days until outcome[2], median (IQR)	189 (178.7–259.7)	189 (180–265)	189 (168–247.5)	0.564
Viral Hepatitis (B and/or C), no. (%)	25 (9.7)	21 (9.48)	4 (10.8)	0.945
Hypertension, no. (%)	21 (8.2)	16 (7.3)	5 (13.5)	0.270
Diabetes, no. (%)	32 (12.5)	28 (12.7)	4 (10.8)	0.946
Previous tuberculosis (%)	64 (25)	52 (23.7)	12 (32.4)	0.355
Complete TB treatment previous, no. (% of previous TB)	44 (68.8)	35 (67.3)	9 (75)	0.742
HAART use before TB, no. (%)	118 (46)	94 (42.9)	24 (64.8)	0.021
HAART during TB treatment, no. (%)	235 (91.7)	202 (92.3)	33 (89.2)	0.763
IRIS upon HAART initiation, no. (%)	12 (4.68)	12 (5.47)	0 (0)	–

To define anemia according to baseline (D0) hemoglobin, the cut-off point of 12 g/dL for women and 13.5 g/dL for men was used. Data are shown as median and interquartile (IQR) range or frequency (percentage). Data were compared between the clinical groups using the Mann–Whitney U test (continuous variables) or the Pearson's χ 2 test (for data on frequency). Complete data at baseline: 256 patients; Complete data at day 60: 239 (93.4%) patients, Complete data at day 180: 191 (74.6%) patients. [1]The physicians also collected information about current use of illicit drugs and alcohol (Y/N to each) during the baseline interview. Potential problematic alcohol use was assessed with the CAGE questionnaire, with scores of 2 or greater indicating clinically significant alcohol problems. [2]Outcomes: Favorable (cure) and Unfavorable (failure, loss follow-up or death). IQR, Interquartile Range; IRIS, Immune reconstitution Inflammatory Syndrome; TB, Tuberculosis; HAART, Highly Active Antiretroviral Therapy.

Figure 1

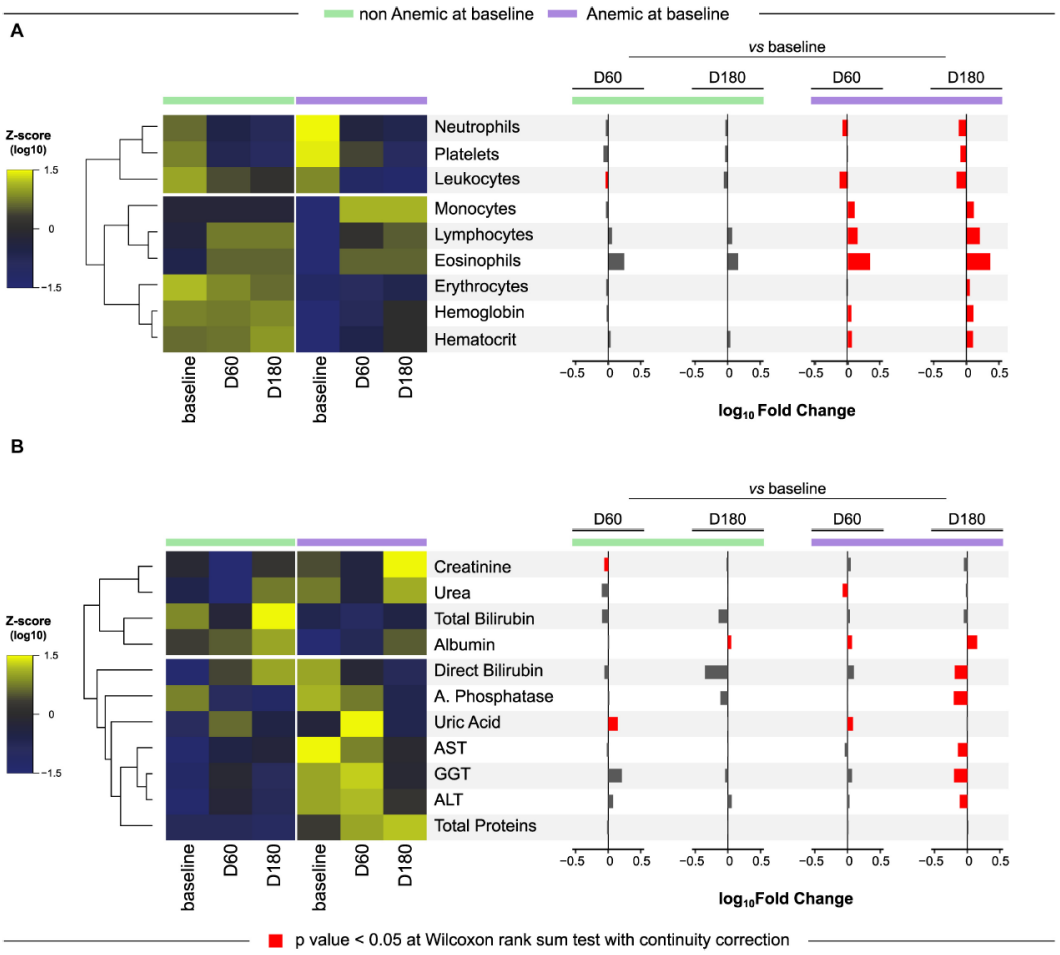

FIGURE 1 | Differential change in biomarkers between anemic and non-anemic patients. A Heatmap was designed to depict the overall pattern of complete blood counts (CBC) **(A)** and biochemical markers **(B)** at all timepoints in anemic and non-anemic at different study timepoints of anti-tubercular treatment. A two-way hierarchical cluster analysis (Ward's method) was performed. Dendrograms represent Euclidean distance. Expression scale represents Z-score normalization from the median at each timepoint and group. To define anemia according to baseline hemoglobin, the cut-off point of 12 g/dL for women and 13.5 g/dL for men was used. A $\log_{10}$ of fold-change was calculated and statistical analyses were performed using the Mann–Whitney *U* adjusted test. Significative differences ($p < 0.05$) between anemic and non-anemic patients for each time point are highlighted in red bars. Data are from 191 patients who had complete information on cell counts and biochemical measurements at all study timepoints.

Figure 2

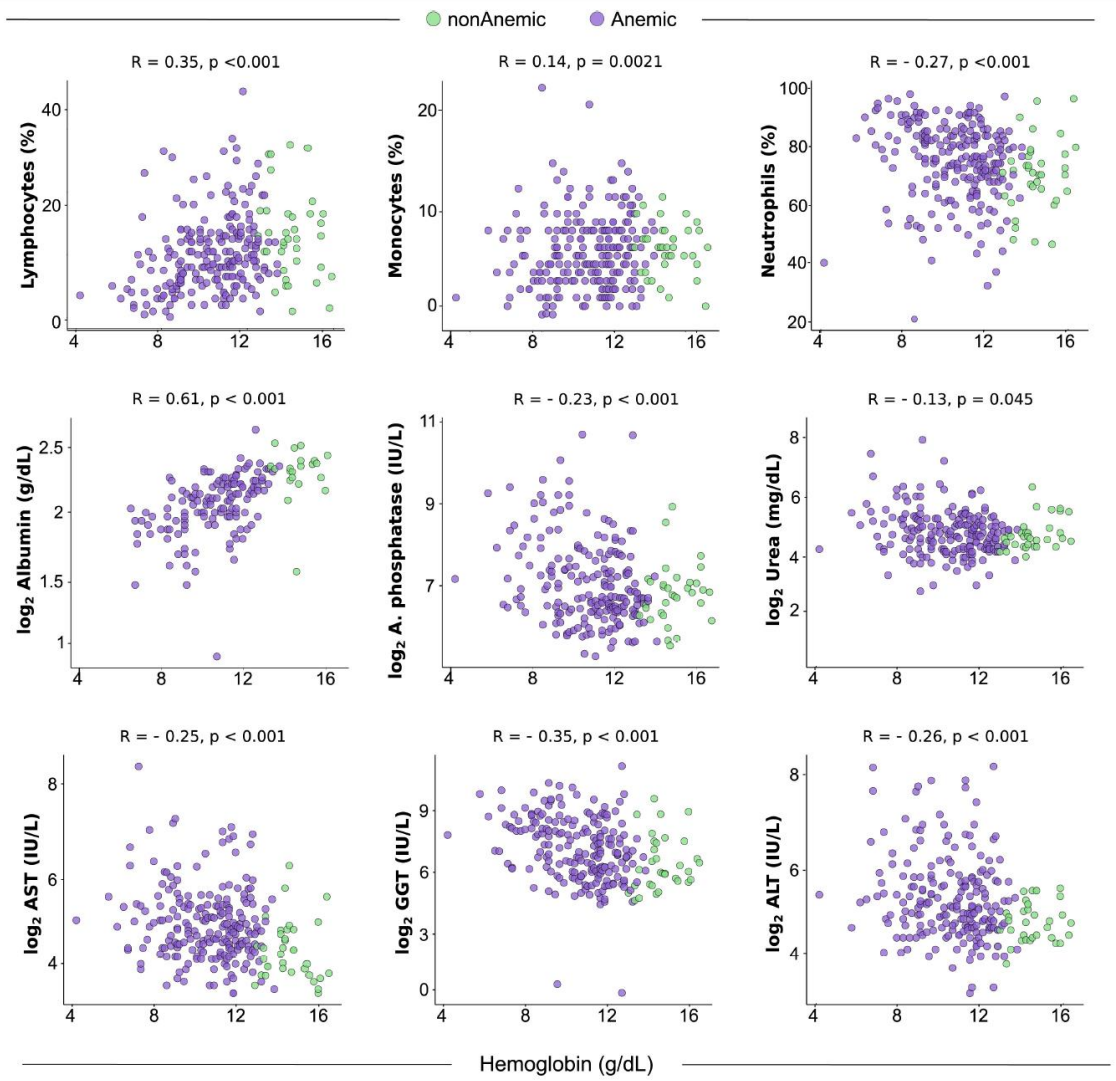

FIGURE 2 | Spearman correlation analysis of cells and biochemical parameters versus hemoglobin in blood of TB-HIV prior to antitubercular treatment initiation. Plots from statistically significant Spearman correlations between biochemical parameters and hemoglobin levels at study baseline (pre-ATT) are shown (n = 256). To define anemia according to baseline hemoglobin, the cut-off point of 12 g/dL for women and 13.5 g/dL for men was used. ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase.

Figure 3

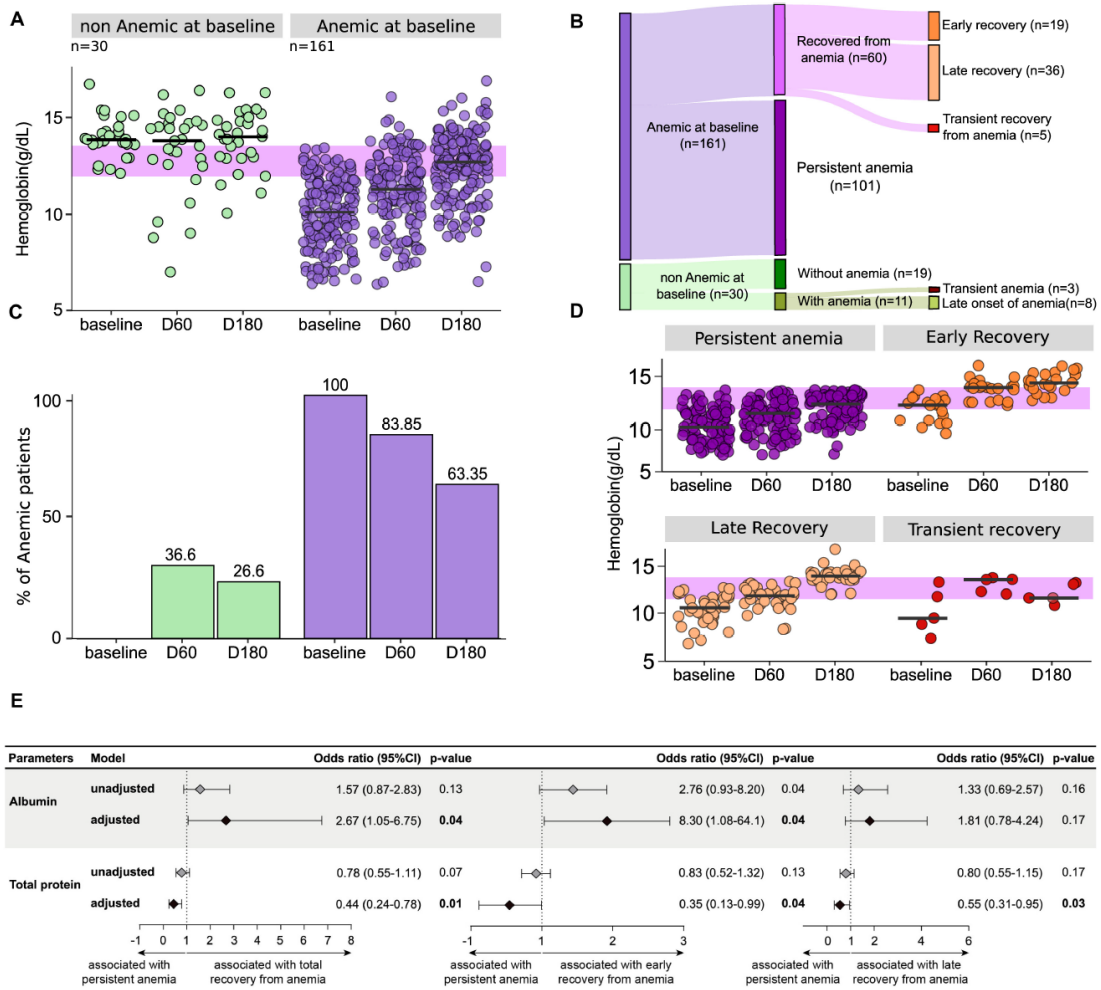

FIGURE 3 | The majority of the anemic patients at baseline persist with low levels of hemoglobin after initiation of anti-tubercular treatment. To define anemia according to baseline hemoglobin, the cut-off point of 12 g/dL for women and 13.5 g/dL for men was used. **(A)** Hemoglobin levels at different time points of antitubercular therapy in the longitudinal population (n = 191) as well as in the groups of patients with or without anemia at baseline of treatment are shown. Anemic group presented statistically significant difference with $p < 0.001$ between baseline and timepoints after 2 months (D60 and D180) using Wilcoxon rank sum test with corrections. On Jonckheere-Terpstra permutation test, where an increase in one variable results in an increase or decrease in another variable, both groups presented $p = 0.001$ to "increase" hypothesis, with number of permutation equal to 1000. Using Jonckheere-Terpstra asymptotic test, p-value of non-anemic group was 0.851 and p-value of anemic group was <2.2e–16. Green dots represent non-anemic TB patients at baseline and purple dots represent anemic TB patients at baseline. **(B)** To define a patient as recovered from anemia, were considered normal levels (above the cut-off) of hemoglobin in any time point after D0. Chi-square test comparing D0 and D180 in both groups returned $p < 0.00001$. Green bars represent non-anemic TB patients at baseline and purple bars represent anemic TB patients at baseline. **(C)** Of the 161 patients who had anemia before starting treatment, 37.3% (n = 60) increased the values to normal hemoglobin levels at some time point. Of these, 95% were completely recovered (n = 55), so that 35% (n = 19) were recovered early (D60), and 60% (n = 36) were recovered late (D180). Finally, 5% (n = 5) of the patients who were anemic at study baseline presented a transient recovery (recovered at day 60 but were once again anemic at D180). 36.6% (n = 11) of the 30 patients without anemia at baseline developed anemia in D60, but three of them recovered normal hemoglobin values at D180. **(D)** Hemoglobin levels at different time points of antitubercular therapy in the in the population of anemic patients at baseline, divided according to the time of recovery. Using Jonckheere-Terpstra asymptotic test and Wilcoxon rank sum test with corrections, only the transient recovery group (that showed higher levels of hemoglobin at time 60 but had anemia at 180) did not exhibit a significant p-value between the timepoints. **(E)** Logistic binomial regression model was used to test independent associations between biochemical and clinical parameters and total recovery from anemia status at baseline, early recovery (recovery from anemia in ≤60 days from baseline) or late recovery (recovery from anemia in >60 days from baseline). The condition persistent anemia (anemia from baseline to day 180) was used as reference to test associations. Only parameters which remained with $p \leq 0.2$ in univariate analysis (**Supplementary Table 2** for details) model were inputted in the adjusted model. (95%CI, 95% confidence interval). Associations reported in **(E)** are for increases in 1 unit in plasma concentrations of the indicated markers. Data are from 191 patients who had complete information on cell counts and biochemical measurements at all study timepoints.

Figure 4

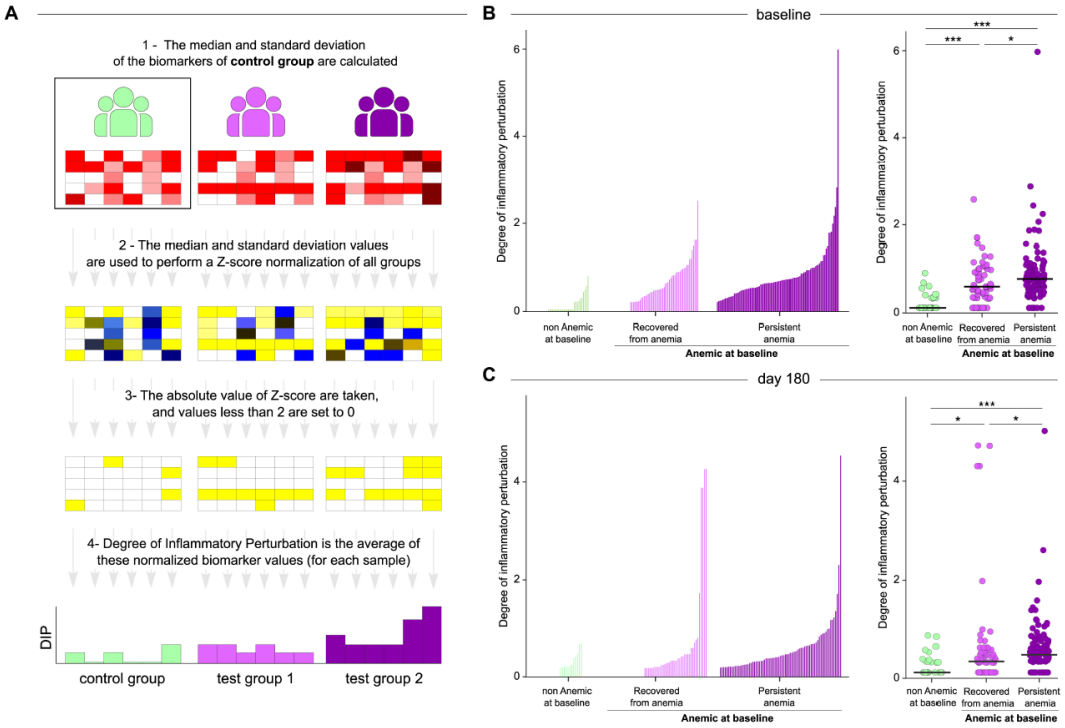

FIGURE 4 | Patients who persists with anemia exhibit substantially higher degree of inflammatory perturbation. **(A)** Degree of inflammatory perturbation (DIP) is based on Molecular Degree of Perturbation (16), but instead of using gene expression, we use biochemical and cellular markers. DIP was calculated was calculated using the median and standard deviation of the control group as a starting point. Then, the Z-score was calculated for all groups, a cut-off point was established and, finally, an average disturbance calculation was performed for each sample. This figure is adapted https://mdp.sysbio.tools/about. **(B,C) Left panels:** Histograms show the single sample degree of inflammatory perturbation (DIP) score values relative to the non-anemic at baseline group between patients recovered from anemia and with persistent anemia at baseline **(B)** and day 180 **(C)**. **Right panels:** Scatter plots of the summary data for each group are shown. DIP score values were compared between non-anemic at baseline (control group), recovered from anemia and persistent anemia at baseline **(B)** and day 180 **(C)**. Lines in the scatter plots represent median values. Data were compared using the Mann–Whitney *U* test. $^{*}P < 0.05$; $^{***}P < 0.0001$. Data are from 191 patients who had complete information on cell counts and biochemical measurements at all study timepoints.

Figure 5

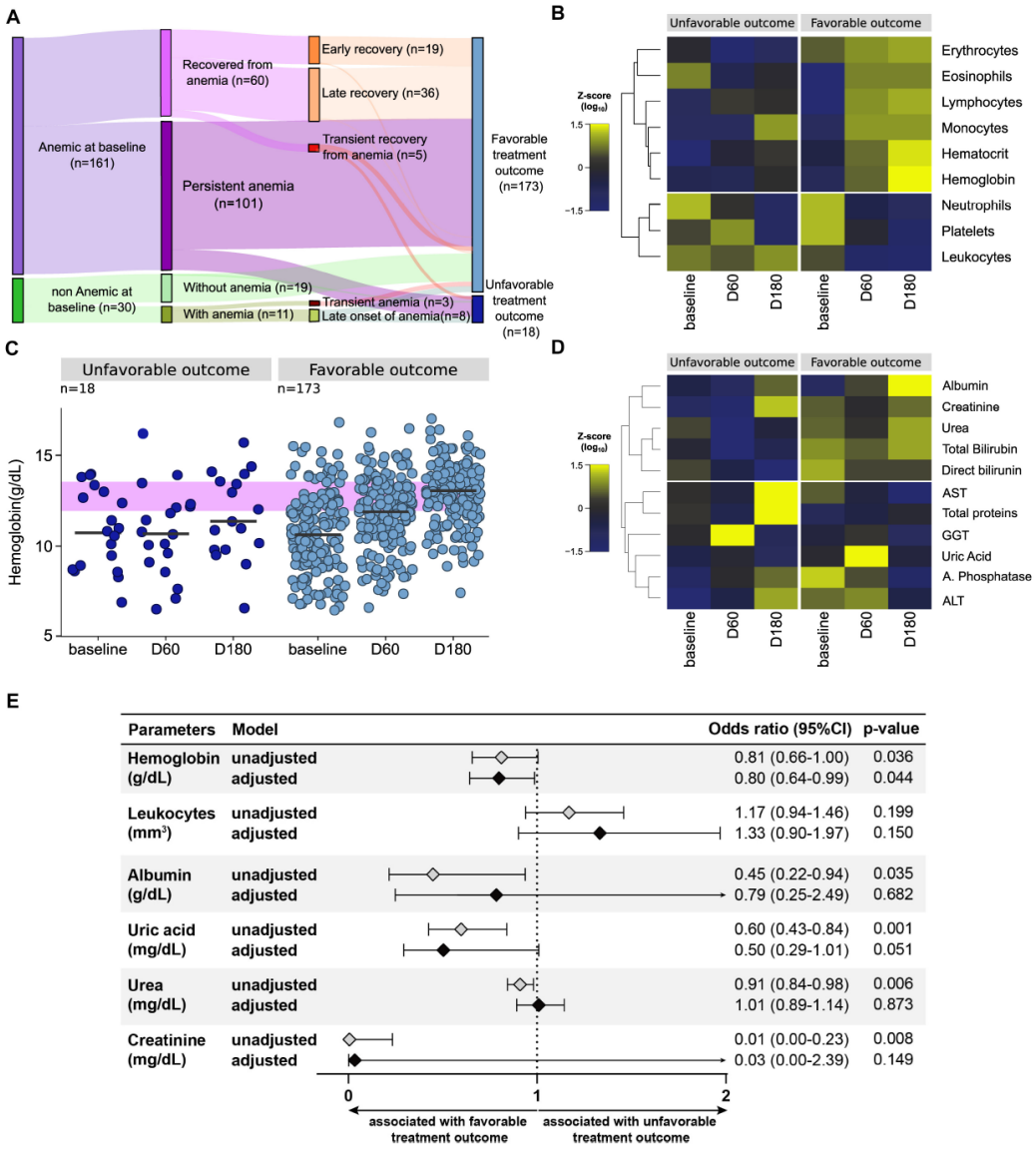

FIGURE 5 | Unfavorable outcome occurs mainly in patients with anemia. **(A)** Among all 191 patients who had complete laboratory data from all the study timepoints, only 18 had unfavorable treatment outcomes (death, failure or loss to follow up), whereas 173 were successfully treated (cure). **(B)** 14 of the 18 patients had anemia at baseline and 14 also had anemia at the end of the treatment. **(C,D)** A Heatmap was designed to depict the overall pattern of cells and biochemical markers at all timepoints in favorable and unfavorable outcome of anti-tubercular treatment. A two-way hierarchical cluster analysis (Ward's method) was performed. Dendrograms represent Euclidean distance. Expression scale represents Z-score normalization from the median at each timepoint and group. It is possible to observe that patients who had an unfavorable outcome have a profile opposite to that of patients with a favorable outcome. In **(C)** we can identify that patients with a favorable outcome had an increase in most of the blood count parameters over time, and also a decrease in neutrophils, platelets and leukocytes, returning to normal levels of these components. In patients with an unfavorable outcome, the parameters remain more similar to the baseline, with the exception of neutrophils and platelets, which also decrease. In **(D)** the biochemical markers of both groups do not seem to change much over time, but they visibly present different profiles when comparing favorable and unfavorable. Patients with a favorable outcome have higher levels of albumin, creatinine, urea and bilirubin throughout the treatment. **(E)** Logistic binomial regression model to significant biochemical parameters was used to test independent associations between biochemical and clinical parameters at baseline and treatment outcome after 180 days. The treatment outcome unfavorable (failure, death or loss to follow up) was used as reference to test associations. Only parameters which remained with $p \leq 0.2$ in univariate analysis model were inputted in the adjusted model. (95%CI, 95% confidence interval). Associations reported in **(E)** are for increases in 1 unit in plasma concentrations of indicated markers.

2.2.6- Discussion

Anemia is a common complication associated with both TB and HIV, and it has been reported to occur in between 16 and 94% of TB patients (21–24); whereas in PLWH the prevalence ranges from 39 to 71% (25–27). These observations were validated by the present study, which was focused on TB-HIV coinfection, and reported that 84.3% of the study participants were anemic at preATT. In addition, our findings demonstrated that anemic patients exhibit higher inflammatory perturbation in the peripheral blood, which is sustained over the course of ATT in those who persisted with low Hb levels. Such condition is shown here to be closely associated with unfavorable outcomes. Early intervention focused on recovery from anemia could be a strategy to optimize the clinical management of PLWH with TB during ATT treatment.

In our cohort, anemic patients more frequently exhibited weight loss, lower CD4+ T-cell counts and higher HIV viral loads than those who were not anemic. These observations reinforce the idea that anemia infers more advanced stage of disease progression. Our results are in agreement with other previously published findings which demonstrated that lower body mass index (27–29), higher HIV viral loads (28), and lower CD4+ T-cell counts are all associated with higher prevalence of anemia (25, 26). As previously reported by us in a different cohort of TB patients, most of the anemia cases are attributed to chronic inflammation rather than to iron deficiency (10). A recent systematic review demonstrated that anemia is related to an increased risk of all-cause mortality and incident TB among PLWH, regardless of the anemia type (30). The magnitude of such effect is thought to be proportional to severity of anemia. Finally, iron supplementation in such cases is still a matter of debate, with inconsistent results reported by clinical trials. The probable determinants of anemia in the context of HIV/AIDS and TB are likely multifactorial and involve several factors including nutritional status (31), chronic inflammation and antibody-mediated erythrophagocytosis (32). Our results demonstrated that anemic patients also exhibit lower counts of other cell types, suggesting that a global effect on the bone marrow may be occurring. Additional mechanistic studies as well as large randomized clinical trials testing different approaches to reduce anemia are necessary to improve our knowledge regarding the molecular targets and to help delineate the best therapeutic schemes.

With regard to the biochemical parameters, our results indicate that low Hb levels accompanied higher values of ALT, AST and GGT, and lower concentrations of albumin. Such findings are similar to those previously published by our group in another cohort of TB patients and reinforce the idea that anemia is related to a distinct biochemical profile and linked to inflammation (10). In our study, the prevalence of hepatitis B or C in anemic patients (9.48%) was very similar to non-anemic patients (10.8%), suggesting that although this comorbidity is present, it is probably not the main factor driving the differences in the levels of liver transaminases. At the end of ATT, none of these biochemical markers demonstrated association with the clinical outcomes. Moreover, out of the 25 patients who had viral hepatitis, 20 (80%) had a favorable outcome, highlighting the low influence of this coinfection on the effectiveness of the treatment.

The results reported here demonstrated that among the study participants with anemia at the baseline, the vast majority persisted with low Hb levels until day 180 of ATT. In addition, within the group of patients who recovered from anemia under the course of ATT, most exhibited a late recovery, occurring between day 60 and day 180 of therapy. Other investigations have reported that anemia frequently has a benign course in TB patients without HIV coinfection, with complete recovery in 64.5% of patients undertaking ATT (5). The discrepancies between the findings presented here and this previous study can be likely explained but the fact that our cohort was composed by PLWH, which may have an additional detrimental effect on inflammation and its related anemia compared to the setting of TB in the absence of HIV. In our study, patients who recovered from anemia presented with relatively higher values of Hb and hematocrit at baseline compared to those who persisted anemic. Individuals who had early recovery from anemia also exhibited higher neutrophil counts and albumin levels. The multivariable logistic regression analysis performed here revealed that albumin was independently associated with recovery from anemia. This observation again reinforces the strong association of albumin levels with recovery from anemia. These findings suggest that the degree of anemia is associated with changes in concentrations of cells and biochemical markers and that more severe anemia before ATT indicates higher odds of persistent anemia for up to 6 months on therapy.

To describe the overall biochemical and cellular disturbances related to anemia in the study population, we used an adaption of the molecular degree of perturbation (18) to estimate the degree of inflammatory perturbation in PLWH and with TB according to anemia status. Our findings indicate that there are important discrepancies in the DIP values between patients with persistent anemia compared to those who recovered during ATT. Individuals who persisted with anemia in the course of ATT exhibited higher DIP values already at pre-ATT, and such profile was sustained ay day 180 of therapy. These findings argue that persistent anemia directly associates with increased disturbances in the biochemical and cellular profiles, which were sustained over the course of ATT. The inverse correlations between DIP values and Hb levels both at pre-ATT and at day 180 indicate that the degree of inflammatory perturbation is proportional to the severity of anemia. Whether anemia sets the stage for persistent inflammation or is just a hallmark of chronic, unfettered, dysregulation of inflammatory responses warrants further investigation. This association between low Hb levels and risk of inflammatory disturbance has been described in PLHW who experience IRIS (33, 34) and also in patients with HIV/TB coinfection (35).

Another important contribution of our study was to test whether lower concentrations of Hb at pre-ATT could be used to predict risk of unfavorable outcomes. We found that the majority of patients who had unfavorable outcomes experienced persistent anemia during the course of ATT. A previous study described that anemia is associated with a 2–3 times increase in the risk of death, recurrence of TB or ATT failure in PLWH/TB (7). Corroborating with these findings, the results from a logistic regression analysis presented here demonstrated that increases in Hb concentrations at pre-ATT play a protective role against unfavorable outcomes independent of other confounding factors.

Our study has some limitations, such as relatively small number of non-anemic participants and of unfavorable outcomes, although the latter is within the expected range in the outpatient clinic from our institution. The small sample size favors a potential bias, as well as the fact that we do not have data on these same patients prior to TB and/or HIV infection, so that we cannot determine whether the anemia was pre-existing or in fact is a consequence of the co-infection. The study population also included few IRIS cases, which precluded additional exploratory analyses. Regardless of such limitations, our study adds to the current knowledge in the field by

demonstrating the relevance of persistent anemia in driving inflammatory disturbances related to worse prognosis of PLWH coinfected with TB. The fact that most patients with an unfavorable outcome persisted with anemia and with a high degree of inflammatory perturbation suggests that early intervention focused on recovery from anemia could be a strategy to optimize the clinical management of PLWH with TB during ATT treatment.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation. ETHICS STATEMENT The studies involving human participants were reviewed and approved by the Institutional Review Board of the Instituto Nacional de Infectologia Evandro Chagas (INI). The patients/participants provided their written informed consent to participate in this study. AUTHOR CONTRIBUTIONS FD, CS, FS'A, and VR contributed to conception and design of the study. FD also collected the data and organized the database. MA-P and MA performed the statistical analysis and data visualization. FD, MA-P, and BA wrote the first draft of the manuscript. VR and BA supervised the project execution. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu. 2020.588405/full#supplementary-material

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3 CONCLUSÃO

3.1- Artigo 1

Os fatores de risco para sobrevida e efetividade do tratamento ARV são diferentes para pacientes VT e PE aos ARVs.

Em relação às análises de efetividade da TARV, as variáveis associadas com pior eficácia em pacientes VT foram: TB prévia e abuso de álcool, enquanto hepatite viral teve uma significância limítrofe. Enquanto para pacientes PE à TARV, os fatores de risco associados ao insucesso do tratamento foram CV elevada e o tratamento com regimes baseados em IP.

Em relação à sobrevida, para os pacientes VT, IRIS é um fator significante para mortalidade precoce (100 dias), e este risco de morrer aumentou significativamente na análise multivariada. Para os pacientes PE à TARV, as análises uni e multivariada não revelaram fatores de risco significativamente associados à sobrevida.

3.2- Artigo 2

Nossos resultados indicaram que os valores de Hb aumentaram gradativamente no decorrer do tratamento de TB nos anêmicos. Enquanto a a maioria dos pacientes que tiveram desfechos desfavoráveis estava com o nível de Hb abaixo da faixa de normalidade no início do tratamento de TB, para aqueles que tiveram desfechos favoráveis a mediana da Hb aumentou significativamente e gradualmente ao longo do tratamento de TB.

Concluímos que aumento nos níveis de Hb em relação ao baseline diminui o risco de desfechos desfavoráveis independentemente de outros fatores, destacando-se a importância da Hb como marcador de prognóstico em PVHA e TB.

Nossos resultados indicaram que existem discrepâncias importantes nos valores de DIP entre os pacientes com anemia persistente em comparação com aqueles que se recuperaram durante a ATT. Mostramos a correlação da anemia persistente com os distúrbios inflamatórios relacionados a desfechos desfavoráveis do tratamento da TB.

As correlações inversas entre os valores de DIP e os níveis de Hb, tanto no início quanto no fim do ATT, indicam que o grau de perturbação inflamatória é proporcional à gravidade da anemia.

4 CONSIDERAÇÕES FINAIS - RECOMENDAÇÕES

IRIS se mostrou como um fator de risco para óbito no grupo VT. Estudos posteriores, que incluam mais pacientes com IRIS, devem ser realizados a fim de avaliar como melhor manejar estes pacientes a fim de reduzir o risco de óbito dos mesmos. Também há uma lacuna na literatura de estudos para avaliar quais os possíveis preditores de mortalidade em pacientes PE, que poderiam contribuir para uma melhor condução destes pacientes.

Considerando que existem discrepâncias importantes nos valores de inflamação entre os pacientes que persistiram com anemia em relação àqueles que se recuperaram ao longo do tratamento para TB, é de extrema importância que seja investigado e diagnosticado esse grau de inflamação no início do ATT para que uma possível intervenção seja feita no quadro anêmico deste paciente, pois o grau de perturbação inflamatória é proporcional à gravidade da anemia. Estudos posteriores são necessários para avaliar a melhor maneira de intervir na recuperação da anemia, para que os pacientes possam ter melhores desfechos do tratamento da TB e melhora na qualidade de vida.

Possivelmente, uma intervenção precoce para promover a recuperação da anemia melhore os desfechos do tratamento de TB nos pacientes co-infectados com HIV. Propomos que estudos adicionais, de preferência ensaios clínicos randomizados testando diferentes abordagens para reduzir a anemia, sejam realizados para melhorar nosso conhecimento sobre como atuar na recuperação da anemia nesses pacientes, com consequente melhoria da morbimortalidade.

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6 ANEXOS

Anexo 1 – Termo do Consentimento Livre e Esclarecido

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Avaliação dos fatores determinantes da sobrevida em indivíduos infectados ou não por HIV

Você está sendo convidado a participar de um estudo sobre a tuberculose. Este estudo visa obter mais conhecimentos sobre esta infecção causada por uma micobactéria chamada *Mycobacterium tuberculosis* que acomete principalmente os pulmões, mas que também afeta outras partes do organismo podendo mesmo ser fatal se não tratada.

Sua participação neste estudo será de comparecer às consultas marcadas e responder a um questionário a cada vez que você vier, além de realizar exames complementares para o diagnóstico e tratamento da tuberculose. Os exames realizados durante este estudo são aqueles necessários para o diagnóstico da sua doença e, portanto, necessários para sua cura.

A obtenção de outros materiais clínicos como lÍquidos pleural, peritoneal, céfalo-raquiano, gânglios e outros só serão feitos mediante a indicação clínica quando o caso o exigir. O objetivo desse estudo será avaliar a sobrevida de pacientes com tuberculose e os fatores que a influenciam. No sangue coletado serão realizados hemograma e dosagens bioquímicas e cultura para micobactérias. Será também pesquisada a presença da micobactéria no escarro e em outros materiais clínicos. Caso seja encontrada essa micobactéria no seu corpo o tratamento específico para a doença lhe será prontamente oferecido.

Os resultados obtidos nesse estudo serão ditos para sua pessoa e considerados estritamente confidenciais, podendo, no entanto, ser divulgados na forma de comunicação científica, mas não será permitida a sua identificação, o que garante a sua privacidade. Os resultados desse estudo poderão não te beneficiar diretamente, mas poderão no futuro beneficiar outras pessoas com essa doença.

Serão coletados na primeira avaliação, 15, 30, 60, 120 e 180 dias após o início do tratamento, 20 ml de sangue (por punção na veia do antebraço) e amostras de escarro. A retirada do sangue poderá ser realizada por médico, enfermeiro ou técnico do INI. A coleta do sangue poderá causar dor e resultar, em alguns casos, numa mancha arroxeada (equimose) que pode durar de 3 a 15 dias.

Todos os cuidados apropriados serão tomados com uso de seringa e gaze descartáveis e álcool, para limpeza local.

As radiologias de tórax serão feitas na avaliação inicial, 30, 60 e 120 dias após o início do tratamento. O médico responsável deve te explicar o conteúdo dessas informações quando você não for capaz de compreender e deve se colocar à disposição para responder todas as suas

perguntas sempre que você tiver novas dúvidas. Você tem a liberdade de consultar outros pesquisadores envolvidos nesse estudo.

Além disso, você poderá ser convidado, no futuro, a participar de um estudo para entender melhor por quais motivos as pessoas deixam de tomar os medicamentos, visando melhorar a adesão ao tratamento e desfechos clínicos em pacientes com TB e HIV. Serão feitas entrevistas com um pequeno

subgrupo da população incluída no estudo (usuários de drogas que são TB-HIV), assim como com médicos e demais profissionais de saúde.

Sua participação é inteiramente voluntaria. E você pode, a qualquer momento, desistir de participar do estudo sem prejuízo para o seu tratamento e acompanhamento.

Você receberá uma via assinada desse termo de consentimento assinado por você e pelo pesquisador. Assim, você está consentindo voluntariamente em participar deste estudo, permitindo, portanto que os procedimentos sejam realizados em você. Ao assinar este documento, você não abrirá mão de nenhum direito legal.

_____ ___/___/_____:_____

Nome do participante (letra legível) Data Hora

_____

Assinatura do participante

_____ ____/_____/_____:___

Nome do representante legal (letra legível) Data Hora

_____

Assinatura do representante legal

_____ ____/_____/_____:___

Nome da testemunha imparcial (letra legível) Data Hora

_____

Assinatura da testemunha imparcial

_____ ____/_____/_____:___

Nome do Profissional que aplicou o termo (letra legível) Data Hora

_____

Assinatura do Profissional que aplicou o termo

Se este termo de consentimento tiver sido lido para o paciente porque ele é incapaz de ler o documento, uma testemunha imparcial e sem vínculo com a pesquisa ou com o investigador deve estar presente durante o consentimento e assinar a seguinte declaração: Declaro que as informações contidas no presente termo de consentimento e outras informações escritas foram explicadas ao paciente de maneira precisa e parecem ter sido por ele compreendidas. O paciente concordou em participar do estudo de livre e espontânea vontade.

_____ __/ /____ :____
Nome da testemunha imparcial Data Hora

_____
Assinatura da testemunha imparcial

Se tiver alguma dúvida sobre a sua participação no estudo ou alguma outra dúvida sobre seus direitos como participante de pesquisa clínica entre em contato com:

Valeria Cavalcanti Rolla MD, PhD.
Instituto Nacional de Infectologia Evandro Chagas - Fiocruz
Laboratório de Pesquisa Clínica em Micobacterioses
Av. Brasil, 4365 Manguinhos
Rio de Janeiro - RJ
CEP 21040-900
Telefone: (21) 3865-9601/07 Telefone de Emergência: (21) 9490-4251
E-mail: valeria.rolla@ini.fiocruz.br
O Comitê de Ética em Pesquisa do INI será capaz de orientar e defender os seus interesses como voluntário da pesquisa. Em caso de duvidas, você poderá consultá-lo:

Coordenadora do CEP: Dra. Lea Camilo Coura
Av. Brasil, 4365 - Manguinhos
Rio de Janeiro- RJ
CEP 21040-900
Telefone: (21) 3865-9585 E-mail: CEP@ini.fiocruz.br

Anexo 2 - Pedido de Autorização para Dispensa de Aplicação do Termo de Consentimento Livre e Esclarecido

À Dra. Léa Camillo Coura

Coordenadora do Comitê de Ética em Pesquisa do INI-Fiocruz,

Venho por meio deste, solicitar à V. Sa. a dispensa da aplicação do Termo de Consentimento Livre e Esclarecido (TCLE) referente ao projeto de pesquisa intitulado "Efetividade e sobrevida dos diferentes regimes terapêuticos usados em pacientes com TB-HIV".

Trata-se de pesquisa retrospectiva **com uso de dados de prontuários de** pacientes incluídos no projeto: "Avaliação dos fatores associados a sobrevida em indivíduos com tuberculose infectados ou não pelo HIV" o qual foi aprovado em sua versão original em 13nov2000 pelo CEP-INI (Projeto CAEE 02/00) com última emenda de 25jun13 aprovada em 08ago13.

O referido termo autoriza a utilização dos dados referentes ao diagnóstico e evolução, permitindo a utilização dos mesmos para a realização do atual projeto.

Não obstante, anexo ao presente o **Termo de Compromisso e Responsabilidade** devidamente assinado, assumindo o compromisso com o sigilo das informações obtidas.

Atenciosamente,

Rio de Janeiro, ____de _____de _____.

_____

Assinatura do Pesquisador responsável

Anexo 3 - Termo de Compromisso e Responsabilidade

Nós, Fernanda de Oliveira Demitto, aluna de Doutorado e Valeria Cavalcanti Rolla, orientadora do projeto de pesquisa intitulado “Efetividade e sobrevida dos diferentes regimes terapêuticos usados em pacientes com TB-HIV”, nos comprometemos manter a confidencialidade assim como a privacidade dos participantes do projeto.
A identidade dos participantes, assim como os resultados obtidos com este projeto, serão mantidos em um banco de dados sob a responsabilidade da orientadora.

Os resultados obtidos com esta pesquisa serão divulgados em comunicações científicas mantendo o anonimato dos participantes e o material utilizado não será empregado em outras pesquisas, a não ser quando abertos novos protocolos.

Rio de Janeiro, _____ de _____ de _____.

_____

Fernanda de Oliveira Demitto Tamogami

_____

Valeria Cavalcanti Rolla

Anexo 4 – Parecer consubstanciado CEP

INSTITUTO NACIONAL DE INFECTOLOGIA EVANDRO CHAGAS - INI / FIOCRUZ

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efetividade e sobrevida dos diferentes regimes terapêuticos usados em pacientes com TB-HIV

Pesquisador: Valéria Cavalcanti Rolla

Área Temática:

Versão: 2

CAAE: 71191417.8.0000.5262

Instituição Proponente: INSTITUTO NACIONAL DE INFECTOLOGIA EVANDRO CHAGAS - INI/FIOCRUZ

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.242.626

Apresentação do Projeto:

Introdução:

O regime de tratamento de primeira linha para TB no Brasil para todos os pacientes com TB sensível desde 2010 é a combinação de rifampicina 600mg, isoniazida 300 mg, pirazinamida 1600 mg e etambutol 1100 mg durante 2 meses na fase inicial ou chamada “fase intensiva” do tratamento, seguido de rifampicina e isoniazida durante 4 meses ou “fase de manutenção”, totalizando 6 meses de tratamento (BRASIL, 2011). A rifampicina afeta diretamente o metabolismo das drogas antirretrovirais (ARV) mais potentes (inibidores da trascriptase reversa não nucleosídeos (ITRNN) e inibidores da protease (IP). Apesar de autorizado pelos EUA e pelo Brasil, a associação dos regimes contendo rifampicina e IP (saquinavir e ritonavir) levaram a reações adversas sérias e descontinuação do tratamento em pacientes virgens de terapia antirretroviral (TARV) (ROLLA et al, 2006). O uso de rifabutina, um derivado da rifamicina, foi recentemente disponibilizado para uso com IPs porque a rifabutina é um
indutor do citocromo P450 menos potente do que a rifampicina. O resultado destas interações é um aumento da concentração sanguínea de rifabutina. A fim de evitar a toxicidade da rifabutina, recomenda-se a redução da dose deste medicamento quando administrado concomitantemente

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INSTITUTO NACIONAL DE INFECTOLOGIA EVANDRO CHAGAS - INI / FIOCRUZ

Continuação do Parecer: 2.242.626

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_952344.pdf	24/08/2017 11:38:26		Aceito
Declaração de Pesquisadores	Resposta.PDF	24/08/2017 11:33:21	FERNANDA DE OLIVEIRA DEMITTO TAMOGAMI	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.PDF	24/08/2017 11:32:25	FERNANDA DE OLIVEIRA DEMITTO TAMOGAMI	Aceito
Outros	T_Compromisso_Fernanda.pdf	13/07/2017 12:30:13	Vera Lucia Ferreira Guimarães Carreira	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_de_Tese_Fernanda_Demitto.pdf	12/07/2017 12:52:02	Valéria Cavalcanti Rolla	Aceito
Folha de Rosto	LapClinTB120717124950.pdf	12/07/2017 12:50:22	Valéria Cavalcanti Rolla	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

RIO DE JANEIRO, 28 de Agosto de 2017

Assinado por:
Léa Ferreira Camillo Coura
(Coordenador)

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