

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO EM PSIQUIATRIA E CIÊNCIAS DO
COMPORTAMENTO

TESE DE DOUTORADO

**TRANSTORNO BIPOLAR E TRANSTORNO DE ESTRESSE
PÓS-TRAUMÁTICO: ASPECTOS CLINICOS E BIOLÓGICOS**

Ives Cavalcante Passos

Orientadora: Profa. Dra. Márcia Kauer-Sant'Anna

Porto Alegre, 2015

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Orientadora: Profa. Dra. Márcia Kauer-Sant'Anna
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Para Francisco Passos Dias, Eurídice Vânia Cavalcante Passos e Sávio Cavalcante Passos.

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Lista de Abreviaturas

BDNF: do inglês, *Brain-derived neurotrophic factor*, Fator Neurotrófico Derivado do Cérebro

IL: Interleucina

IL-1RA: do inglês, *Interleukin-1 receptor antagonist*, antagonista do receptor da interleucina

sIL-2R: do inglês, *Soluble Interleukin-2 Receptor*, receptor solúvel da IL-2

sIL-6R: do inglês, *Soluble Interleukin-6 Receptor*, Receptor solúvel da IL-6

TB: Transtorno bipolar

TEPT: Transtorno do estresse pós-traumático

TNF- α : do inglês, *Tumor necrosis factor- α* , Fator de Necrose Tumoral

RESUMO

O transtorno de humor associado ao transtorno de estresse pós-traumático (TEPT) tem, em geral, desfechos clínicos mais graves. Embora essa associação esteja consolidada no transtorno depressivo, esse não é o caso no transtorno bipolar (TB). Os poucos estudos realizados acerca dessa comorbidade demonstraram piora na qualidade de vida e aumento no número de tentativas de suicídio associado aos pacientes com TEPT e TB. Nenhum estudo, entretanto, avaliou o impacto do TEPT em características centrais do TB, como o número de episódios de humor ou funcionamento psicossocial. Por outro lado, estudos pré-clínicos sugerem que existe um fenômeno de sensibilização cruzada entre o TB e o TEPT. Foi proposto que a redução do brain-derived neurotrophic factor (BDNF) e alterações de marcadores inflamatórios poderiam ser mecanismos associados a esta sensibilização. Com relação a última hipótese, as alterações em marcadores inflamatórios estão bem consolidadas no TB, porém esse não é o caso no TEPT. Com a finalidade de aprofundar o estudo dessas questões, os dois primeiros estudos que compõem essa tese abordaram aspectos clínicos e biológicos relacionados a esses transtornos. O primeiro é uma metanálise e metaregressão que demonstrou que o TEPT está associado a níveis aumentados de IL-6, IL-1 β , TNF- α e interferon- γ na circulação periférica. Além disso, o tempo de doença foi positivamente associado aos níveis de IL-1 β , enquanto a gravidade dos sintomas foi positivamente associada aos níveis de IL-6. Esses achados podem não só apresentar um novo mecanismo biológico para explicar o fenômeno de sensibilização cruzada entre TEPT e TB, mas também abre novos horizontes na busca de novas estratégias terapêuticas e diagnósticas para o TEPT. O segundo foi um estudo caso-controle que avaliou características clínicas centrais do TB e demonstrou pela primeira vez que indivíduos com TB e comorbidade com TEPT apresentam mais episódios maníacos e pior funcionamento psicossocial. Ademais, o início dos episódios maníacos e o uso de substâncias de abuso surgiram de maneira mais precoce nesses indivíduos. Além desses dois estudos, optamos por abordar a influência da comorbidade com os transtornos ansiosos (como o TEPT) em um desfecho clínico trágico em pacientes com

transtorno de humor: o suicídio. Esse estudo foi realizado em uma população diferente da do estudo clínico anterior, incluindo pacientes com depressão maior ou com TB, e foi focado em apenas um desfecho. Nosso objetivo era criar uma ferramenta que pudesse gerar um escore de probabilidade para cada paciente com transtorno de humor que refletisse o risco de tentar suicídio. Utilizando técnicas de *machine learning*, demonstramos que o risco de suicídio em indivíduos com transtorno de humor pode ser estimado objetivamente a partir de variáveis clínicas facilmente obtidas e variáveis demográficas. Embora tenhamos encontrado uma boa acurácia (72%) e área (*area under the curve*) (77%), futuros estudos podem integrar dados de variáveis de marcadores biológicos (genética, neuroimagem, neurocognição, etc.), usando também técnicas de *machine learning*, para atingir uma acurácia ainda maior. O uso de um instrumento objetivo é o primeiro passo na busca de um tratamento mais personalizado para aqueles pacientes com alto risco de se suicidar.

Palavras-chave: Transtorno bipolar. Transtorno de estresse pós-traumático. Inflamação. Suicídio. Machine learning. Medicina personalizada. Big data.

ABSTRACT

The comorbidity between mood disorders and posttraumatic stress disorder (PTSD) is associated with poorer clinical outcomes. Although this association is well established in major depressive disorder, fewer studies included patients with bipolar disorder (BD). These studies report that patients with BD and PTSD show increased number of suicide attempts and worse quality of life. However, no clinical studies so far have reported the impact of comorbid PTSD on core features of BD, such as number of mood episodes and functional impairment. On the other hand, preclinical studies showed that there is a cross-sensitization between BD and PTSD. Accordingly, it was proposed that the brain-derived neurotrophic factor (BDNF) and changes of inflammatory markers might be the biological underpinnings of this cross-sensitization. Although the changes in inflammatory markers are well established in BD, this is not the case for PTSD. In view of the above, the first two studies of this thesis addressed clinical and biological issues related to BD and PTSD. The first is a meta-analysis and metaregression study that showed increased levels of IL-6, IL-1 β , TNF- α and interferon- γ in peripheral circulation among patients with PTSD. Furthermore, illness duration was positively associated with IL-1 β levels, while the severity of the symptoms was positively associated with IL-6 levels. These findings may not only provide a new biological mechanism to explain the cross-sensitization between PTSD and BD, but also opens a new avenue in the search for new therapeutic targets and diagnostic strategies for PTSD. The second was a case-control study that assessed core clinical features of BD showed increased manic episodes and functional impairment among patients with BD and comorbid PTSD. In addition, those patients were younger when they started the manic episodes and substance use. Moreover, we chose to address the influence of comorbid anxiety disorders (such as PTSD) in suicide in patients with mood disorders. This study was conducted in a different population, including patients with major depressive disorder or BD, and it was focused on only one outcome. Suicide is a tragic clinical outcome, but highly preventable. Our aim was to develop a clinical tool using machine learning techniques to estimate a probability score at individual level to stratify the risk of a

patient with a mood disorder attempts suicide. Therefore, our study showed that the risk of suicide in individuals with a mood disorder can be objectively estimated from easily assessed clinical and demographic variables. Although we have found a good accuracy (72%) and area under the curve (77%), future studies may integrate data from biological markers (genetic, neuroimaging, neurocognition, etc.) also using machine learning techniques to achieve a higher accuracy. This objective instrument is the first step towards a more personalized treatment for those patients at high risk of suicide.

Keywords: Bipolar disorder. Posttraumatic stress disorder. Inflammation. Suicide. Machine learning. Personalized medicine. Big data.

1 - APRESENTAÇÃO

Este trabalho surgiu do interesse nos efeitos da associação entre transtorno do estresse pós-traumático (TEPT) e transtorno bipolar (TB), por ser uma situação de difícil manejo clínico. Ao olhar este tema a partir da percepção clínica de uma vulnerabilidade dos pacientes com TB aos efeitos do trauma, percebemos a importância de estudar os marcadores inflamatórios e os efeitos do TEPT nos desfechos clínicos do TB. Embora no campo do TB já existam fortes evidências científicas do envolvimento da resposta inflamatória, no TEPT os estudos são mais escassos e controversos. Isso motivou o primeiro artigo de revisão e metanálise, para entendermos o ponto de partida em relação à resposta inflamatória no TEPT. Em seguida, investigamos o impacto clínico da associação TB e TEPT, pois apesar de hipóteses e ensaios pré-clínicos demonstrarem que o TEPT poderia levar a um curso mais grave do TB, poucos estudos clínicos abordaram tal questão diretamente. Os achados de piores desfechos associados ao TB comórbido com TEPT são descritos no segundo artigo. Entre os desfechos associados à comorbidade no TB, o suicídio é um dos mais desafiadores. A comorbidade com transtornos ansiosos, como o TEPT, pode ter um papel importante na piora do risco de suicídio. Para investigar esta associação optamos por uma abordagem inovadora, que utilizou análise de *machine learning* para estratificar objetivamente o risco de suicídio. Conforme esperado, entre os fatores clínicos, a presença de TEPT foi um dos fatores com maior peso para identificar pacientes que tentaram o suicídio.

Os trabalhos que compõem esta tese foram desenvolvidos entre os anos de 2013 e 2015 em duas localidades: no Laboratório de Psiquiatria Molecular, localizado no Centro de Pesquisas Experimentais do Hospital de Clínicas de Porto Alegre, UFRGS, sob a orientação da Profa. Dra. Márcia Kauer-Sant'Anna e no Center for Molecular Psychiatry e Center of Excellence on Mood Disorders localizados no Behavioral and Biomedical Sciences Building da University of Texas Health Science Center at Houston (Texas, USA), sob a orientação do Prof. Dr. Flávio Kapczinski (Doutorado Sanduíche).

A presente tese de doutorado resultou em três artigos: 1) "Inflammatory markers

in posttraumatic stress disorder: a meta-analysis and meta-regression study”; 2) “Clinical outcomes associated with comorbid posttraumatic stress disorder among patients with bipolar disorder”; 3) “Identifying a clinical signature of suicide attempts among patients with mood disorders”. O primeiro artigo foi aceito na revista “The Lancet Psychiatry”. O artigo foi comentado por duas pesquisadoras especializadas em exposição ao trauma e TEPT: Dra. Vasiliki Michopoulos e Dra. Tanja Jovanovic do Department of Psychiatry and Behavioral Sciences da Emory University School of Medicine, Atlanta, Georgia (USA). O comentário se encontra na seção de anexos. O segundo artigo foi aceito na revista “Journal of Clinical Psychiatry” e selecionado para a seção “Early Career Psychiatrists”. O terceiro artigo foi submetido na revista “The American Journal of Psychiatry”. Na sessão anexos, também se encontrar os artigos que foram produzidos durante o período de doutorado.

2 – Introdução

2.1. O transtorno bipolar

A prevalência do transtorno bipolar (TB) tipo 1 e 2 é de aproximadamente 2% da população mundial, com as outras formas de TB afetando mais 2% (1). As taxas de suicídio completo entre pacientes com TB são de 7.8% em homens e 4.9% em mulheres (2), o que o torna a sexta causa de anos perdidos por doença entre adultos jovens (3). O foco do tratamento convencional do TB é a estabilização dos sintomas agudos e prevenção de recaídas, porém 37% dos pacientes recaem em depressão ou mania dentro de um ano e 60% dentro de dois anos (4). Adicionalmente, o diagnóstico do TB tem um atraso médio de 10 anos entre os primeiros sintomas e o diagnóstico formal (5) e somente 20% dos pacientes que apresentam um episódio depressivo são corretamente diagnósticos com TB (6).

Evidências recentes sugerem que determinados preditores estão associados a um curso mais grave da doença (7,8). Foi proposto que a densidade do número de episódios de humor, a exposição ao estresse (comorbidade com TEPT ou trauma precoce) e o uso de cocaína podem apresentar sensibilização a si mesmo e sensibilização cruzada entre si levando a progressão da morbidade do TB (9) (Figura 1). A progressão da morbidade do TB está associada a tentativas de suicídio, redução do intervalo entre os episódios, refratariedade, prejuízo do funcionamento e elevadas taxas de hospitalização (9). Os fundamentos biológicos desse fenômeno ainda são pouco conhecidos e a maior parte das evidências provém de estudo pré-clínicos (9). Supõe-se, entretanto, que o decréscimo das neurotrofinas, como o “brain derived neurotrophic factor” (BDNF), e alterações no sistema imune desempenhem um papel fundamental (9).

Guiados pela hipótese acima,

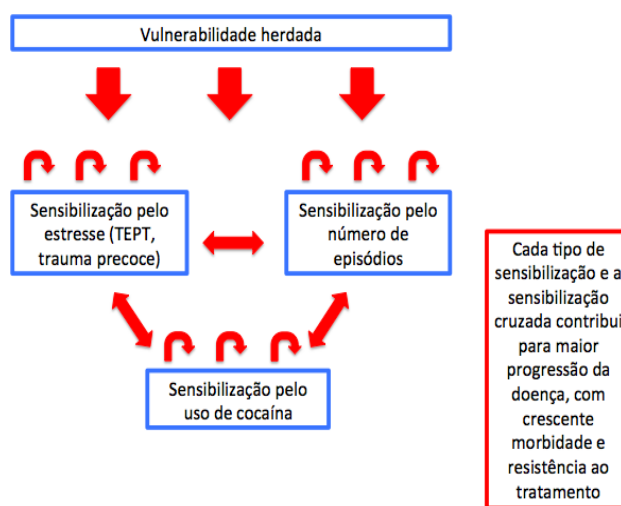


Figure 1. Mecanismo de progressão do transtorno bipolar. }

optamos por estudar primeiramente os aspectos biológicos e os desfechos clínicos associados à comorbidade entre TEPT e TB. Em um segundo momento, discutiremos como a presença de comorbidades, sobretudo a comorbidade com transtornos ansiosos e uso de substâncias, podem influenciar um desfecho de grande relevância médica e social nos transtorno de humor: o suicídio.

2.2. A comorbidade entre transtorno bipolar e transtorno do estresse pós-traumático

Embora ensaios pré-clínicos sugerem que a comorbidade com TEPT leve a um pior prognóstico em pacientes com TB, o quadro clínico em humanos e os fundamentos biológicos são poucos conhecidos.

2.2.1. Fundamentos biológicos

Alterações nas neurotrofinas

A principal neurotrofina implicada no TB é o BDNF. Ele é responsável pela proteção, sobrevivência e proliferação neuronal. Foi demonstrado que o BDNF reduz durante os episódios maníacos e depressivos da doença (10). Além disso, foi observado que com a evolução para estágios mais tardios da doença os níveis de BDNF se reduzem (8). Por outro lado, também é sabido que os níveis de BDNF encontram-se reduzidos no soro de pacientes com TEPT (11). Ainda nessa linha, foi também relatado que pacientes com TB expostos à trauma apresentam níveis de BDNF ainda mais baixos comparados àqueles sem trauma (12). Todas essas evidências levaram a criação de uma hipótese de que a redução do BDNF seria um provável mediador entre a comorbidade de TEPT e TB, levando a um pior prognóstico do TB (figura 2) (13).

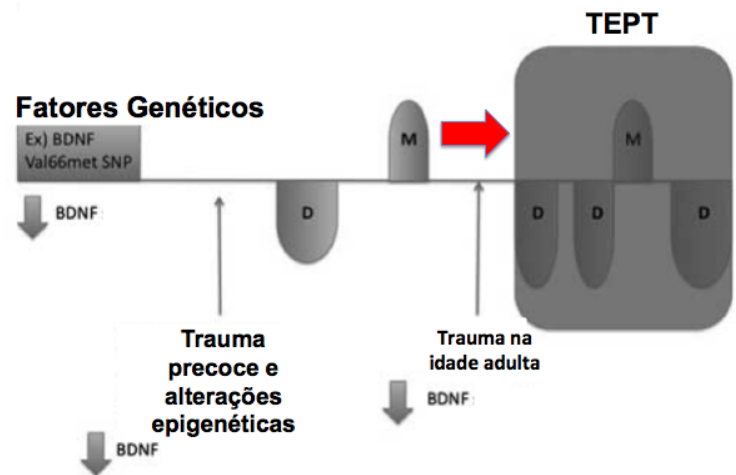


Figure 2. Redução nos níveis do BDNF resultantes de fatores genéticos, exposição ao trauma precoce e transtorno do estresse pós-traumático levariam a um pior prognóstico no transtorno bipolar, com maior densidade de episódios de humor. Adaptado da referencia 13. Abreviações: BDNF, brain derived neurotrophic fator; D, episódio depressivo; M, episódio maníaco; TEPT, transtorno do estresse pós-traumático.

Alteração de marcadores inflamatórios no transtorno bipolar

As alterações nos marcadores inflamatórios no TB têm sido demonstradas tanto na circulação sanguínea periférica como no sistema nervoso central (14). Estudos recentes têm relatado a ativação de algumas células que compõe o sistema imune, como a micróglia (15,16), os macrófagos e os linfócitos T (17,18), que levam à liberação de citocinas. Citocinas são pequenas proteínas responsáveis pela regulação do sistema imune tanto na resposta inata como na adaptativa. A ação dessas moléculas, entretanto, pode ser ainda mais abrangente, com efeitos no sistema neuroendócrino, na neurogênese e no metabolismo dos neurotransmissores (19). Embora seu papel já esteja estabelecido nas doenças cardiovasculares e autoimunes por exemplo, o interesse no estudo dessas proteínas nas doenças psiquiátricas e neurodegenerativas está apenas começando (20).

Uma metanálise recente com 30 estudos demonstrou elevação dos níveis do fator da necrose tumoral α (TNF- α), receptor solúvel do TNF- α 1 (sTNF-R1), interleucina (IL)-4, IL-6, receptor solúvel da IL-6 (sIL-6R), IL-10, antagonista do receptor da interleucina 1 (IL-1RA) e receptor solúvel da IL-2 (sIL-2R) no sangue de pacientes com TB (21). Outra metanálise com objetivos similares, mas com critérios de inclusão mais estritos, demonstrou elevação dos níveis de TNF- α , sTNFR1, sIL-2R, sIL-6R e IL-4 em pacientes com TB (22) comparado com controles saudáveis. Uma terceira metanálise avaliou os marcadores periféricos de acordo com o estado de humor, relatando que pacientes em episódio maníaco apresentam níveis elevados de sTNF-R1 e TNF- α comparado com pacientes eutímicos (23). Por outro lado, um recente estudo caso-controle demonstrou níveis diminuídos de sTNF-R1 em pacientes com TB em episódio depressivo comparado com pacientes no estado maníaco ou eutímico (24). Por fim, com relação a evolução do TB, parece haver uma redução dos níveis de IL-6 juntamente a uma elevação dos níveis de TNF- α à medida que o paciente atinge os estágios mais tardios do transtorno (8).

Achados recentes também demonstraram que existe um processo neuroinflamatório no cérebro de pacientes com TB. Células da glia, como a micróglia e a astroglia, são responsáveis pela manutenção da homeostase e proteção dos

neurônios no cérebro (25). As células da micróglia, por exemplo, são as principais células do sistema imune no cérebro, tornando-se ativadas durante a neuroinflamação (26). Estudos post-mortem do córtex frontal do cérebro demonstraram níveis elevados de marcadores de ativação da micróglia e da astroglia (glial fibrillary acidic protein, inducible nitric oxide synthase, c-fos e CD11b) nos pacientes bipolares, bem como aumentos na proteína e no RNA mensageiro de marcadores inflamatórios, como a IL-1 β , receptores de IL-1 β , fator de diferenciação mielóide 88, subunidades do fator nuclear kappa- β (NF κ β) e enzimas da cascata do ácido araquidônico (ciclooxigenase e prostaglandina-E sintetase) (27,28). Adicionalmente, estudos com positron emission tomography (PET) scan demonstraram ativação microglial no hipocampo direito de pacientes com TB (16).

Em linha com esses achados, foi relatado que estabilizadores do humor regulam a apoptose de células da micróglia. O ácido valpróico, por exemplo, atenuou a superativação microglial em ratos (29). O lítio, por sua vez, inibe a ativação microglial através da cascata PI3K/Akt/FoxO1 (30). Diante desses novos achados em relação ao mecanismo de ação dos estabilizadores do humor, alguns grupos de pesquisa realizaram ensaios clínicos com drogas imunomoduladoras e anti-inflamatórias nos transtornos de humor com resultados satisfatórios. Um estudo duplo-cego, randomizado e controlado por placebo usando celecoxib (medicação antiinflamatória que age inibindo a ciclooxigenase 2) como estratégia para potencializar o tratamento com estabilizadores do humor demonstrou um rápido início dos efeitos antidepressivos no grupo de pacientes com episódios mistos ou depressivos que utilizaram essa medicação (31). Finalmente, um ensaio randomizado controlado por placebo demonstrou eficácia do infliximab, um medicamento imunomodulador que age antagonizando o TNF- α , no tratamento da depressão resistente nos pacientes que apresentavam elevados níveis de proteína C reativa no início do estudo (32).

Alterações dos marcadores inflamatórios no transtorno do estresse pós-traumático

Embora as alterações nos marcadores inflamatórios no TB esteja estabelecida,

inclusive com a realização de alguns ensaios clínicos com medicamentos antiinflamatórios, esse não é o caso no TEPT. É sabido que o TEPT está associado com doenças onde a ativação imune assume um papel central, como doenças coronarianas (33), diabetes (34) e doença aterosclerótica (35). Além disso, sabe-se que o TEPT está associado com a incidência aumentada de doenças autoimunes (36) e pode desencadear ou piorar doenças inflamatórias (37). A persistências dos sintomas de TEPT estão também associados ao envelhecimento precoce, um processo fisiológico associado à alteração dos marcadores inflamatórios (38). Enquanto esses achados apontam para um papel sistêmico da ativação imune no TEPT, o que não é sabido é que marcadores inflamatórios estão alterados nessa doença e qual o papel deles como biomarcadores.

Estudos investigando marcadores inflamatórios no TEPT levaram a resultados contraditórios (39,40). Esses achados inconsistentes poderiam ser justificados por variáveis confundidoras como uso de psicotrópicos, doença inflamatória concomitante, tempo que se passou entre o evento traumático e a coleta do exame, gravidade dos sintomas de TEPT e o tipo de ensaio realizado para quantificar os marcadores inflamatórios. Diante disso, o objetivo do primeiro artigo da presente tese foi buscar definir quais marcadores inflamatórios estavam alterados no TEPT e qual era o papel deles como potenciais marcadores de gravidade da doença e de tempo de doença. Portanto, foram utilizadas técnicas de metanálise e de meta-regressão, que até então não tinham sido realizadas no campo do TEPT. O esclarecimento de que marcadores inflamatórios estão alterados no TEPT é o primeiro passo na busca da compreensão de como a inflamação pode mediar a sensibilização cruzada entre TEPT e TB.

2.2.2. Desfechos clínicos

Apesar de hipóteses e ensaios pré-clínicos demonstrarem que o TEPT poderia levar a um curso mais pernicioso do TB, poucos estudos clínicos haviam tentado abordar tal questão. O TEPT tem prevalência ao longo da vida de 7.6% na população

em geral (41). Assim como o TB, o TEPT tem sido independentemente associado à prejuízo da produtividade no trabalho (42,43), aumento da incidência da comorbidade com doenças cardiovasculares (33,44) e endócrinas (34,44), tentativas de suicídio (45,46) e alterações estruturais nos exames de neuroimagem (47–49). Surpreendentemente, alguns estudos têm demonstrado que a comorbidade entre TEPT e TB é muito frequente, mesmo comparado com a comorbidade de TEPT com esquizofrenia e transtorno depressivo maior (50–52). Em uma amostra de pacientes adultos em atendimento primário, aqueles com TB tiveram 2,9 vezes mais probabilidade de apresentar sintomas atuais de TEPT comórbido comparados àqueles sem TB (53). Corroborando esses achados, a “National Comorbidity Survey Replication” demonstrou que a prevalência ao longo da vida de TEPT entre pacientes com TB é de 24% (1).

Apesar dessa frequente coocorrência, a relevância clínica da comorbidade com TEPT entre os paciente com TB é praticamente desconhecida (54). O TEPT é frequentemente não reconhecido na prática clínica entre os pacientes com TB (55). Dois estudos transversais avaliaram especificamente essa questão (51,56). Um deles demonstrou que a comorbidade com TEPT em adolescentes com TB está associada com tentativas de suicídio (51). O outro relatou que a comorbidade com TEPT está associada com piora na qualidade de vida e aumento do risco de suicídio (56). Um terceiro estudo analisou o impacto de todos os transtornos de ansiedade em pacientes com TB e demonstrou, em uma subanálise, que pacientes com TEPT apresentavam mais tentativas de suicídio (57). Não existe, entretanto, um estudo clínico que demonstre que o TEPT é capaz de piorar características centrais do TB, como o número de episódios maníacos por exemplo. Portanto, em virtude da alta prevalência de TEPT em paciente com TB, nosso grupo conduziu o segundo artigo avaliando as alterações em desfechos clínicos de pacientes bipolares naqueles que apresentavam os dois transtornos. Um segundo desfecho avaliado nesse estudo foi a piora do funcionamento psicossocial em quem possuía os dois transtornos comparado a quem só possuía o TB.

Funcionamento psicossocial no transtorno bipolar

Pacientes com TB podem sofrer com prejuízo no funcionamento mesmo quando eutímicos, apresentando disfunção nas atividades sociais e na produtividade no trabalho (58). Um estudo de 24 meses de seguimento achou que praticamente 100% dos pacientes com TB obtiveram recuperação sindrômica após 2 anos da internação pelo primeiro episódio maníaco, porém somente um terço apresentou recuperação funcional (59). Portanto, a recuperação funcional e sintomática não estão necessariamente associadas e podem necessitar de diferentes estratégias de tratamento.

Uma forte associação linear foi achada entre uma escala que avalia o funcionamento em pacientes com TB, The Functioning Assessment Short Test (FAST) scores, e os estágios clínicos proposto por Kapczinski et al (60), sugerindo um progressivo declínio funcional do estágio I até o estágio IV nos pacientes com TB (61). De maneira similar, utilizando análise de classe latente em uma amostra de 106 pacientes com TB em remissão, um estudo identificou dois subtipos de pacientes: um apresentando “bom” e outro apresentando “mal” prognóstico com relação ao funcionamento (62). Inteligência verbal estimada, controle inibitório, densidade de episódios e nível dos sintomas depressivos residuais apareceram como os mais significativos preditores para determinar os subtipos (62). Digno de nota, o tempo de doença não foi um preditor de mal funcionamento psicossocial, uma vez que ambos os grupos eram comparáveis com relação a idade, idade de início e duração da doença (62). O impacto de comorbidades psiquiátricas, como o TEPT, também não tinha sido avaliado.

2.3. Suicídio nos transtornos de humor

Quando um paciente com um episódio depressivo se apresenta para a primeira entrevista com um psiquiatra qual a porcentagem de chance de ele ter um comportamento suicida? Essa questão é um tema fundamental no tratamento dos

transtornos de humor e apresenta grande importância social, visto que o suicídio é um evento trágico, mas altamente prevenível (63). Em 2010, o suicídio foi responsável por 4,8% e 5,7% do total de mortes no mundo em indivíduos do sexo feminino e masculino de 15-49 anos respectivamente (64). Sabe-se também que aproximadamente 90% dos indivíduos que morrem por suicídio são diagnosticadas com um transtorno psiquiátrico - sobretudo transtorno depressivo maior, TB, esquizofrenia, transtornos relacionados ao uso de substâncias e transtornos de personalidade (66). Apesar da relevância clínica, o suicídio ainda é amplamente negligenciado e pouco se progrediu na busca de ferramentas para estratificar seu risco objetivamente ou na busca de tratamentos específicos. Em virtude disso, alguns autores chamam o suicídio de "a epidemia silenciosa" (63). Este quadro é particularmente preocupante nos transtornos do humor, em virtude da alta prevalência e da forte associação de suicídio e sintomas depressivos (67).

O suicídio era considerado uma resposta extrema a um evento catastrófico, como a perda de um parente próximo ou mesmo a perda de um emprego importante. Vários indivíduos, entretanto, e mesmo alguns pacientes com transtorno de humor, passam por esses estressores e mesmo assim não tentam se suicidar. Diante dessas observações, um conjunto de evidências vem demonstrando fatores clínicos e biológicos associados aos pacientes que tentam suicídio (65,66). Esses fatores foram relacionados como preditores associados ao desenvolvimento do comportamento suicida (66). Portanto, além do estressor agudo, é importante identificar que tipo de paciente possui um padrão mais propício a tentar suicídio.

Com relação aos fatores clínicos, um recente estudo demonstrou que as comorbidades psiquiátricas desempenham um papel fundamental no desenvolvimento do um comportamento suicida (67). Sabe-se também que os fatores de risco clínicos para a tentativa de suicídio compartilhados pelo TB e pelo transtorno depressivo maior são semelhantes. A maioria se refere a comorbidades com transtornos ansiosos e por uso de substâncias, mas também alguns fatores demográficos (45,57,68-91). O que não se sabe, entretanto, é como agrupar tais fatores na construção de uma ferramenta que possa objetivamente identificar o padrão de um paciente que possa tentar suicídio.

Tal ferramenta poderia estratificar o risco de suicídio numa escala de probabilidade de 0 a 100%. A falta de tal informação é importante porque a atual estratificação do risco de suicídio é subjetiva por natureza, sobretudo naqueles que ainda não tentaram suicídio. Buscando desvendar essa questão, nosso grupo utilizou uma técnica chamada *machine learning* que vem ganhando espaço no meio científico e que tem o potencial de avançar significativamente o campo da psiquiatria em direção a uma medicina personalizada.

2.4 Métodos Avançados para Análise de Dados

2.4.1. Meta-regressão

Metanálise é um método estatístico consolidado capaz de agrupar tamanhos de efeito (de um mesmo desfecho) oriundos de múltiplos estudos independentes na busca de um tamanho de efeito geral. Uma limitação desse modelo estatístico, entretanto, é como explicar as diferenças nos desenhos do estudo. Especificamente nessa tese, buscamos metanalisar os marcadores inflamatórios no TEPT. Uma grande questão, entretanto, era como lidar com o fato de que alguns estudos incluíram pacientes com depressão comórbida ao comparar os marcadores inflamatórios em paciente com TEPT e controles saudáveis. Essa questão era pertinente em virtude de uma metanálise recente ter demonstrado que pacientes com transtorno depressivo maior apresentam alteração nos marcadores inflamatórios (92). Com a finalidade de abordar esse fato, buscamos realizar um estudo com meta-regressão.

Em linhas gerais, a meta-regressão é uma espécie de regressão múltipla aplicada a estudos de metanálise. Com esse modelo, podemos determinar se variáveis contínuas ou categóricas relacionadas ao desenho do estudo (moderadores) podem influenciar o tamanho de efeito do estudo. Especificamente nessa tese, consideramos que variáveis como comorbidade com depressão maior, uso de medicação, tipo de ensaio realizado (ELISA ou outro) e a hora da coleta do sangue (em jejum ou não)

poderiam explicar porque alguns estudos achavam resultados positivos e outros negativos em determinados marcadores inflamatórios. Digno de nota, esses moderadores foram selecionados, pois eles estão associados a alterações inflamatórias (92–94).

Além de explicar porque alguns estudos apresentam resultados diferentes usando moderadores relacionados ao desenho dos estudos, a meta-regressão pode também estabelecer relações entre os moderadores e o tamanho de efeito geral. Por exemplo, alguns marcadores inflamatórios podem aumentar em função da gravidade dos sintomas de TEPT ou ainda em função do tempo de doença. A importância desse último aspecto está na busca de biomarcadores que possam estar associados a algumas características da doença.

2.4.2 Machine learning

Para o terceiro estudo usamos técnicas de *machine learning*. Tipicamente, os algoritmos utilizados no *machine learning* são mais bem aplicados nas situações em que inúmeros atributos devem ser considerados simultaneamente para estimar a probabilidade de que um evento ocorra (95). Enquanto muitas técnicas estatísticas ignoram efeitos pequenos, técnicas de *machine learning* utilizam toda a evidência disponível para executar previsões ou identificar padrões. Se um grande número de características tem relativamente um efeito pequeno quando analisadas em separado, tomadas em conjunto e combinadas podem apresentar um tamanho de efeito bem maior. Quando o modelo é significativo, um escore de probabilidade entre 0 a 100 pode ser inferido para um determinado paciente. Quanto maior a probabilidade, maior será a chance que aquele evento ocorra (ou que aquele padrão seja identificado) naquele indivíduo (96).

Essa técnica de análise fundamentou as bases para responder a questão levantada no primeiro parágrafo da sessão 2.3. Embora o TEPT estivesse associado a tentativas de suicídio em pacientes com TB e transtorno depressivo grave (57),

somente a presença desse transtorno não é capaz de determinar o padrão de um paciente com transtorno de humor que tenta suicídio. Portanto, teríamos que integrar mais variáveis e combiná-las a fim de obter um modelo com acurácia suficiente para identificar um paciente com um perfil de quem tenta suicídio entre os pacientes com transtorno de humor, mesmo que ele não tenha tentado suicídio antes. Escolhemos, portanto, variáveis clínicas e demográficas baseadas na literatura científica para compor esse modelo. Digno de nota, a escolha da população de pacientes com transtorno de humor e não apenas aqueles com TB se deu por dois motivos: 1. As variáveis associadas ao suicídio nos dois transtornos são semelhantes; 2. Nosso objetivo era criar uma ferramenta que pudesse abranger a grande quantidade de pacientes com transtornos de humor que se apresentam na atenção primária.

Ao final, o principal resultado de um estudo com *machine learning* é a produção de um instrumento que é capaz de dar um escore entre 0 e 100 para cada paciente. Quanto mais próximo do 100 maior a probabilidade daquele paciente ter um padrão suicida.

3 - Justificativa

Embora esteja presente em quase 1/4 dos pacientes com TB, **o estudo do TEPT em pacientes com TB** permanece incipiente. Novas descobertas nesse campo podem avançar significativamente o tratamento de subgrupos de pacientes com TB em direção a uma medicina mais personalizada. Dessa forma, **o estudo do impacto clínico, assim como dos fundamentos biológicos** relacionados aos dois transtornos pode levar ao desenvolvimento de novos alvos terapêuticos, bem como à descoberta de novos biomarcadores que possam ser utilizados como instrumentos na avaliação da gravidade do transtorno, da resposta terapêutica, etc.

Ainda, o estudo do suicídio no TB e o peso das comorbidades com transtornos ansiosos (como o TEPT) e com transtornos do uso de substâncias nas tentativas de suicídio é muito relevante. Em particular, a busca de uma abordagem que possa gerar um instrumento prático e útil na **identificação do padrão do paciente que tenta suicídio** tem alto valor clínico. Tal instrumento pode mudar a forma como estratégias terapêuticas para prevenir suicídio são aplicadas na prática clínica de uma forma subjetiva para uma forma mais objetiva.

4 – Objetivos

4.1. Objetivo Geral

Estudar o impacto do transtorno do estresse pós-traumático comórbido em pacientes com transtorno bipolar.

4.2. Objetivos Específicos

- Determinar os desfechos clínicos associados à comorbidade com transtorno do estresse pós-traumático em paciente com transtorno bipolar.
- Determinar se marcadores inflamatórios estão alterados nos pacientes com transtorno

do estresse pós-traumático, bem como seus papéis como biomarcadores de gravidade e duração do transtorno.

- Desenvolver um instrumento clínico capaz de identificar o padrão do paciente com transtorno de humor que tenta suicídio e que possa também estratificar a probabilidade de um determinado paciente apresentar esse padrão em um nível individualizado.

5 – Aspectos éticos e legais

O estudo “Clinical outcomes associated with comorbid posttraumatic stress disorder among patients with bipolar disorder” foi aprovado pelo Institutional Review Boards da University of Texas Health Science Centers at San Antonio and University of North Caroline at Chapel Hill. Os participantes assinaram o consentimento informado antes de quaisquer procedimentos relacionados com o estudo, após uma descrição completa do estudo com tempo adequado para perguntas.

O estudo “Identifying a clinical signature of suicide attempts among patients with mood disorders” foi aprovado pelo Institutional Review Boards da University of Texas Health Science Center at Houston. Todos os participantes assinaram o consentimento informado antes de quaisquer procedimentos relacionados ao estudo com tempo amplo para perguntas.

6 – Artigos

6.1. Artigo 1

Carta de aceite:

29/06/2015

Dear Flavio,

I am pleased to tell you that your submission, INFLAMMATORY MARKERS IN POSTTRAUMATIC STRESS DISORDER: A META-ANALYSIS AND META-REGRESSION STUDY, has been accepted for publication in The Lancet Psychiatry.

Within the next 1-2 weeks you will receive, electronically, a set of PDF page proofs of your article. Please note that because of our varied and international readership, The Lancet Psychiatry edits heavily to a strict house style. Thus, changes will inevitably be made to your manuscript at this time. There will also probably be a few final editorial queries at this stage. Please correct and return these pages by the deadline stated in the covering email. Because your paper is entered into our fast-track publication process this deadline will usually be between 24 and 48 hours.

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Please contact me if you have any further queries regarding your manuscript.

Best wishes,

Joan

Dr. Joan Marsh

Deputy Editor, The Lancet Psychiatry

Email: joan.marsh@lancet.com

Versão do manuscrito aceita:

INFLAMMATORY MARKERS IN POSTTRAUMATIC STRESS DISORDER: A META-ANALYSIS AND META-REGRESSION STUDY

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ABSTRACT

Background: Studies investigating inflammatory markers in Posttraumatic Stress Disorder (PTSD) have yielded mixed results.

Methods: Meta-analysis and meta-regression of studies comparing inflammatory markers between subjects with PTSD and healthy control subjects (HCs). We also performed subgroup meta-analysis using “presence of comorbid Major Depressive Disorder (MDD)” and “use of psychotropic medication” as predictors. We searched PubMed, Embase, Scopus, Web of Science, and PsycInfo.

Findings: A total of 8057 abstracts were identified and twenty studies were included. IL-6 (SMD=0.88;p<0.001), IL-1 β (SMD=1.42;p=0.045), and interferon- γ (SMD=0.49;p=0.002) levels were higher in PTSD group. Subgroup meta-analysis of unmedicated subjects showed higher TNF- α (SMD=0.69;p<0.001) in PTSD group in addition to the aforementioned cytokines. TNF- α (SMD=1.32;p<0.001), IL-1 β (SMD=2.35;p=0.048), IL-6 (SMD=1.75;p<0.001) levels remained increased in PTSD group in subgroup meta-analysis of studies that excluded comorbid MDD. Illness duration was positively associated with IL-1 β levels (b=0.33,p<0.001) and severity with IL-6 (b=0.02,p=0.042). A model composed by “presence of comorbid MDD”, “use of psychotropic medications”, “assay performed”, and “time of the day the blood was collected” variables explained large amount of heterogeneity of IL-1 β , IL-6, and CRP studies.

Interpretation: PTSD is associated with increased IL-6, IL-1 β , TNF- α , and interferon- γ levels. This information may be useful to deal with chronic low-grade of inflammation as a potential target or biomarker in PTSD treatment. “Use of psychotropic medications” and “presence of comorbid MDD” were important moderators that explain the lack of consistency on results of previous studies.

Key Words: Posttraumatic stress disorder, Meta-analysis, Inflammatory markers.

INTRODUCTION

Posttraumatic Stress Disorder (PTSD) has a lifetime prevalence of 10–12% in women and 5–6% in men¹. PTSD has been associated with suicide attempts², missed workdays^{3,4}, and cognitive impairment⁵. It is known that PTSD is associated with illnesses where the immune activation plays a key role, such as coronary heart disease⁶, diabetes⁷ and atherosclerosis⁸. It is also known that PTSD is associated with increased incidence of autoimmune diseases⁹, and can trigger or worsen inflammatory disorders¹⁰. Furthermore, persistence of PTSD symptoms has been associated with accelerated aging, a physiological process associated with inflammation¹¹. While these findings point to a systemic role of immune activation among subjects with PTSD, what is not known is which inflammatory markers are increased in subjects with PTSD and their potential role as biomarkers.

Inflammatory markers, such as cytokines and interferon (IFN), are key signaling molecules of the immune activation that exert effects in the periphery and in the brain¹². Recently published meta-analyses have shown increased levels of these markers in subjects with major depressive disorder (MDD)¹³, schizophrenia¹⁴ and bipolar disorder¹⁵. Studies investigating inflammatory markers in subjects with PTSD have yielded mixed results^{16,17}. These inconsistent findings might be explained by use of psychotropic or anti-inflammatory medications, comorbidity with other psychiatric disorders, concurrent inflammatory disease, time elapsed since the traumatic event, severity of PTSD symptoms, and type of assay performed to assess the inflammatory markers. The aim of the present study is to compare levels of inflammatory markers among subjects with PTSD and HCs using meta-analysis from available studies in the literature. We will also explore sources of heterogeneity among studies using meta-regression and subgroup meta-analysis.

METHODS AND MATERIALS

Search strategy and selection criteria

This study was performed according to the “Preferred reporting items for systematic reviews and meta-analyses (PRISMA)”¹⁸. We searched PubMed, Embase, Scopus, Web of Science, and PsycInfo for articles published until April 7th, 2015 using the following keywords: (“Inflammation” OR “Immune Activation” OR “Interleukin” OR “Cytokine” OR “Interferon” OR “Lymphocyte” OR “Macrophage” OR “Microglia” OR “Tumor Necrosis Factor-alpha” OR “C-Reactive Protein” OR “Transforming growth factor”) AND (“Posttraumatic Stress Disorder” OR PTSD). Mesh terms were used in PubMed and Emtree terms were used in Embase. We also searched the reference lists of included studies and contacted experts in the field of PTSD for unpublished data. Two researchers (I.C.P. and M.P.V.M.) independently selected the studies and extracted the data from articles.

The inclusion criteria were a) Cross-sectional studies or baseline of data from longitudinal studies of peripheral blood cytokine levels comparing adult subjects (>18 years) with PTSD to healthy controls (HCs); b) Studies that used well-validated diagnostic criteria for PTSD; c) Studies that showed the mean and standard deviation (SD) of peripheral blood cytokines (authors were contacted if there is missing information). Exclusion criteria were a) Subjects with severe comorbid axis 1 disorder (bipolar disorder and psychotic disorder), severe medical illness or autoimmune/inflammatory disease; b) Use of anti-inflammatory or immunomodulatory drugs; c) review articles; d) Inflammatory markers concentrations assessed in cerebrospinal fluid or brain tissue; e) peripheral inflammatory markers assessed after stimulation; f) Assessments of gene polymorphisms. If the study assessed peripheral inflammatory markers and gene polymorphisms, it was included, but only the data related to the inflammatory markers were analyzed in the meta-analysis. Since several studies assessed subjects with PTSD and comorbid MDD, we included these studies. However, a subgroup meta-analysis was performed to differentiate the effect size of PTSD subjects with and without MDD. We didn’t limit our search to English language publications.

Data extraction

The following characteristics were extracted from each study: name of the first author; publication year; number, gender and age of the subjects; mean and SD of peripheral blood cytokines (if instead of mean and SD, other values were reported, such as median, we requested data from the corresponding author); type of diagnostic instrument used; time of the day the blood was collected; type of sample (serum or plasma); type of assay; whether the study allowed subjects with comorbid MDD in PTSD group; whether subjects are drug free; illness duration; and severity of PTSD symptoms assessed by Clinician-Administered PTSD Scale (CAPS). We classified subjects with PTSD as drug free if they were not in current use of psychotropic medications.

Data Analysis

We used the package metafor from R to conduct the meta-analysis and meta-regression analysis¹⁹. First, we performed meta-analyses whenever values of inflammatory markers were available in two or more studies¹⁹. A random-effects model with restricted maximum-likelihood estimator (REML) was used to synthesize the effect size across studies. Random-effects model with REML can incorporate both within-study variability and between-study variability^{19,20}. Because studies used different assessment methods, the standardized mean difference (SMD) was used to assess the effect size¹⁹. SMD was calculated by means of Cohen's d. The significance level for this meta-analysis model was 0.05¹⁹. An effect size of 0.2 is considered as indicating a low effect, while 0.5 is a moderate effect and 0.8 or more is a large effect²¹. Egger's test was used to assess publication bias²². This is a test for asymmetry of the funnel plot, and it was performed whenever three or more studies were included²². For this specific test p-values < 0.1 shows significant asymmetry and therefore publication bias²². If Egger test revealed a potential publication bias, we used Trim and fill method to test the data²². We also used the "leave-one-out" function¹⁹ for conducting sensitivity analyses. This method consists in removing one study at a time from the dataset to run the meta-analysis without it. This analysis tests if the effect size of the meta-analysis is driven by one study. Of note, the method should not be regarded as a way of yielding a more "valid" estimate of the overall effect or outcome, but as a way of examining the sensitivity of the results to one particular selection mechanism. We used the Q-statistic to test the existence of

heterogeneity and I² to assess the proportion of total variability due to heterogeneity²³. A value of the I² around 25%, 50%, and 75% could be considered as low-, moderate-, and high-heterogeneity, respectively²³. τ^2 was used to estimate the total amount of heterogeneity²³.

Second, we performed subgroup meta-analysis of inflammatory marker levels to determine the impact of “use of psychotropic” and “presence of comorbid MDD” in inflammatory markers levels. We performed subgroup analysis for these two specific moderators, given their well-know effects on inflammatory markers and their clinical implications^{13,24}. On one hand, psychotropic medications, such as antidepressants and antipsychotics, was associated with decreased levels of proinflammatory cytokines in patients with PTSD^{14,24}. On the other hand, a recent meta-analysis showed abnormal inflammatory markers in patients with MDD¹³.

Third, we explored other sources of heterogeneity among studies using meta-regression analysis^{25,26} and linear mixed effects model. In addition to “presence of comorbid MDD”¹³ and “use of psychotropic”²⁴, we selected other variables to compose a multiple meta-regression model that are reported in all included studies and may influence the association between PTSD and changes in inflammatory markers: “type of assay performed” (ELISA or other than ELISA) and “time of the day the blood was collected” (fasting or postprandial state)²⁷. Both variables are more related to methodological procedures. Meta-regression was performed whenever the number of included studies for a specific cytokine was larger than the number of moderators¹⁹. The ‘variance accounted for’ (VAF), a pseudo-R² statistic, is given for the model, indicating the percentage of the total heterogeneity in the true effects (heterogeneity) that is accounted for the model with all covariates included.

Since “illness duration” and “PTSD symptoms assessed by CAPS” were not available in all included studies, we performed a separate univariate meta-regression analysis using these variables whenever at least three studies assessed them for a specific cytokine¹⁹. The aim was to explore the role of inflammation as biomarkers of severity and illness duration.

Role of Funding Source

No sponsor was involved in any aspect of the study, including design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript. The corresponding author (FK) had access to all data used in study and had final responsibility for decision to submit for publication.

RESULTS

Figure 1 shows the flow chart describing the study selection process. Study selection, quality of studies, and characteristics of included studies (table S1) were described in supplemental material.

Meta-analyses

Concentrations of interleukin (IL)-1 β , IL-6, and IFN- γ were higher in subjects with PTSD compared to HCs (Table 1), with moderate to high effect sizes (SMD ranging from 0.49 to 1.42). No between group differences were observed for IL-2, IL-4, IL-8, IL-10, sIL-2R, sIL-6R, CRP, and TNF- α (Table 1). Egger test revealed a potential publication bias for IL-1 β . Trim and fill method resulted in the same parameter estimates for IL-1 β , given that there was no need to input studies according to the non-parametric algorithm implemented in metafor package. In addition, for all inflammatory markers study heterogeneity was found to be high ($I^2 > 75\%$). The significance remains robust when leave-one-out models were carried out for IL-6. However, for IL1- β , significance testing revealed to be non-significant after removing the following studies: Tucker P et al., 200424 (SMD=0.88; $p=0.11$), Spivak B et al., 199728 (SMD=1.46; $p=0.08$), and Oganesyanyan LP et al., 200929 (SMD=1.07; $p=0.14$).

Subgroup meta-analysis for MDD comorbidity and psychotropic medication

Levels of IL-1 β and IL-6 remained significantly different in subgroup meta-analysis of unmedicated subjects with PTSD (Figure 2 and 3). Unmedicated subjects showed higher TNF- α levels in the PTSD group, with a moderate effect size (Figure 4). IL-1 β , IL-6, TNF- α levels remained increased in PTSD group in subgroup meta-analysis of

studies that excluded comorbid MDD (Figures 2,3, and 4). Subgroup meta-analysis using “presence of comorbid MDD” and “use of psychotropic medication” as predictors variables were not performed for IL-2, IL-4, IL-8, IL-10, sIL-2R, sIL-6R, CRP, IFN- γ since there weren't at least two studies in each group to be meta-analyzed.

Investigating other sources of heterogeneity (meta-regression analyses)

We found that the multiple models with four predictors significantly explained between study heterogeneity for IL-1 β , IL-6, and CRP (Table 2). For IL-1 β and CRP, the moderators explained all model heterogeneity with no significant residual heterogeneity. “Use of psychotropic” and “time of the day the blood was collected” decreased between-group differences on both markers, whereas “type of assay performed” increased (Table 2). For IL-6 the moderators significantly explained a large amount of the heterogeneity, but residual heterogeneity was still detected. “Use of psychotropic” increased between group differences for IL-6, whereas “presence of MDD” decreased group differences. We detected an interaction between “use of psychotropic” and “presence of MDD” variables (estimate=-1.52; p=0.016) for IL-6. Post-hoc stratified analysis showed that the effect of the medication in reducing IL-6 levels is significant only for studies that excluded patients with comorbid MDD (k=6, estimate=2.06; p<0.0001), but not for studies that allowed patients with MDD (k=9, estimate=0.56, p=0.2011). Meta-regression analyses for IL-4, IL-8, sIL-2R, sIL-6R, and INF- γ were not performed because of the limited number of studies.

Supplemental analysis to investigate the potential role of inflammatory markers

Separate univariate meta-regression model using the variable “illness duration” as a predictor was significant (b=0.33, p<0.001) for IL-1 β , which means that the duration of the disorder was positively associated with IL-1 β levels. Of note, coincidentally all studies that assessed IL-1 β and reported illness duration included only drug-free subjects with PTSD. No significant association was found for IL-6 and TNF- α . This analysis was not performed for other inflammatory markers because of the limited number of studies that assessed such variable.

A separate univariate meta-regression using “PTSD symptoms assessed by CAPS” as a predictor was significant ($b=0.02$, $p=0.042$) for IL-6, which means that severity of the disorder was positively associated with IL-6 levels. This analysis was not performed for other inflammatory markers because of the limited number of studies that assessed such variable.

DISCUSSION

This is the first meta-analysis and meta-regression of inflammatory markers in PTSD. It reports that IL-1 β , IL-6, and IFN- γ levels were higher in subjects with PTSD. The effect size was large for IL-1 β and IL-6, and it was moderate for IFN- γ . Subgroup meta-analysis shows that TNF- α levels were also increased in drug free subjects with PTSD (moderate effect size). Even when subjects with PTSD and comorbid MDD were excluded from the analysis, levels of IL-1 β , IL-6, and TNF- α remained significant. Moreover, duration of PTSD symptoms was positively associated with higher levels of IL-1 β in unmedicated subjects, and severity was positively associated with IL-6. In meta-regression analysis, “presence of comorbid MDD”, “use of psychotropic medications”, “type of assay performed”, and “time of the day the blood was collected” variables explained 100%, 79,7%, and 100% of the heterogeneity of studies that assessed IL-1 β , IL-6, and CRP, respectively. However, the test for residual heterogeneity remained significant for IL-6, which would suggest the presence of unmeasured moderators. These findings have pathophysiological, clinical, and therapeutic implications.

Regarding pathophysiological implications, IL-6, IL-1 β , and TNF- α can decrease neurogenesis, potentially leading to central nervous system volume loss³⁰⁻³², which could explain partly the association of PTSD and volume reductions of hippocampus, prefrontal cortex, and middle temporal gyrus³³. Furthermore, several studies reported the effects of IL-1 β on memory formation and consolidation³⁴, a physiological process that is dysregulated in PTSD³⁵. For instance, central administration of IL-1 β in the rat can impair memory, and prior consumption of eicosapentaenoic acid can block this cognitive effect³⁴. It seems that the immune activation³⁴ occurs in absence of infection or

autoimmune primary pathophysiological process in PTSD¹². The psychological stress can be a trigger for a process that probably perpetuates and takes to a systemic chronic sterile low-grade of inflammation, as happens in atherosclerosis and in neurodegenerative disorders^{12,36,37}. This may explain the association between PTSD and diseases where the immune activation plays a key role³⁸, such as cardiovascular, metabolic and autoimmune diseases¹². Moreover, systemic chronic inflammation can accelerate aging via Reactive Oxygen Species (ROS)-mediated exacerbation of telomere dysfunction and cell senescence³⁹, which explains the association between PTSD and aging¹¹. Finally, although both MDD and PTSD seem to have immune activation, some pathophysiological differences may explain in part the differences in clinical picture: 1) the meta-analysis of inflammatory markers in MDD didn't found increased levels of IL-1 β in patients with MDD; 2) a study showed higher levels of serum cortisol and lower peak thyroid-stimulating hormone (TSH) response to thyrotropin-releasing hormone (TRH) in the MDD patients than in either the PTSD patients or controls⁴⁰.

The clinical implications are related to the potential use of inflammatory mediators as biomarkers of severity, illness duration, and illness activity. Our findings point to a potential use of IL-1 β as a biomarker of illness duration and IL-6 as a biomarker of severity. Previously, a cross-sectional study with a small sample size (19 subjects with PTSD) has shown a positive correlation of length of symptoms and IL-1 β in drug free subjects²⁸, but no study has reported the association of IL-6 and symptom severity. However, the potential role of IL-6 as a biomarker of illness activity was showed in a cross-sectional study⁴¹. Women that recovered from PTSD showed plasma IL-6 levels significantly lower than those of women with persistent PTSD, and comparable to those of never-traumatized controls⁴¹. Moreover, a recent cross-sectional study showed that higher sIL-6R levels differentiated persistent versus remitted PTSD¹⁷.

The development of medications with anti-inflammatory properties that address chronic inflammation, lowering IL-6, TNF- α , INF- γ and IL-1 β levels, might be of value in PTSD treatment. The IL-1 β inhibitors canakinumab and anakinra, the IL-6 inhibitor tocilizumab, the TNF- α inhibitor infliximab, and the antagonist of IL-6 and TNF- α methotrexate are

promising alternatives. For instance, large-scale Phase III trials are now underway with canakinumab and methotrexate to target such chronic low degree of inflammation in atherosclerosis^{42,43}. Moreover, interventions such as smoking cessation and aerobic exercise program are linked to reductions in peripheral inflammation, as well as reduced PTSD symptoms^{44,45}.

An important strength of our systematic review was the search strategy, since we have used a variety of databases and made an exhaustive effort to acquire data by contacting the authors. Our study has some limitations. High levels of between-study heterogeneity were observed for the majority of cytokine parameters measured in our analysis. However, meta-regression analysis could explain a large amount of this heterogeneity. Residual unexplained heterogeneity could be related to a) body mass index (BMI); b) smoking status; c) physical activity; d) blood pressure; e) alcohol consumption; f) genetics. All these variables are related to inflammatory changes, but the majority of studies didn't report them. For instance, a recent study showed that genetic variability in the CRP gene is associated with serum CRP level and PTSD symptom severity, including that of hyperarousal symptoms⁴⁶. Unfortunately this study didn't meet the inclusion criteria for the present meta-analysis, since there is no comparison of a group diagnosed with PTSD versus a control group. Another limitation was the number of studies to perform meta-regression and subgroup analysis for some cytokines. For instance, meta-regression analysis of CRP indicated that "use of psychotropic medication" was an important moderator to explain between-study heterogeneity, however subgroup analysis was not carried out because only one study excluded medicated patients¹⁶. Moreover, some of the meta-regression models may be subjected to over fitting given the number of model parameters relative to the number of included studies. Over fitting would lead to an overstatement of the variance explained by the moderator variables. Of note, three studies^{17,47,48} included in our meta-analysis allowed healthy controls possibly exposed to trauma but without PTSD in the control group. Although those controls don't have any psychiatric or physical illness, we do not know to what extent exposure to trauma may trigger long-term changes in inflammatory markers in healthy subjects, even if they have not developed PTSD. Finally, although serum

levels of interleukin 1 β are elevated in patients with PTSD in our meta-analysis, “leave-one-out” method showed that the significant effect size might be driven by a small number of studies. Therefore, more studies are needed for a definitive conclusion in this issue.

In summary, the contribution of the present study was to clarify results reported previously, showing that PTSD is associated with a pattern of immune activation. Future longitudinal studies examining the mechanism of how traumatic events are linked to inflammatory markers and its potential consequences may offer new insight on how to treat and how to prevent the emergency of PTSD in subjects exposed to psychological trauma⁴⁹. Interventions that address chronic low-grade inflammation may be potentially useful in PTSD patients.

RESEARCH IN CONTEXT

Evidence before this study

We searched Pubmed, Embase, Scopus, Web of Science, and PsycInfo using the search terms: ("Inflammation" OR "Immune Activation" OR "Interleukin" OR "Cytokine" OR "Interferon" OR "Lymphocyte" OR "Macrophage" OR "Microglia" OR "Tumor Necrosis Factor-alpha" OR "C-Reactive Protein" OR "Transforming growth factor") AND ("Posttraumatic Stress Disorder" OR PTSD). Mesh terms were used in Pubmed and Emtree terms were used in Embase. Articles published between 1960 and April 7th, 2015 were included. The inclusion criteria were original studies and reviews about inflammatory markers in PTSD. We didn't limit our search to English language publications.

Added value of this study

This is the first meta-analysis and meta-regression of inflammatory markers in PTSD. Our study clarify that PTSD is associated with a pattern of immune activation, including increased IL-6, IL-1 β , TNF- α , and interferon- γ levels. Meta-regression analysis and subgroup meta-analysis showed that "use of psychotropic medications" and "presence of comorbid MDD" were important confounders that explain the lack of consistency on results of previous studies.

Implications of all the available evidence

Our study showed that immune dysregulation takes place in PTSD. This raises the interesting possibility of using anti-inflammatory strategies as potential therapeutic targets or biomarkers in PTSD. Moreover, the findings of the present study suggest chronic low-grade inflammation as potential mediator of the association between PTSD and cardiovascular, metabolic, and immune diseases.

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AUTHORS' CONTRIBUTIONS

Dr Passos participated in the study design, data collection, data analysis, interpretation of findings, figures, literature search, writing, and approval of final manuscript. Dr Moreno participated in the study design, data collection, writing, and approval of final manuscript. Dr Costa participated in the study design, data collection, literature search, and approval of final manuscript. Dr Kunz participated in the study design, literature search, writing, and approval of final manuscript. Dr Brietzke participated in the study design, literature search, writing, and approval of final manuscript. Dr Quevedo participated in the study design, interpretation of findings, writing, and approval of final manuscript. Dr Salum participated in the study design, data analysis, interpretation of findings, writing, and approval of final manuscript. Dr Magalhães participated in the study design, data analysis, interpretation of findings, writing, and approval of final manuscript. Dr Kapczinski participated in the study design, data analysis, interpretation of findings, literature search, writing, and approval of final manuscript. Dr Kauer-Sant'Anna participated in the study design, data analysis, interpretation of findings, literature search, writing, and approval of final manuscript.

CONFLICT OF INTEREST STATEMENTS

Dr Passos reported no biomedical financial interests or potential conflicts of interest. Dr Vasconcelos-Moreno reported no biomedical financial interests or potential conflicts of interest. Dr Costa reported no biomedical financial interests or potential conflicts of interest. Dr. Brietzke reported no biomedical financial interests or potential conflicts of interest. Dr Kunz reported no biomedical financial interests or potential conflicts of interest. Dr Quevedo reported no biomedical financial interests or potential conflicts of interest. Dr Magalhaes reported no biomedical financial interests or potential conflicts of interest. Dr Salum reported no biomedical financial interests or potential conflicts of interest. Dr Kapczinski has received grants/research support from AstraZeneca, Eli Lilly, Janssen-Cilag, Servier, NARSAD, and the Stanley Medical Research Institute; has been a member of speakers' boards for AstraZeneca, Eli Lilly, Janssen and Servier; and has served as a consultant for Servier. Dr Kauer-Sant'Anna is on speaker/advisory boards for, or has received research grants, from NARSAD, Stanley Medical Research Institute, and Eli-Lilly.

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FIGURES:

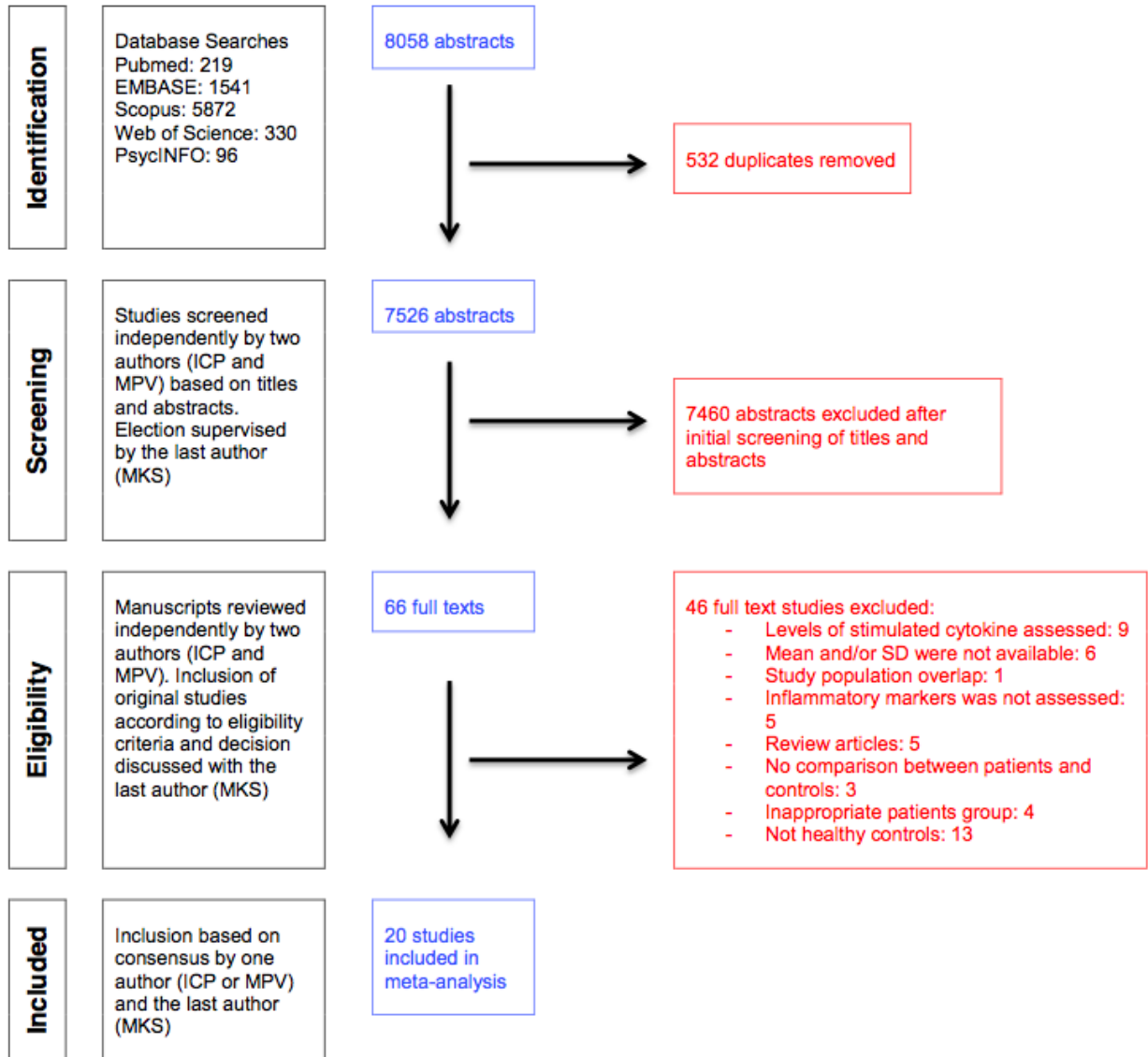


Figure 1. Flowchart of review process and study selection

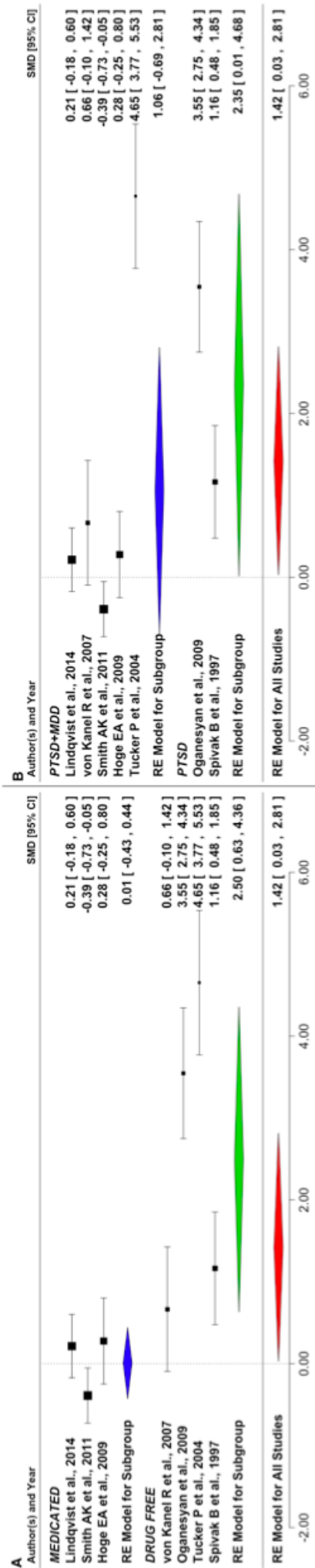


Figure 2. Meta-analysis of Interleukin-1β. A. Subgroup meta-analysis using “use of psychotropic medication” as a predictor. B. Subgroup meta-analysis using “comorbid MDD” as a predictor. PTSD, Posttraumatic Stress Disorder; RE, Random-effects model; SMD, standardized mean difference.

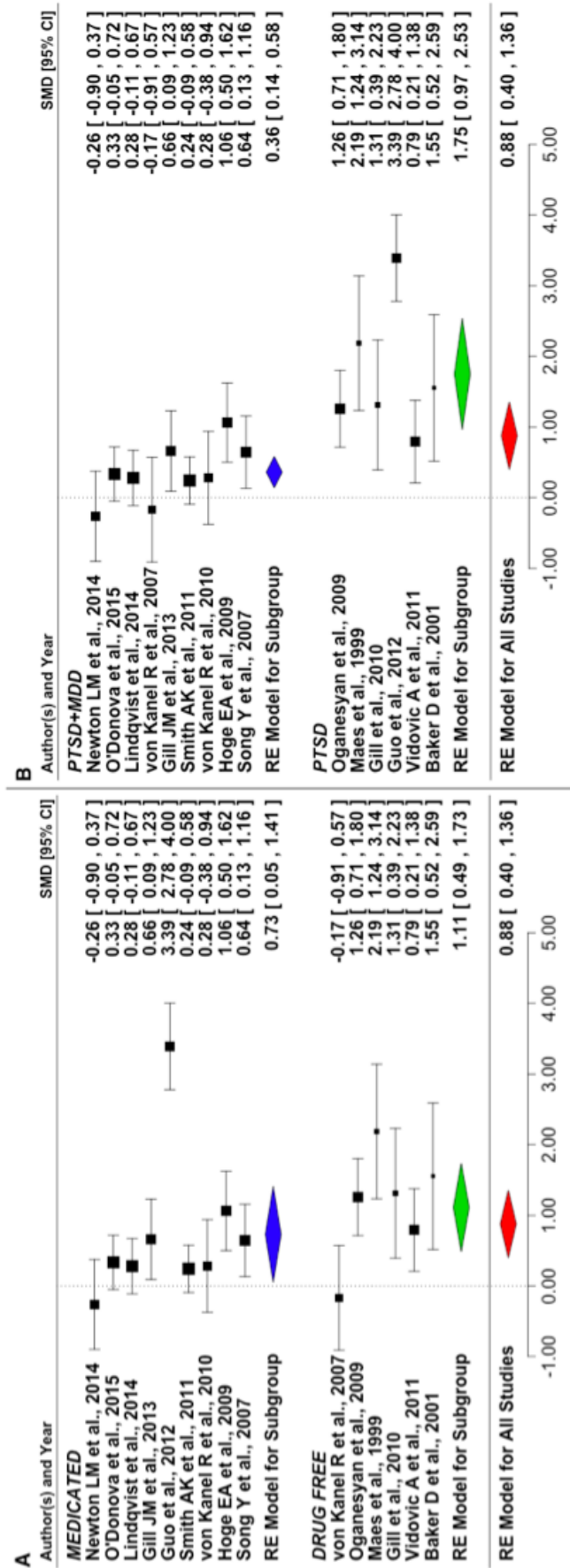


Figure 3. Meta-analysis of Interleukin-6. A. Subgroup meta-analysis using "use of psychotropic medication" as a predictor. B. Subgroup meta-analysis using "comrbid MDD" as a predictor. PTSD, Posttraumatic Stress Disorder; RE, Random-effects model; SMD, standardized mean difference.

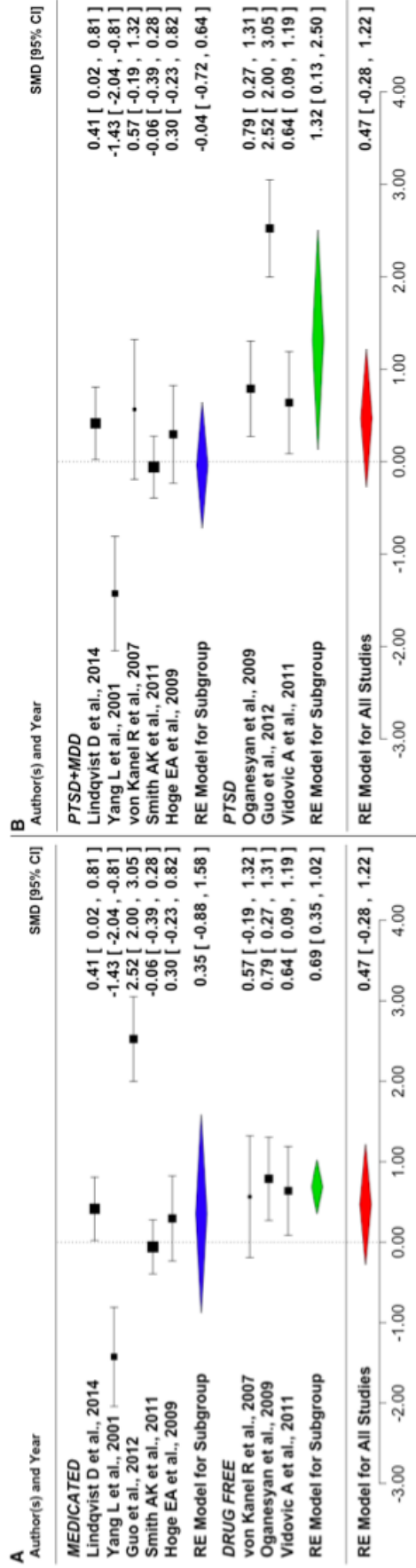


Figure 4. Meta-analysis of TNF- α . A. Subgroup meta-analysis using “use of psychotropic medication” as a predictor. B. Subgroup meta-analysis using “comrbid MDD” as a predictor. PTSD, Posttraumatic Stress Disorder; RE, Random-effects model; SMD, standardized mean difference.

TABLES:

Table 1. Meta-analysis of inflammatory markers in Posttraumatic Stress Disorder

	N: PTSD / HC	SMD (95% CI)	p Value	Heterogeneity			Egger's test: z; p
				Q-statistic (df; p)	τ^2	I^2	
IL-1β	248 / 294	1.42 (0.03 to 2.81)	0.045	174.62(6; p<0.001)	3.43	97.7%	3.41; <0.001
IL-2	177 / 273	0.08(-1.10 to 1.25)	0.896	104.83(4; p<0.001)	1.74	96.7%	-0.27; 0.780
IL-4	139 / 222	0.35(- 0.89 to 1.59)	0.581	83.18(3; p<0.001)	1.53	96.0%	-0.10; 0.914
IL-6	398 / 615	0.88 (0.40 to 1.35)	<0.001	124.37(14;p<0.001)	0.78	90.8%	1.50; 0.133
IL-8	112 / 112	0.70 (-1.11 to 2.49)	0.451	77.34(2; p<0.001)	2.47	97.3%	-0.10; 0.918
IL-10	190 / 273	0.46 (-0.22 to 1.15)	0.184	42.53(4; p<0.001)	0.54	91.0%	-0.29; 0.770
sIL-2R	77 / 40	-2.64(-7.19 to 1.90)	0.255	65.65(1; p<0.001)	10.62	98.4%	NA
sIL-6R	22 / 58	1.54 (-0.33 to 3.42)	0.106	10.20(1; p=0.001)	1.65	90.2%	NA
CRP	131 / 136	-0.14(-0.84 to 0.56)	0.692	29.42(4; p<0.001)	0.55	86.5%	-0.66; 0.504
IFN-γ	79 / 79	0.49 (0.18 to 0.81)	0.002	0.52(1; p=0.468)	0	0%	NA
TNF- α	278 / 356	0.47 (-0.28 to 1.22)	0.218	106.61(7; p<0.001)	1.09	94.5%	-0.09; 0.920

Abbreviations: CI, confidence interval; CRP, C-Reactive Protein; df, degrees of freedom; GM-CSF, Granulocyte Macrophage Colony Stimulating Factor; HC, Healthy controls; I^2 , I squared; IL, interleukin; IFN- γ , interferon- γ ; N, number of subjects; NA, not applicable; PTSD, Posttraumatic Stress Disorder; sIL-2R, soluble IL-2 receptor; sIL-6R, soluble IL-6 receptor; SMD, standardized mean difference; TNF- α , Tumor Necrosis Factor- α ; X², chi squared.

Table 2. Multiple meta-regression model of inflammatory markers in posttraumatic stress disorder

	Test of Moderators	VAF	Test for Heterogeneity	Residual	Estimated	SE	p-value	Lower	Upper
IL-1β	QM(df:4)=173.21, p<0.001	100%	QE(df:2)=1.41, p=0.493						
<i>Use of psychotropic</i>					index				
No (reference)									
Yes					-4.80	0.43	<0.001	-5.65	-3.95
<i>Assay performed</i>					index				
ELISA (reference)									
Other					0.70	0.22	0.002	0.26	1.14
<i>Blood Collection</i>					index				
Fasting (reference)									
Postprandial state					-3.49	0.42	<0.001	-4.31	-2.67
<i>Presence of comorbid MDD</i>					index				
No (reference)									
Yes					0.59	0.42	0.16	-0.23	1.40
IL-2*	QM(df:3)=6.62, p= 0.084	49.3%	QE(df:1)=18.04, p<0.001						
<i>Assay performed</i>					index				
ELISA (reference)									
Other					0.42	1.19	0.723	-1.91	2.76
<i>Blood Collection</i>					index				
Fasting (reference)									
Postprandial state					-0.34	1.38	0.804	-3.06	2.37
<i>Presence of comorbid MDD</i>					index				
No (reference)									
Yes					-2.90	1.19	0.015	-5.23	-0.57
IL-6	QM(df:4) = 36.40, p<0.001	79.7%	QE(df:10)=27.09, p=0.002						
<i>Use of psychotropic</i>					index				
No (reference)									
Yes					1.21	0.44	0.006	0.34	2.08
<i>Assay performed</i>					index				
ELISA (reference)									
Other					0.12	0.29	0.683	-0.46	0.70
<i>Blood Collection</i>					index				
Fasting (reference)									
Postprandial state					-0.17	0.32	0.595	-0.81	0.46
<i>Presence of comorbid MDD</i>					index				
No (reference)									
Yes					-2.29	0.42	<0.001	-3.12	-1.46
IL-10**	QM(df:3)=5.21, p=0.156	39.7%	QE(df:1)=6.49, p=0.010						
<i>Use of psychotropic</i>					index				
No (reference)									
Yes					0.30	0.91	0.745	-1.49	2.09
<i>Assay performed</i>					index				
ELISA (reference)									
Other					0.62	0.74	0.402	-0.83	2.08
<i>Presence of comorbid MDD</i>					index				
No (reference)									
Yes					-1.59	0.86	0.064	-3.28	0.09
CRP***	QM(df:3)=28.58, p<0.001	100%	QE(df:1) = 0.84, p=0.358						
<i>Use of psychotropic</i>					index				
No (reference)									
Yes					-2.51	0.62	<0.001	-3.72	-1.29
<i>Assay performed</i>					index				
ELISA (reference)									
Other					1.72	0.35	<0.001	1.03	2.40
<i>Blood Collection</i>					index				
Fasting (reference)									
Postprandial state					-1.16	0.38	0.002	-1.91	-0.42
TNF-α	QM(df:4)=3.98, p=0.408	0%	QE(df:3)=43.67, p<0.001						
<i>Use of psychotropic</i>					index				
No (reference)									
Yes					0.55	1.11	0.616	-1.62	2.73
<i>Assay performed</i>					index				
ELISA (reference)									
Other					-0.80	1.10	0.465	-2.96	1.35
<i>Blood Collection</i>					index				
Fasting (reference)									
Postprandial state					-0.51	1.11	0.643	-2.70	1.67
<i>Presence of comorbid MDD</i>					index				
No (reference)									

Yes

-0.94

1.18

0.426

-3.26

1.38

* "Use of psychotropic" didn't enter in this model since all studies included allowed the use of psychotropic medications. ** "Blood Collection" didn't enter in this model since all studies included allowed the use of psychotropic medications. *** "Presence of comorbid MDD" didn't enter in this model since all studies allowed patients with comorbid PTSD.

Abbreviations: CRP, C-reactive protein; IL, interleukin; MDD, major depressive disorder; TNF- α , tumor necrosis factor α ; VAF, variance accounted for;

SUPPLEMENTAL MATERIAL:

Study selection

The initial literature search identified 8058 abstracts from databases. A total of 532 duplicates were removed, and 7460 abstracts were excluded after initial screening of titles and abstracts. Sixty-six potentially eligible articles remaining, and their full text were reviewed for more detailed analysis¹⁻⁶⁶. Forty-six studies were excluded (see Figure 1 for reasons). Twenty studies (see supplemental material - Table S1) with a total of 1342 participants (603 subjects with PTSD and 739 HCs) were included in the meta-analysis. Peripheral blood levels of eleven inflammatory markers were assessed in 2 studies or more.

Characteristics and Quality of Studies

Most studies reported age and gender of subjects and HCs, showing a similar distribution between groups (Table S1). Eleven studies excluded subjects with PTSD medicated with psychotropic drugs (Table S1). Seven studies excluded subjects with comorbid MDD from PTSD group (Table S1). Most studies assessed serum or plasma concentrations of inflammatory markers using enzyme-linked immune sorbent assay (ELISA). Coefficients of variation were reported by 13 studies^{1,3,4,13,14,19,21,35,43,44,56,57,66}. PTSD was diagnosed using DSM III or IV in 17 studies; International Classification of Disorders (ICD)-10 in two studies^{27,43}; and Chinese Classification of Mental Disorders (CCMD)-2-R in one study⁵⁴. Fasting blood samples were collected in 13 studies^{2-4,14,19,21,24,27,35,36,43,56,57}.

Table S1. Characteristics of studies included in the meta-analysis of inflammatory markers levels in Post-traumatic Stress Disorder

Study, Year	Inflammatory Markers Assessed	N (PTSD, HC)	Gender: % Male (PTSD, HC)	Age (PTSD, HC)	CAPS score	Blood Fraction	Illness Duration (years)	Excluded MDD*	Medicated
Azcurra DS et al., 2010 ³	ICAM1/ VCAM1/ eSelectin	48 (24/24)	50/50	45±10/44±11	NR	Plasma	NR	No	No
Baker DG et al., 2001 ⁴	IL-6	19 (11/8)	100/NR	42.2±3/41.3±3	NR	Plasma	NR	Yes	No
Gill J et al., 2010 ¹³	IL-6	32 (18/14)	72/29	NR/ 34±11	69±10	Plasma	NR	Yes	No
Gill JM et al., 2013 ¹⁴	IL-6/CRP	77 (53/24)	0/0	NR/ 34.79±8.5	NR	Plasma	1.7	No	Yes
Guo M et al., 2012 (30)	IL-2/IL-4/IL-6/IL-8/IL-10 /TNF-α	100 (50/50)	44/50	42±12/41±14	NR	Serum	9.5±6.2	Yes	Yes
Hoge EA et al., 2009 ¹⁹	MIP-1α/MCP-1/IL-1α/IL-1β/IL-2/IL-4/IL-5/L-6/IL-7/IL-8/IL-10/IL-12p40/IL-13/IL-15/Eotaxin/GM-CSF/IFN-γ/IP-10/IL-12p70/TNF-α	56 (28/28)	NR/NR	41.2±11.3/41.7±11	NR	Plasma	NR	No	Yes
Lindqvist et al., 2014 ⁵⁶	IL-6, IL-1β, TNF-α, IFN-γ, CRP	102 (51/51)	100/100	34.1±8.7/33.7±9.0	67.8±16.9	Serum	NR	No	No
Maes M et al., 1999 ⁴⁹	IL-6/sIL-6R/sgp130/IL-1RA/CC16/sCD8	39 (7/32)	44/16	44.7±11.5/45.3±7.7	NR	NR	NR	Yes	No
Muhtz C et al., 2011 ²⁴	CRP	50 (25/25)	36/36	71±0.5/71±0.4	NR	NR	NR	No	Yes
Newton LM et al., 2014 ⁶⁶	sIL-6R	41 (15/26)	0/0	53.55±2.64 / 54.92±3.43	32±13	Plasma	12.1±13.1	No	Yes
O'Donovan et al., 2015 ⁵⁷	IL-6 e sTNFRII	172 (40/132)	(77/77)	42.12±8.63 / 45.02±10.18	63.23 ±15.38	NR	NR	No	No
Oganesyan LP et al., 2009 ²⁷	TNF-α/IL-1β/IL-6	62 (31/31)	87/87	42±4.6/39±43.1	NR	Serum	13	Yes	No
Smith AK et al., 2011 ⁶³	IL6/IFN-α/IL1β/IL-2/TNF-α/IL-4/IL-10	177 (47/130)	NR	NR	NR	Plasma	NR	No	Yes
Song Y et al., 2007 ³⁵	IL-2/IL-6/IL-8	68 (34/34)	38/38	40.4±10.9/37.6±11	NR	Serum	NR	No	Yes
Spivak B et al., 1997 ³⁹	IL-1β/sIL-2R	38 (19/19)	100/100	25.3±10.9/31.7±10.4	NR	Serum	7.5±6.9	Yes	No
Tucker P et al., 2004 ²	IL-1β/sIL-2R	79 (58/21)	26/NR	NR	88.74 ±14.20	Serum	NR	No	No

Vidović A et al., 2011 ⁴³	TNF- α /IL-6	64 (39/25)	100/100	38.5 \pm 9.1/ 32.6 \pm 8.6	69 \pm 3	Serum	5.8 \pm 0.3	Yes	No
von Kanel R et al., 2007 ¹	CRP/IL-1 β /IL-6/TNF- α /IL-4/IL-10	28 (14/14)	64/64	33 \pm 10/ 33 \pm 11	NR	Plasma	4 \pm 2.9	No	No
von Kanel R et al., 2010 ⁴⁴	IL-6/CRP	44 (15/29)	73/83	58.38 \pm 7.4/ 58.68 \pm 6.9	43 \pm 10	Plasma	2.7 \pm 0.4	No	Yes
Yang L et al., 2001 ⁵⁴	GM-CSF, TNF- α , IL-2	52 (21/31)	NR/NR	NR	NR	Serum	NR	No	Yes

* Some studies did not report standard deviation for years of disease. CRP, C-Reactive Protein; CCMD, Chinese Classification of Mental Disorders; DSM, Diagnostic and Statistical Manual of Mental Disorders; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; GM-CSF, Granulocyte-macrophage colony-stimulating factor; HC, Healthy controls; ICAM-1, Intercellular Adhesion Molecule 1; ICD, International Classification of Diseases; IFN- γ , interferon- γ ; IL, interleukin; IL-1RA, interleukin-1 receptor antagonist; IP-10, Interferon gamma-induced protein 10; MCP-1, monocyte chemotactic protein 1; MDD, Major Depressive Disorder; MIP-1 α , Macrophage Inflammatory Protein 1 α ; NR, not reported; sIL-2R, soluble IL-2 receptor; sIL-6R, soluble IL-6 receptor; Sgp130, soluble glucoprotein 130; sTNFRII, soluble receptor for tumor necrosis factor type II; TNF- α , Tumor Necrosis Factor- α ; VCAM-1, Vascular cell adhesion protein 1.

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6.2. Artigo 2

Carta de aceite:

Dear Dr. Kapczinski:

RE: J15-M09935R

CLINICAL OUTCOMES ASSOCIATED WITH COMORBID POSTTRAUMATIC STRESS
DISORDER AMONG PATIENTS WITH BIPOLAR DISORDER

I am pleased to inform you that your manuscript has been accepted for publication in The Journal of Clinical Psychiatry. I am forwarding it to our publisher, Physicians Postgraduate Press, for final editing, and their office will contact you about a month before your article is published. <http://www.psychiatrist.com>

Congratulations to you and your co-authors!

Thank you.

Sincerely,

Erika F. H. Saunders, MD

Special Section Editor, Early Career Psychiatrists

Versão do manuscrito aceita para publicação:

CLINICAL OUTCOMES ASSOCIATED WITH COMORBID POSTTRAUMATIC STRESS DISORDER AMONG PATIENTS WITH BIPOLAR DISORDER

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ABSTRACT

Objective: To assess clinical outcomes associated with the presence of lifetime history of comorbid posttraumatic stress disorder in subjects with bipolar disorder.

Method: Cross-sectional study of 284 subjects with bipolar disorder assessing the association between lifetime comorbid posttraumatic stress disorder and clinical characteristics. Participants were included from January 2006 to June 2009. We assessed age of onset and number of mood episodes, presence of rapid cycling, first drug use, suicide attempts, hospitalizations, functional impairment, and quality of life. Diagnostic, clinical, and functional assessments were carried out using the Structured Clinical Interview for DSM Disorders (SCID), the Functioning Assessment Short Test (FAST), and the World Health Organization Quality of Life scale (WHOQOL).

Results: The prevalence of lifetime comorbid posttraumatic stress disorder was 19.7% (56 subjects). Subjects with bipolar disorder and posttraumatic stress disorder presented an accelerated course of illness, with a lower age of onset of manic/hypomanic episodes ($p=0.009$), and earlier initiation on illicit drug use ($p=0.008$). In addition, they were more likely to be younger at the age they received the diagnosis of bipolar disorder ($p=0.036$), and presented a higher number of manic/hypomanic episodes ($p=0.01$). Quality of life was worse in all domains among subjects who presented the comorbidity, as well as higher rates of functional impairment.

Conclusion: Comorbid posttraumatic stress disorder was associated with increased morbidity and accelerated illness progression among subjects with bipolar disorder.

Keywords: bipolar disorder; posttraumatic stress disorder; manic episode; functional impairment; drug use.

INTRODUCTION

Lifetime prevalence of bipolar disorder (BD) is 2.1% worldwide, with subthreshold forms affecting another 2.4%¹. Posttraumatic stress disorder (PTSD) has an estimated lifetime prevalence of 7.6% in general population². Both disorders have been independently associated with missed workdays^{3,4}, comorbid cardiovascular^{5,6} and endocrine diseases^{6,7}, suicide attempts^{8,9}, and structural brain changes in magnetic resonance imaging¹⁰⁻¹². In a sample of adult primary care patients, those with BD were 2.9 times as likely to screen positive for current PTSD compared with patients without BD¹³. Moreover, the National Comorbidity Survey Replication has shown that the lifetime prevalence of PTSD among patients with BD is 24%¹. When compared to patients with major depressive disorder (MDD) or schizophrenia, patients with BD have a greater risk for PTSD¹⁴⁻¹⁶.

Despite of its frequent co-occurrence, the clinical relevance of PTSD comorbidity among BD patients is largely unknown¹⁷. PTSD is often unrecognized in clinical practice among patients with BD¹⁸. Cross-sectional studies have shown that comorbid PTSD is associated with worse quality of life and higher rates of suicide attempts among patients with BD^{15,19}. In addition, it has been proposed that traumatic stress and number of mood episodes may show sensitization to themselves and cross-sensitization to one another leading to residual vulnerabilities to further occurrences of mood episodes, faster illness stage progression, and early drug misuse²⁰. Therefore, one could hypothesize that the stress-induced behavioral sensitization related to PTSD may be associated with a more pernicious course of BD illness.

Given the high prevalence of comorbid PTSD among patients with BD and the dearth of studies in this field, we set forth to study correlates of accelerated illness progression such as: a) age of onset of first manic/hypomanic and depressive episodes, age of first drug use, and age at first BD diagnosis²¹; b) number of manic/hypomanic and depressive episodes, lifetime hospitalization, rapid cycling, suicide attempts, and drug abuse²²; c) quality of life and functional impairment.

METHOD

We performed a cross-sectional study to assess subjects with BD who presented lifetime PTSD diagnosis vs. those with BD without PTSD. The study was approved by the Institutional Review Boards of the University of Texas Health Science Centers at San Antonio and University of North Carolina at Chapel Hill. Subjects signed informed consent before any study-related procedures after a complete description of the study with ample time for questions. Participants were included from January 2006 to June 2010.

Participants

Subjects were recruited from the community and psychiatric clinics through flyers, radio, and newspaper advertisements. Inclusion criteria were subjects with BD types I, II, or NOS according to DSM-IV, and age between 18 and 65. Exclusion criteria were head trauma with residual effects, neurological disorder, and uncontrolled major medical conditions.

Assessments

Subjects were evaluated through a socio-demographic history form to assess age, gender, years of education, and occupational status. Axis-I diagnoses and clinical characteristics were assessed with the Structured Clinical Interview for DSM-IV axis-I Disorders (SCID-I)²³, which was administered by fully trained staff. In addition, we used SCID to assess psychiatric history, current mood status, number of mood episodes (depressive and manic/hypomanic), age of onset of first episode (depressive and manic/hypomanic), age of first drug use, age of first PTSD symptoms, age first diagnosed with BD (when subject received the first diagnosis by a physician). Current dimensional mood symptoms were assessed with the Hamilton Depression Rating Scale (HDRS)²⁴, the Young Mania Rating Scale (YMRS)²⁵, and Hamilton Anxiety Rating Scale (HARS)²⁶. Data from all instruments were collected regardless of treatment or mood status.

The Functioning Assessment Short Test (FAST) was performed to assess functional impairment²⁷. FAST is a 24-item scale and assesses six functional domains: autonomy, occupational functioning, cognitive, financial issues, interpersonal relationships, and leisure time. Higher scores indicate higher degrees of functional impairment. This scale has been widely used in order to assess functional impairment among BD subjects²⁸. We also performed The World Health Organization Quality of Life (WHOQOL-BREF) to assess quality of life. It was developed by the World Health Organization and validated in several studies²⁹. Apart from the first 2 items of general nature, the remaining 24 items of the instrument comprise four domains: “physical health”, “psychological”, “social relationships”, and “environment”. Higher scores show a higher quality of life.

Statistical Analyses

Statistical analyses were conducted using SPSS software (version 21). Descriptive analyses were reported as means (standard deviations), median (interquartile range) or absolute and relative frequencies. We have used Chi-Square, t Student test or Mann-Whitney to analyze demographic and clinical variables. We divided participants in two groups: subjects with BD and lifetime comorbid PTSD diagnosis and subjects with BD without PTSD across lifespan. Number of mood episodes, FAST, age of first mood episode, age first diagnosed with BD, and age of first drug use were analyzed with Mann-Whitney test since it had non-parametric distribution. The Linear Regression adjusted for current mood and anxiety symptoms was performed to verify the effect of comorbid PTSD in functional impairment and quality of life. P values <0.05 were considered significant. Number of manic/hypomanic episodes was adjusted for age of onset of manic/hypomanic episodes.

RESULTS

A total of 284 subjects with BD were included in all mood states. Among them, 80 (28.2%) were euthymic, 147 (51.8%) were depressed, 33 (11.6%) were manic/hypomanic, and 24 (8.4%) were mixed. Moreover, 42% of the recruited patients were receiving outpatient psychiatric care, whereas 58% were not. Fifty-six (19.7%)

subjects showed lifetime comorbid PTSD diagnosis and twenty-five subjects (8.8%) have current PTSD symptoms. PTSD occurred prior to BD in 68% of subjects with BD and PTSD. Prevalence of PTSD comorbidity among subjects with BD was 22.2% in BD type 1, 16.0% in BD type 2, and 10.0% in BD NOS. However, there was no difference among groups in prevalence ($p=0.273$). Table 1 shows demographics and clinical characteristics.

The median and interquartile range of age of onset of PTSD symptoms was 14(11-23) years among subjects with lifetime comorbid PTSD diagnosis. Figure 1 shows that subjects with BD and PTSD had an earlier onset of manic/hypomanic episodes [BD+PTSD: 16(13-20) vs BD: 18(15-25); $p=0.009$] and first drug use [15(14-18) vs 18(15-21); $p=0.008$]. Moreover, subjects with BD and PTSD were younger at age first diagnosed with BD [24(18-32) vs 28(21-39); $p=0.036$]. The onset of depressive episodes ($p=0.548$) was not significantly different between groups.

Subjects with BD and lifetime PTSD reported higher number of manic/hypomanic episodes than subjects with BD without PTSD [7(2-20) vs 3(1-7); $p=0.012$] (Figure 2B), even when adjusted for age of onset of manic/hypomanic episodes ($p=0.016$). However, number of depressive episodes was not significantly higher in subjects with BD and PTSD [6(4-12) vs 5(3-10); $p=0.278$] (Figure 2A).

Subjects with BD and PTSD showed higher functional impairment [46(33-50) vs 24(16-38); $p=0.022$] (Figure 3). Quality of life was poorer in subjects with BD and PTSD regarding physical health (37.3 ± 16.4 vs 49.1 ± 14.1 ; $p=0.005$), psychological health (40.3 ± 13.8 vs 52.3 ± 14.3 ; $p=0.004$), social relationships (37.9 ± 23.1 vs 53.2 ± 26.4 ; $p=0.039$), and environment (48.2 ± 20.4 vs 70.9 ± 15.1 ; $p=0.001$). Subjects with BD and PTSD showed higher scores of depressive ($p<0.001$), manic ($p=0.002$) and anxiety ($p=0.012$) symptoms when compared to subjects without PTSD. The difference among groups in FAST and WHOQOL-BREF scores remained statistically significant after adjustment for severity of depressive, manic and anxiety symptoms, except for social relationships (Figure 4).

DISCUSSION

The present study showed that subjects who have BD comorbid with PTSD presented a lower age of onset of manic/hypomanic episodes and first drug use, and were also more likely to be younger at the age they received the diagnostic of BD. This group also shows more manic/hypomanic episodes, worse quality of life, and more functional impairment. Our study suggests that comorbid PTSD is associated with accelerated illness progression and increased morbidity in BD³⁰. The lifetime prevalence of PTSD among subjects with BD was 19.7% in our sample. This rate is similar to that found in the Systematic Treatment Enhancement Program for Bipolar Disorder (18.8%)³¹.

The association of comorbid PTSD among subjects with BD regarding the clinical variables assessed (age of onset of manic episode and first drug use, age at first diagnosis of BD, and number of manic/hypomanic episodes) has not been reported so far. This finding is in line with the notion that the stress sensitization related to PTSD leaves residual vulnerabilities to further occurrences of mood episodes and accelerated illness progression^{20,32}. A cohort study has shown similar results among 651 BD patients, but studying another kind of stress^{33,34}. Patients with early childhood trauma also presented earlier onset of bipolar illness, a greater number of subsequent manic episodes, faster cycling pattern, more suicide attempts, and higher incidence of substance abuse^{33,34}. Although in our sample the age of onset of PTSD symptoms precedes the age of first BD diagnosis, we cannot exclude the possibility that number of manic/hypomanic episodes leads to increased risk to develop PTSD since this is a cross-sectional study.

Our study also showed that PTSD comorbidity among BD subjects was associated with worse quality of life and functional impairment. A previous study with 405 patients with BD has found worse quality of life in three domains of WHOQOL (psychological, social relationships, and environment) in comorbid PTSD group¹⁹. No study so far has reported the association of PTSD comorbidity with functioning impairment among subjects with BD. Functional impairment is a key clinical feature in PTSD and BD^{35,36}. A previous study with 3345 BD patients has shown that multiple mood episodes were associated

with worse functioning and lower quality of life³⁷. Even young subjects with BD have shown functional impairment, which get worse with illness progression^{36,38,39}. It was proposed that PTSD stress and manic episodes may have sensitization to themselves and cross-sensitization to one another contributing to a pernicious course among BD patients with even more functional impairment^{30,32}.

PTSD and BD share some biological underpinnings, which may explain the interaction of both disorders contributing to accelerated illness progression among BD patients^{30,40}. Serum concentrations of BDNF are decreased in drug free BD patients during manic episodes⁴¹, as well as drug-naïve patients with PTSD⁴². Also, exposure to traumatic events may induce epigenetic modification leading to further reductions of BDNF^{43,44}. Moreover, levels of tumor necrosis factor- α (TNF- α) and soluble tumor necrosis factor receptor type 1 are elevated in BD during manic episodes⁴⁵. In addition, increased levels of TNF- α were reported in patients with PTSD^{46,47}. Both decreased BDNF levels and increased TNF- α levels are associated with a more severe course of BD^{48,49}.

Considering all the findings indicating an association of comorbid PTSD and increased morbidity among subjects with BD, an important clinical question emerges regarding current treatment practices. Antidepressants are the mainstay in the treatment of patients with PTSD. However, among patients with BD its clinical usefulness seems to be restricted to some cases to the acute treatment of bipolar depression⁵⁰. Psychosocial interventions that have been shown to improve both PTSD and BD may offer an important alternative in this subset of patients. Also, prazosin is another useful alternative in the treatment of individuals with PTSD and BD. Prazosin was effective for trauma nightmares, sleep quality, functioning, and hyperarousal symptom cluster in patients with PTSD⁵¹.

It should be mentioned in the present cross-sectional study that reverse causality could not be discarded. Whether PTSD predispose individuals with BD to a worse course, or whether it is the characteristics of the severe BD itself that determine PTSD onset remains unclear. Recall bias and the influence of current disorder may interfere in our findings. Specifically, our reliance on retrospective self-report for lifetime disorders and

severity of illness at this cross-sectional assessment does not protect from the possible bias that patients with greater severity of illness may have been more likely to acknowledge a history of PTSD in a structured interview. Also, we do not know the extent to which comorbid PTSD motivates subjects to participate in researches or seek care. However, inclusion of patients at all levels of symptom severity, treatment, and phase of illness allows for a broad generalizability. Future longitudinal studies including prospective follow up will be needed to confirm the present findings and provide further information regarding the phenomenological changes in the course of BD with PTSD.

POTENTIAL CONFLICT OF INTEREST:

Dr Kauer-Sant'Anna has received grants from CNPQ, CAPES, and FINEHCPA from Brazil. Also, Dr Kauer-Sant'Anna has been a speaker for Eli-Lilly. Dr Jair C Soares has received grants/research support from Forrest, BMS, Merck, and has been a consultant for Roche and Abbott. The other authors report no financial or other relationship relevant to the subject of this article.

CLINICAL POINTS:

1. Comorbid PTSD was associated with increased morbidity among subjects with bipolar disorder, including more manic/hypomanic episodes, worse quality of life, and more functional impairment.
2. Comorbid PTSD was associated with a lower age of onset of manic/hypomanic episodes and first drug use.
3. Psychosocial interventions and prazosin may offer an important alternative to antidepressants in subjects with comorbid PTSD.

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FIGURES:

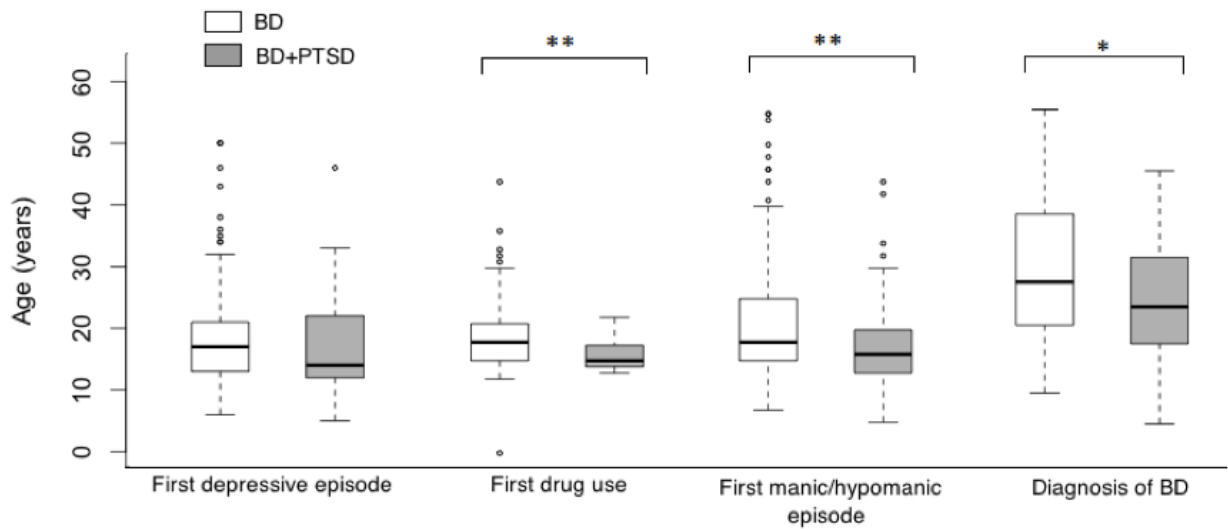


Figure 1. Age of onset of mood episodes, drug use, and diagnosis of bipolar disorder among patients with and without PTSD comorbidity. * $p < 0.05$; ** $p < 0.01$.

Abbreviations: BD, Bipolar disorder without PTSD; BD+PTSD, Bipolar disorder with PTSD.

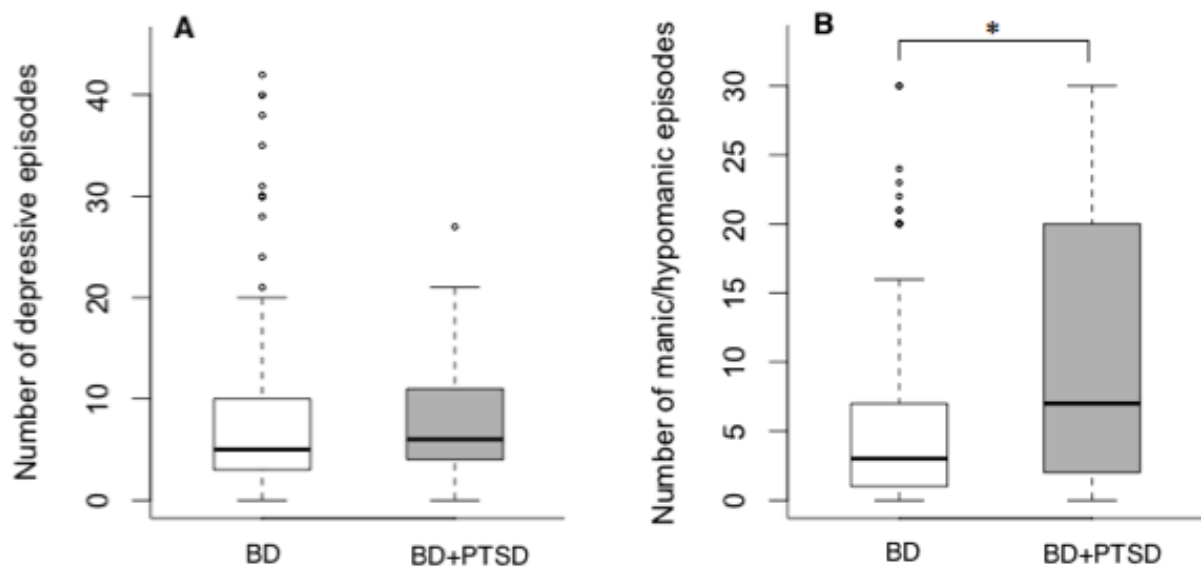


Figure 2. Number of depressive and manic/hypomanic episodes in bipolar disorder patients with and without lifetime PTSD. * $p < 0.05$; Adjusted for age of onset of first manic/hypomanic episode.

Abbreviations: BD, Bipolar disorder without PTSD; BD+PTSD, Bipolar disorder with PTSD.

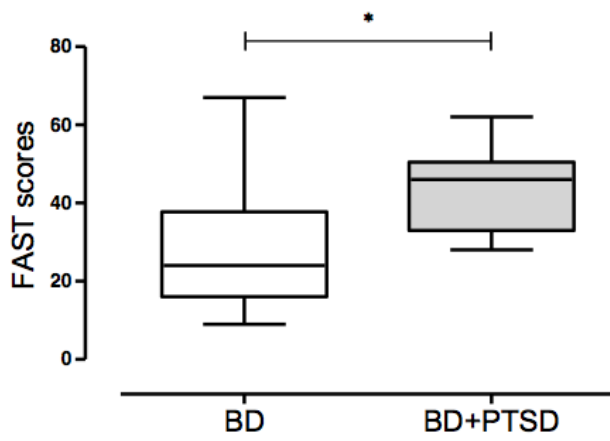


Figure 3. Functional impairment in bipolar disorder patients with and without lifetime PTSD.

* $P < 0.05$; Adjusted for severity of depressive, manic and anxiety symptoms.

Abbreviations: BD, Bipolar disorder without PTSD; BD+PTSD, Bipolar disorder with PTSD.

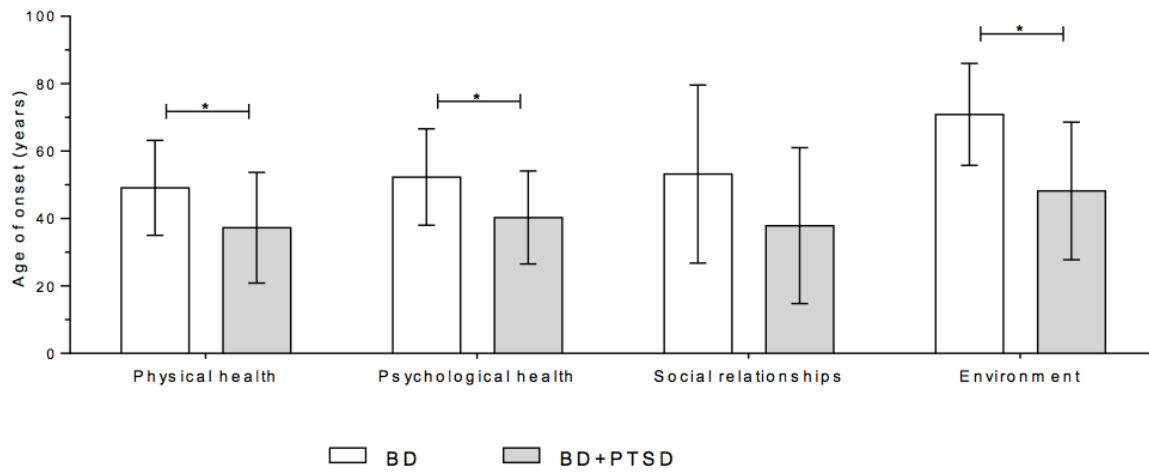


Figure 4. Quality of life in bipolar disorder patients with and without lifetime PTSD. * $p < 0.05$; Adjusted for current severity of depressive, manic and anxiety symptoms

Abbreviations: BD, Bipolar disorder without PTSD; BD+PTSD, Bipolar disorder with PTSD.

TABLE:

Table 1. Demographic and clinical characteristics

Characteristics	BD (n=228)	BD+PTSD (n=56)	p-value
Age (years) ^a	37.00 ±12.52	35.39 ±10.40	0.373
Years of education ^a	14.15±2.98	13.60 ±4.03	0.357
Manic symptoms (YMRS scores) ^b	4 (1 - 8)	7 (3 - 11)	0.002
Depressive symptoms (HDRS scores) ^b	11 (5 - 18)	18 (12 - 21)	<0.001
Anxiety symptoms (HARS scores) ^b	11 (5 - 17)	15 (8 - 21)	0.012
Gender ^c			0.280
Male	35.5% (81)	26.8%(15)	
Female	64.5%(147)	73.2%(41)	
Currently employed ^c	43.8% (96)	43.4% (23)	1.000
Diagnosis of BD ^c			0.273
BD 1	64.5%(147)	75.0%(42)	
BD 2	27.6%(63)	21.4%(12)	
BD NOS	7.9%(18)	3.6%(2)	
Rapid cycling ^{c,d}	40.2%(72)	48.9%(22)	0.377
Prevalence of prior mixed episodes ^{c,d}	14.2%(24)	26.8%(11)	0.052
Comorbid medical illness ^c	15.8%(36)	14.5%(8)	0.982
Comorbid substance abuse or dependence ^c	37.7%(86)	44.6%(25)	0.425
Lifetime suicide attempts ^{c,d}	34.5%(60)	42.1%(16)	0.483
Lifetime hospitalization ^c	46.3%(99)	51.9%(27)	0.563
Lifetime psychotic symptoms ^c	27.0%(60)	32.1%(17)	0.572
Current Medication ^c			
Antidepressants	18.4%(40)	13.7%(7)	0.426
Lithium	6,4%(14)	2.0%(1)	0.209
Anticonvulsants	22.6%(49)	15.7%(8)	0.279
Atypical antipsychotics	13.7%(30)	13.7%(7)	0.985
Typical antipsychotics	0.9%(2)	0%(0)	0.491
	17.0%(37)	21.6%(11)	0.449

Benzodiazepines**Comorbid anxiety disorders^c**

GAD	12.71%(29)	17.8%(10)	0.317
OCD	8.7%(20)	10.7%(6)	0.652
Social Phobia	12.7%(29)	8.9%(5)	0.243
Panic Disorder	19.7%(45)	26.8%(15)	0.247

Table 1. ^aMean and standard deviation, p value according to t Student test; ^bMedian and 25th/75th quartiles, p value according to Mann-Whitney U test; ^cRelative (%) and absolute (n) frequencies, p value according to Chi-square test.
^dPresence of missing data.

Abbreviations: BD, Bipolar disorder without PTSD; BD+PTSD, Bipolar disorder with PTSD; GAD, Generalized Anxiety Disorder; HARS, Hamilton Anxiety Rating Scale; HDRS, Hamilton Depression Rating Scale; OCD, Obsessive Compulsive Disorder; YMRS, Young Mania Rating Scale

6.3. Artigo 3

July 22, 2015

Dear Editor,

I would like to submit a new manuscript entitled “Identifying a clinical signature of suicide attempts among patients with mood disorders: a machine learning approach” for consideration by The American Journal of Psychiatry. I confirm that this work is original and has not been published elsewhere nor is it currently under consideration for publication elsewhere. I affirm that all authors had access to all data from the study, both what is reported and what is unreported, and also that they had complete freedom to direct its analysis and its reporting, without influence from the sponsors. I also affirm that there was no editorial direction or censorship from the sponsors. All authors approved manuscript submission all authors in addition to transfer of copyright to the American Psychiatric Association.

Suicide attempt is usually viewed as an extreme response to a catastrophic event, such as loss of a close relative or the loss of an important job. However, many individuals, including patients with mood disorders, go through these kind of stressors and yet they do not necessarily attempt suicide. Consequently, a growing body of knowledge has put forward several clinical risk factors associated with patients that attempt suicide. They are developmental and distal factors (as opposed to precipitating factors, such as loss of a close relative) associated to the suicide behavior. These efforts have largely reported average group-level differences between suicide attempters and non-attempters. However, what is not known is how to integrate these clinical variables into a predictive clinical signature able to objectively estimate the probability of an individual patient with mood disorder attempting suicide. Lack of such knowledge is an important question since suicide is highly preventable but the assessment of this tragic event is subjective and inaccurate, especially in patients who have not attempted suicide yet. Therefore, In the current study, we used advanced machine learning algorithms to objectively identify individual patients probability of attempting suicide. Machine learning in psychiatric

research is an emerging field with great potential for innovation and paradigm shift as these algorithms integrate multiple measurements simultaneously in order to estimate the probability of an outcome.

The relevance vector machine learning algorithm correctly predicted 103 out of 144, translating into 72% accuracy (72.1% sensitivity and 71.3% specificity) and an area under the receiver operating characteristic curve (ROC) of 0.77 ($p < 0.0001$). Also, the main outcome of the machine learning algorithm was a probability score able to quantify the risk of a subject being a suicide attempter. An important strength of our study is the high area under the ROC curve of 0.77 for a machine learning approach. Notably, a recent study reported that most breast cancer prediction algorithms, including the widely-studied 'Gail score' report area under ROC curves below 0.7. This is the first study to use a machine learning algorithm in predicting suicide attempts among patients with mood disorders at an individual subject level.

Many thanks for your consideration on this manuscript.

Yours sincerely,

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Identifying a clinical signature of suicide attempts among patients with mood disorders: a machine learning approach

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ABSTRACT

Objective: A growing body of evidence has put forward several clinical risk factors associated with patients with mood disorders that attempt suicide. These efforts have reported average group-level differences between suicide attempters and non-attempters. However, what is not known is how to integrate clinical variables into a clinically useful tool in order to estimate the probability of an individual patient attempting suicide.

Method: A total of 144 patients with mood disorders were included in the study. Clinical variables associated with suicide attempts among patients with mood disorders (such as comorbid anxiety and comorbid drug use) and demographic variables were used to 'train' a multivariate machine learning algorithm. The resulting algorithm was utilized in identifying novel or 'unseen' individual subjects as either suicide attempters or non-attempters. Three machine learning algorithms namely: the least absolute shrinkage selection operator (LASSO), support vector machines (SVM), and relevance vector machine (RVM) were implemented.

Results: All algorithms distinguished individual suicide attempters from non-attempters with prediction accuracy ranging between 65%-72% ($p < 0.05$). In particular, the RVM algorithm correctly predicted 103 out of 144 subjects translating into 72% accuracy (72.1% sensitivity and 71.3% specificity) and an area under the receiver operating characteristic curve (ROC) of 0.77 ($p < 0.0001$).

Conclusion: Risk for suicide attempt among patients with mood disorders can be estimated at an individual subject level by incorporating both demographic and clinical variables. Future studies should examine the performance of this model in other populations and its subsequent utility in facilitating selection of interventions to prevent suicide.

Keywords: suicide, bipolar disorder, major depressive disorder, machine learning, personalized medicine.

INTRODUCTION

When a patient with a depressive episode and no known previous history of suicide attempts first presents for treatment, what is the likelihood that this individual patient will attempt suicide? This question is a major unmet challenge in mood disorder treatment and also an important question for society at large, as suicide is a tragic but highly preventable event (1). Suicide accounted for 4.8% and 5.7% of total global deaths in female and male subjects aged 15-49 years respectively in 2010 (2). In the same year, suicide was reported as the sixth leading cause of life years lost in North-America (2). Furthermore, it has been reported that approximately 90% of individuals who die through suicide are diagnosed with a mental disorder prior to their death (3). However, there is little awareness in medical practice on objective stratification of suicide risk, which has led suicide to be referred to as “the quiet epidemic” (4). The current situation is particularly worrisome in major depressive disorder (MDD) and bipolar disorder (BD), given the high prevalence of these disorders and the strong association between suicide and depressive symptoms.

Suicide is usually viewed as an extreme response to a catastrophic event, such as loss of a close relative (5). However, many individuals, including patients with mood disorders, go through these kinds of stressors and yet they do not attempt suicide (6). Consequently, a growing body of knowledge has put forward several risk factors associated with patients that attempt suicide (4; 6; 7). They are developmental and distal factors (as opposed to precipitating factors, such as loss of a close relative) associated to the suicidal behavior (4; 8). These efforts have largely reported average group-level differences between suicide attempters and non-attempters. However, what is not known is how to integrate these variables to build a signature of suicide attempt and estimate the probability of an individual patient with mood disorder attempting suicide while faced with a stressful event.

In this study we set out to investigate whether a predictive clinical signature derived from easily accessible clinical and demographic variables can objectively identify individual patients likely to attempt suicide. Predictor variables were selected using a priori

knowledge. Notably, the majority of clinical variables selected were related to psychiatric comorbidities (9–35), given the recent findings that effects of mental disorders on the risk of suicide attempt were exerted almost exclusively through a general psychopathology factor representing the shared effect across all mental disorders (36). As a result, we implemented a set of algorithms that are able to integrate the information from multiple variables to subsequently identify an individual patient's probability or risk of being a suicide attempter.

Machine learning in psychiatric research is an emerging field with a great potential for innovation and paradigm shift as these algorithms facilitate integration of multiple measurements as well as allows objective predictions of previously 'unseen' observations. Typically, these algorithms are implemented in three key stages. First, subjects' data are separated into algorithm 'training' and 'testing' sets with the former being used to 'train' a machine learning model. Second, the predictive accuracy of the 'learnt' model is evaluated using the 'testing' set that consists of observations unseen by the model, and algorithm accuracy, sensitivity and specificity on this testing set is reported. In this study, we implemented three machine learning algorithms: the least absolute selection shrinkage and selection operator (LASSO) (37), support vector machines (SVM) (38) and relevance vector machine (RVM) (39). A detailed discussion of these algorithms in the context of psychiatric research is given elsewhere (40).

In summary, the main objective of the current study was to establish a clinically useful predictive signature able to accurately determine individual patients likely for committing suicide. We used easily accessible clinical variables to achieve our aim.

METHODS

The Institutional Review Board of the University of Texas Health Science Center at Houston approved the study. All subjects signed informed consent before any study-related procedures with ample time for questions. Participants included in the current study were recruited from January 2006 to June 2010.

Participants

A total of 144 subjects were recruited from the community and psychiatric clinics through flyers, radio, and newspaper advertisements. Inclusion criteria were subjects with MDD or BD types I or II (DSM-IV), and aged between 18 and 65. Exclusion criteria were head trauma with residual effects, neurological disorder, and uncontrolled major medical conditions.

Assessments

Subjects were evaluated using a socio-demographic history form to assess age, gender, years of education, and occupational status. Axis-I diagnoses and clinical characteristics were assessed with the Structured Clinical Interview for DSM-IV axis-I Disorders (SCID-I), which was administered by fully trained staff. The criterion for suicidality was one or more documented actual suicide attempts assessed by the suicide history form from the Conte Center for the Neuroscience of Mental Disorders (New York State Psychiatric Institute, Columbia University). Current dimensional mood and anxiety symptoms were assessed with the Hamilton Depression Rating Scale (HDRS), the Young Mania Rating Scale (YMRS), and Hamilton Anxiety Rating Scale (HARS). Data from all instruments were collected regardless of treatment status.

Selection of predictor variables and machine learning

Selection of predictor variables to be utilized in ‘training’ an algorithm is a challenge in machine learning. However, a recommended method of selecting relevant predictor variables is the use of expert domain knowledge – largely from previously published literature (41; 42). Also, we have performed a structured search on Pubmed to find relevant studies that shows the association of the clinical and demographic variables

included in this study with suicide attempts (Table 1). Of note, we chose patients with mood disorders because a) the developmental variables associated with suicide in both BD and MDD are similar (table 1); and b) our goal was to create a tool that could be used with the large number of patients with mood disorders treated in primary care settings (Table 1).

Three machine learning algorithms (LASSO, SVM, and RVM) were implemented in MATLAB (The Mathworks, Inc., Natick, Massachusetts, United States). Specifically, predictor variables (see Table 1) were normalized by z-scoring and together with corresponding categorical labels (1 – suicide attempt, 0 – non-attempt) input into the machine learning algorithm. Here, we briefly describe these machine learning algorithms but detailed and technical discussions are given elsewhere (37–39). SVM and RVM are ‘kernel-learning’ algorithms which transform a set of input variables (feature vectors) into a ‘kernel’ or similarity matrix through a ‘kernel function’ and subsequently used in the algorithm ‘learning’ process. These algorithms can utilize multiple types of kernel functions (e.g. linear, polynomial, Gaussian) but in the current study a linear function was utilized in training both SVM and RVM algorithms as described elsewhere (40). In contrast, the LASSO utilizes a typical linear regression formulation but introduces a ‘penalization’ procedure by assigning some predictor variables zero coefficients or weighting factors - a process also referred to as regularization. The penalization or regularization process reportedly improves the linear regression algorithm prediction accuracy and generalization ability but entails identifying an optimal regularization parameter (37). In the current study, the regularization parameter was identified through a 10-fold cross-validation process using training data only.

Class imbalance problem

Class imbalance is a common problem in machine learning where observations (subjects) in one class (e.g. suicide non-attempters) exceed observations in the other class (e.g. suicide attempters). A typical machine learning algorithm trained using an

imbalanced data set assigns new observations to the majority class (e.g. suicide non-attempters) (43). In this study, the class imbalance problem was circumvented by ‘under-sampling’ the majority class (suicide non-attempters) followed by training an algorithm with a balanced sample – a process which was repeated until all observations in the majority class were selected at least once and predictions aggregated as shown in Figure 1.

Model testing and validation

A unique characteristic of machine learning algorithms is the ability to utilize a cross-validation approach to separate data into model ‘training’ and ‘testing’ sets. In this study, a leave-one-out cross-validation (LOOCV) approach was used. LOOCV involves training a machine learning algorithm with all subjects except one while the ‘left out’ subject is used for algorithm testing. This iterative process is repeated until all subjects are left out for algorithm testing at least once as shown in Figure 1 and described elsewhere (44). Cross-validation is used in machine learning to establish the generalization ability of an algorithm to new or previously ‘unseen’ subjects. The validity of the algorithms in predicting individual suicide attempters from non-attempters was evaluated using prediction accuracy, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). In this study, sensitivity and specificity represented correctly predicted suicide attempters (true positives) and correctly predicted non-attempters (true negatives) respectively. A ‘classical’ prediction accuracy was calculated as the sum of true positives, true negatives and divided by the total sample. In contrast, to account for the class imbalance problem, a ‘balanced’ accuracy was calculated as the average of predicted sensitivity (true positives) and specificity (true negatives) as reported elsewhere (45). In addition, PPV was calculated as the proportion of subjects predicted as suicide attempters that were actual positives (attempters) and NPV as the proportion of subjects predicted as non-attempters but were actual non-attempters (true negatives). Receiver operating characteristic curves and the corresponding area under curve were computed and reported. Lastly, chi-square statistical test between actual and machine

learning predicted labels were calculated and the results were reported. The outcome of a chi-square test was considered significant at $p < 0.05$.

RESULTS

A total of 144 subjects with BD or MDD were included in the study. Table 2 summarizes subjects' clinical and demographic characteristics. All algorithms (RVM, SVM and LASSO) distinguished individual suicide attempters from non-attempters with prediction accuracy ranging (64.7%-72%) and all models were significant (chi-square $p < 0.05$) as detailed in Table 3. In particular, the RVM algorithm correctly identified 103 out of 144 as either attempters or non-attempters. This translated into 72% accuracy (72.1% sensitivity and 71.3% specificity) and significant at $\chi^2 p < 0.0001$. The RVM receiver operating characteristic (ROC) curve and the 'confusion matrix', which were used in calculating the sensitivity, specificity and area under ROC curve values, are shown in Figure 2.

The most relevant predictor variables in distinguishing suicide attempters from non-attempters that were assigned positive coefficients or weighting factors by RVM – indicating an increase in suicide attempters included; 1) a high number of previous hospitalizations for depression, 2) a history of psychosis, 3) cocaine dependence, and 4) PTSD. In contrast, patients' age and mood diagnosis (0 - bipolar disorder or 1 - unipolar depression) were assigned negative weighting factors indicating a reduction of these variables was relevant in identifying suicide attempters. Figure 3 shows predictor variables with corresponding weighting factors.

An additional RVM algorithm was evaluated by removing previous number of hospitalizations in the analysis and the algorithm was able to identify individual subjects with accuracy = 68.9%, sensitivity = 74%, specificity = 63%, $p < 0.05$ and area under ROC curve = 0.71. This is an indication that past hospitalization variable was not the only contributor to the algorithm performance.

DISCUSSION

Although risk factors for suicide are widely known, synthesizing easily accessible clinical information to optimize suicide risk identification in subjects with mood disorders has been an elusive goal up to now. This is the first study to evaluate the feasibility of using a clinical tool developed with advanced machine learning algorithms to identifying an individual's risk of being a suicide attempter among patients with mood disorders. All algorithms achieved greater than chance (>50%) accuracy in distinguishing attempters from non-attempters. Most notably, the RVM algorithm achieved the best prediction accuracy 72% and an area under receiver operating characteristic curve of 0.77. Notably, the prediction accuracy, sensitivity and specificity of the machine learning algorithm in identifying previously 'unseen' suicide attempters was determined through a robust cross-validation approach. The main outcome of the machine learning algorithm was a probability score able to quantify the risk of a subject being a suicide attempter.

In contrast with other branches of medicine (e.g. cardiology, oncology) – the lack of risk stratification approaches in psychiatric clinical practice is notable (46). Noteworthy risk stratification applications include, prediction of cardiac arrest risk, cancer screening, and prediction of mortality risk in intensive care units (47; 48). Indeed, in personalized medicine, risk stratification plays an important role in treatment planning from the time of diagnosis. For instance, in acute care of myocardial infarction efforts to develop treatment algorithms on the basis of clinical presentation are increasingly integrated in clinical practice (5).

One important question is how this suicide risk stratification tool can be applied in a clinical practice to optimize individual patient treatment? For example, it is possible that individual patients with a high risk of attempting suicide as identified by the algorithm would benefit from preventive strategies or antisuicidal drugs. For instance, a randomized clinical trial of suicidal patients reported that a selective serotonin-reuptake inhibitor led to a greater acute improvement in suicidal ideation compared to a

noradrenergic-dopaminergic drug. Notably, this therapeutic advantage was greater in patients with the most severe suicidal ideation (49). Furthermore, evidence from meta-analysis reported that lithium reduces suicide attempts (50). In addition, clozapine recently emerged as one option for treatment-resistant patients with BD and also showed a protective effect against suicidal behavior compared with olanzapine (51). These studies further highlight the need to develop novel suicide stratification algorithms as presented in this study and allow individualized treatments and care for patients predicted with a high risk of attempting suicide.

The current study has some potential limitations. The study sample was from Texas region and may not fully represent the entire United States population. However, the current study serves as a proof-of-concept and future studies will collect larger samples from multiple centers to replicate current findings. In line with our hypothesis, our findings are related to patients with mood disorder and may not be generalized to patient with other mental disorders. However, future studies may utilize a similar approach to accommodate other mental disorders. Of note, borderline personality disorder that is also shown to be associated with suicide attempts in patients with mood disorders (30) was not included in the algorithm due to missing data in our database. Notwithstanding these limitations, this study is the first to describe and implement a risk stratification tool using machine learning algorithms to identify an individual's probability of attempting suicide among patients with mood disorders. This algorithm could be used in a primary care setting by general practitioners to identify the risk of a mood disorder patient attempting suicide. Future work will focus on validating the algorithm using a larger sample as well as developing a web-based suicide risk calculator. Importantly, the strengths of our study include the application and testing of a multivariate machine learning algorithm in suicide stratification leading to a high area under the ROC curve of 0.77. Notably, a recent study reported that most breast cancer prediction algorithms, including the widely-studied 'Gail score' report area under ROC curves below .70 (48).

In summary, we report a highly accurate algorithm able to identify suicide attempts in patients with mood disorders using clinical and demographic data. These results

suggest that it is possible to utilize clinical measures in identifying individual patients at greater risk of attempting suicide. Lastly, we hypothesize that the combination of genetics, blood markers, neuroimaging, and the clinical signature together with a machine learning algorithm could generate an even more accurate multi-modal signature.

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Tables:

Table 1. Clinical and demographic variables selected to develop to ‘train’ the model

Variable	Type	Study	Results
Age	Continuous variable.	Gonzalez A et al., 2006	Suicidal risk was higher in young patients with BD.
		Galfalvy H et al., 2006	Younger age predicted suicide attempts among patients with BD.
		Perlis RH et al., 2007	Younger age was associated with suicide risk in patients with MDD.
Gender	Categorical variable: 0 – male; 1 – female.	Schaffer A et al., 2015	Female gender was associated with suicide attempts among patients with BD.
		Schaffer A et al., 2014	Female gender was associated with suicide attempts among patients with BD.
		Perlis RH et al., 2007	Female gender was associated with suicide risk among patients with MDD.
Race	Categorical variable: 0 – Caucasian 1 – Non-	Bhui KS et al., 2012	Black African men have the highest rates of suicide compared to the white group.

	Caucasian		
Years of education	Continuous variable	De Araújo RM et al., 2015	Elementary school as highest education level completed was associated with suicide attempt.
Current employed	Categorical variable: 0 – no; 1 – yes.	Blackmore ER, 2008	Being unemployed was significantly associated with a suicidal act.
Mood Diagnosis	Categorical variable: 0 – BD; 1 – MDD.	Holma KM et al., 2014	Patients with BD had higher cumulative incidence of suicide attempts compared to those with MDD.
		Bottlender R, 2000	Patients with BD had higher past suicide attempts compared to those with MDD.
Social Phobia	Categorical variable: 0 – no; 1 – yes.	Simon et al., 2004	Comorbid social phobia was associated with greater likelihood of suicide attempts in patients with BD.
		Stein MB, 2006	Social anxiety disorder is associated with an increased risk for MDD and a more malignant course, characterized by increased likelihood of suicide

			attempts
Specific Phobia	Categorical variable: 0 – no; 1 – yes.	Goes FS et al., 2012	Subject with specific phobia was associated with increased suicide attempts among patients with BD.
Panic Disorder	Categorical variable: 0 – no; 1 – yes.	Simon et al., 2004	Comorbid panic disorder was associated with greater likelihood of suicide attempts in patients with BD.
		Katz C et al., 2011	Comorbid panic disorder was associated with greater likelihood of suicide attempts in patients with MDD.
Generalized Anxiety Disorder	Categorical variable: 0 – no; 1 – yes.	Simon et al., 2004	Comorbid generalized anxiety disorder was associated with greater likelihood of suicide attempts in patients with BD.
		Foley DL et al., 2006	Comorbid generalized anxiety disorder was associated with greater likelihood of suicide attempts in patients with MDD.
		Lenze EJ et al., 2000	Comorbid generalized anxiety disorder was associated with

			greater likelihood of suicide attempts in depressed elderly patients.
Obsessive Compulsive Disorder	Categorical variable: 0 – no; 1 – yes.	Simon et al., 2004	Current obsessive compulsive disorder was associated with greater likelihood of suicide attempts in patients with BD.
		Torres AR et al., 2011	MDD was associated with suicide attempts in patients with obsessive compulsive disorder.
Posttraumatic Stress Disorder	Categorical variable: 0 – no; 1 – yes.	Simon et al., 2004	Comorbid posttraumatic stress disorder was associated with greater likelihood of suicide attempts in patients with BD.
		Morina N et al., 2013	Comorbid posttraumatic stress disorder was associated with greater likelihood of suicide attempts in patients with MDD.
Alcohol/Cocaine dependence	Categorical variables: 0 – no; 1 – yes.	Oquendo MA et al., 2010	Comorbid alcohol use disorders were associated with suicide risk among individuals with BD
		Blanco C et al., 2012	Substance use disorder was associated with higher rates of

			suicide attempts in patients with MDD.
		Webb RT et al., 2014	Comorbid alcohol or drug use disorders predicted suicide attempts among patients with BD.
Number of depressive episodes and prior hospitalization by depressive episodes	Continuous variable.	Goldstein TR et al., 2012	Severity and number of depressive symptoms was associated with suicide attempts among patients with BD
		Isometsa et al., 2014	Patients with BD and prior hospitalization by depressive episode had increased suicide risk.
		Gonzalez A et al., 2006	Number of depressive episodes and hospitalization was associated with suicidal risk in patients with BD.
Psychotic symptoms	Categorical variable: 0 – no; 1 – yes.	Johnson J et al 1991	Comorbid psychotic symptoms were associated with suicide attempts among patients with MDD.
		Bottlender R et	Psychotic symptoms were

al., 200

associated with suicidality among
patients with BD.

BD, bipolar disorder; MDD, major depressive disorder.

Table 2. Demographic and clinical characteristics.

	Attempters (n=43)	Non-attempters (n= 101)	p-value
Age (years)	35.26 ± 11.14	37.53 ± 12.96	0.317
Years of education	13.22 ± 3.56	14.55 ± 3.098	0.026
Gender			
Male	14 (32.5%)	39 (38.6%)	0.616
Female	29 (67.5%)	62 (61.4%)	
Currently employed	13 (30.2%)	50 (49.5%)	0.051
Race			0.037
Caucasian	22 (51.2%)	72 (71.3%)	
African American	7 (16.2%)	6 (5.9%)	
Hispanic or Latino	14 (32.6%)	23 (22.8%)	
Number of hospitalization	1.58 ± 1.8	0.29 ± 0.82	<0.001
Mood disorder diagnosis			0.014
BD	40 (93%)	74 (73.2%)	
MDD	3 (7%)	27 (26.7%)	

Comorbid anxiety**disorders**

GAD	6 (13.9%)	14 (13.9%)	1.000
OCD	4 (9.3%)	11 (10.9%)	1.000
Social Phobia	7 (16.3%)	14 (13.9%)	0.905
Panic Disorder	13 (30.2%)	18 (17.8%)	0.150
PTSD	13 (30.2%)	16 (15.8%)	0.081
Specific Phobia	5 (11.6%)	6 (5.9%)	0.404

Comorbid**substance use****disorder**

Cocaine	10 (23.3%)	9 (8.9%)	0.039
Alcohol	23 (53.5%)	31 (30.7%)	0.016
Lifetime psychotic	18 (41.9%)	19 (18.8%)	0.007

symptoms

Independent t-test was performed to numeric variables, while chi-square test with Yates correction was performed to categorical variables. BD, bipolar disorder; GAD, generalized anxiety disorder; MDD, major depressive disorder; OCD, obsessive-compulsive disorder; PTSD, posttraumatic stress disorder.

Table 3. Algorithm performance in identifying individual suicide attempters from non-attempters among patients with mood disorders. The balanced accuracy (sensitivity + specificity/2) was used to identify the best performing algorithm due to the class imbalance problem.

Algorithm	RVM	SVM	LASSO
Area Under Curve	0.77	0.65	0.73
Balanced accuracy (%) (Specificity+sensitivity/2)	72	64.7	68
Classical accuracy (%)	71.5	67.4	72.9
Specificity (%)	71.3	71.3	80.2
Sensitivity (%)	72.1	58.1	55.8
PPV (%)	51.7	46.3	54.6
NPV (%)	85.7	80.0	81.0

LASSO, least absolute selection shrinkage and selection operator; NPV, negative predictive value; PPV, positive predictive value; RVM, relevance vector machine; SVM, support vector machines.

Figures:

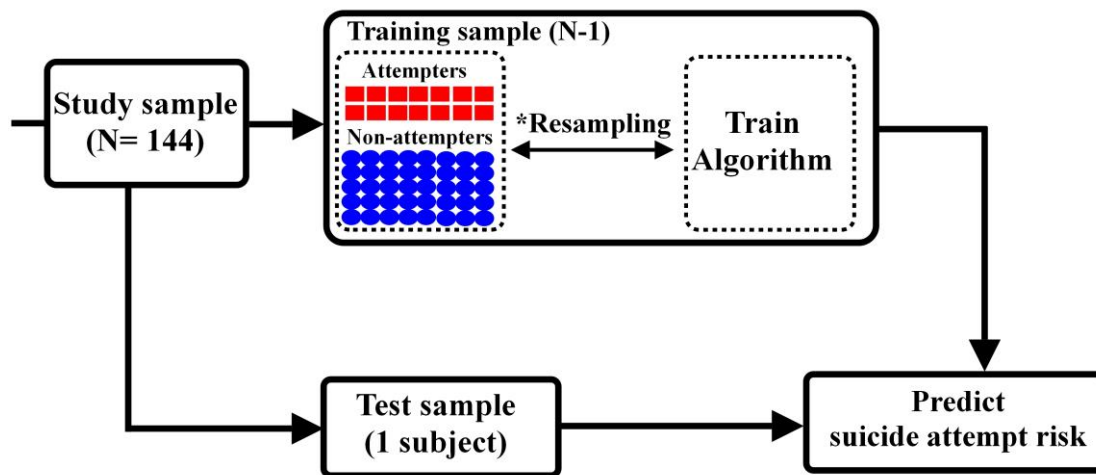


Figure 1. Flow diagram showing algorithm training and testing process. *The class imbalance problem was addressed through a resampling step, which entailed under-sampling the majority class (suicide non-attempters) followed by algorithm training. This process was repeated 1000 iterations and algorithm predicted probabilities averaged over the resampling iterations. Class imbalance is a recurrent challenge in machine learning as recently highlighted by Libbrecht and colleagues (98). A leave-one-out cross-validation approach was used to separate algorithm training and testing data.

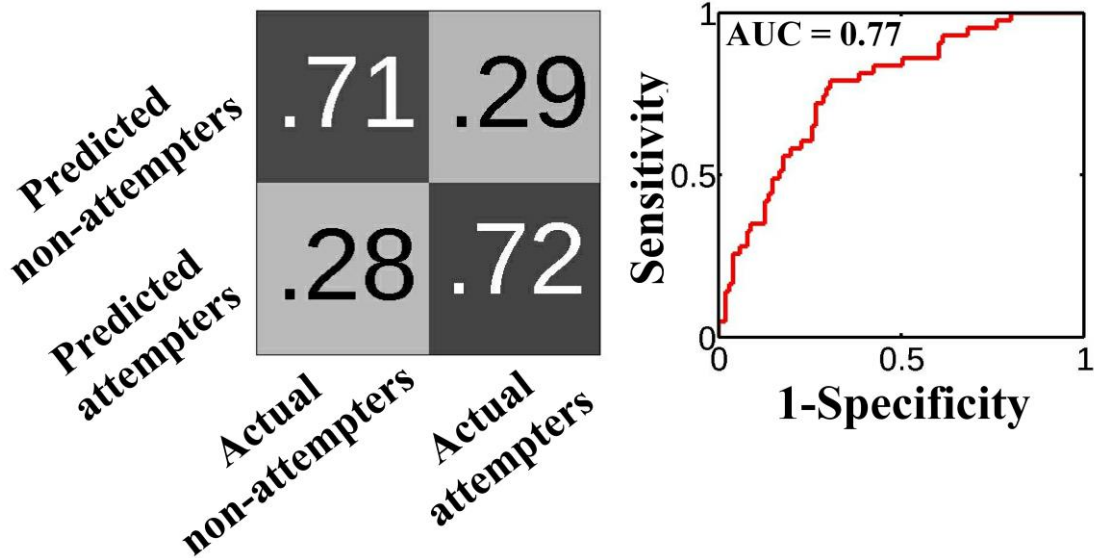


Figure 2. 'Confusion matrix' and receiver operating characteristic (ROC) curve.

'Confusion matrix' and receiver operating characteristic (ROC) curve were used in computing the algorithm accuracy, sensitivity, specificity and area under the ROC curve.

The RVM algorithm performed best as compared to SVM and LASSO.

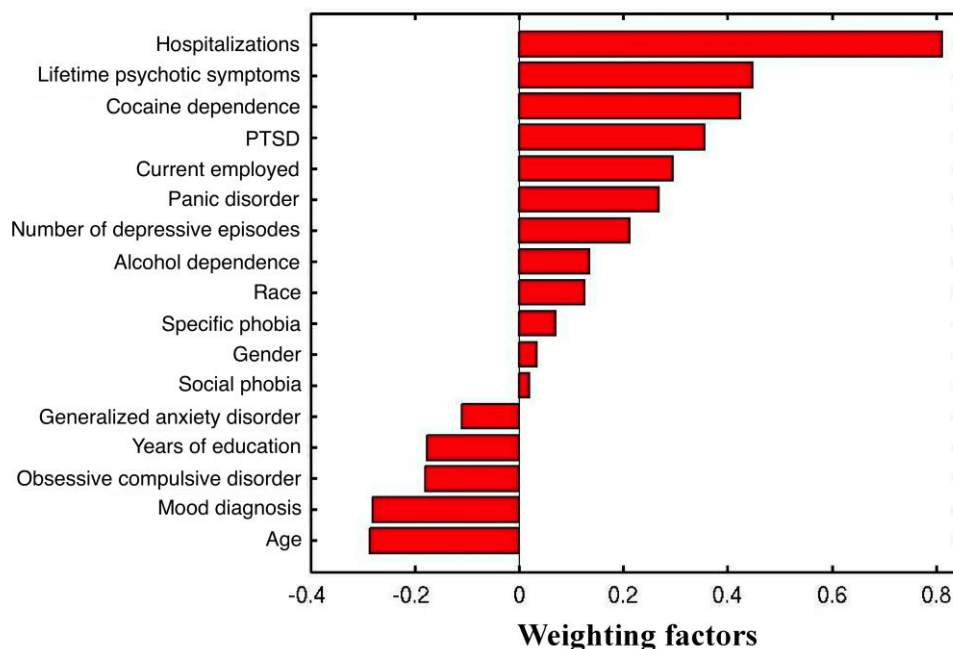


Figure 3. Clinical signature. Bar graph showing weighting factors assigned to each clinical variable by RVM based on their relevance in distinguishing suicide attempters from non-attempters. Clinical variables which increase the probability of an individual patient being a suicide attempter were assigned positive weighting factors whilst those that decrease the probability of a patient being a suicide attempt were assigned negative weighting factors. Variables related to anxiety and substance use disorder are lifetime comorbidities. PD, panic disorder; PTSD, posttraumatic stress disorder.

7 - Considerações Finais

A partir dos dados apresentados, nossos resultados sugerem que a presença da comorbidade com TEPT está associada a um curso mais grave nos pacientes com TB.

Em síntese, os achados desta tese são:

- 1) O TEPT está associado a um padrão de alteração dos marcadores inflamatórios, com níveis aumentados de IL-6, IL-1 β , TNF- α e interferon- γ . Além disso, o tempo de doença foi positivamente associado aos níveis de IL-1 β , enquanto a gravidade dos sintomas foi positivamente associado aos níveis de IL-6.
- 2) Indivíduos com TB e comorbidade com TEPT apresentam mais episódios maníacos, pior funcionamento psicossocial e pior qualidade de vida. Além disso, eles eram mais novos quando iniciaram os episódios maníacos e o uso de substâncias.
- 3) O risco de suicídio em indivíduos com transtorno de humor pode ser estimado objetivamente com um escore de probabilidade construído a partir de variáveis relacionadas à comorbidades com transtornos ansiosos, uso de substâncias e variáveis demográficas.

Ao demonstrar que o TEPT está associado a um padrão de alteração dos marcadores inflamatórios, abrimos mais uma possível via para explicar o fenômeno de sensibilização cruzada entre o TEPT e o TB. Esse é o primeiro passo para estudar a inflamação como um modelo de mediação da sensibilização entre os dois transtornos. Futuros estudos poderão aferir o padrão inflamatório em pacientes com TB com TEPT e sem TEPT e relacionar com desfechos clínicos. A utilidade desses achados, entretanto, extrapola os objetivos dessa tese, apresentando implicações fisiopatológicas, clínicas e terapêuticas.

Em relação às implicações fisiopatológicas, IL-6, IL-1 β e TNF- α podem diminuir a neurogênese (99–101), o que pode explicar a associação de TEPT e reduções de volume do hipocampo, córtex pré-frontal, e giro temporal médio (102). Além disso, vários estudos relataram os efeitos da IL-1 β sobre a formação e consolidação da

memória (103), um processo fisiológico que se encontra alterado no TEPT (104). O nível alterado de citocinas no TEPT pode também explicar a associação entre TEPT e doenças em que a ativação imune desempenha um papel chave, tais como doenças cardiovasculares, metabólicas e doenças autoimunes. As implicações clínicas estão relacionadas ao uso potencial de citocinas inflamatórias como biomarcadores de gravidade, duração da doença e atividade da doença. Os nossos resultados apontam para um uso potencial de IL-1 β como um biomarcador da duração da doença e da IL-6 como um biomarcador da gravidade. Por fim, o desenvolvimento de intervenções que diminuam os níveis de IL-6, TNF- α , INF- γ e IL-1 β pode ser útil no tratamento do TEPT. Nesse sentido, intervenções como a cessação do tabagismo e programas de exercícios aeróbicos estão ligados a reduções na inflamação periférica, bem como a redução dos sintomas de TEPT (105).

No segundo artigo, demonstramos que a comorbidade com TEPT pode piorar características centrais do TB. A associação entre comorbidade com TEPT e piores desfechos clínicos em pacientes com TB (idade de início do episódio maníaco e uso primeira droga, idade ao primeiro diagnóstico de TB, e o número de episódios maníacos / hipomaníacos) não havia sido relatado previamente. Este resultado está em linha com a hipótese de que a sensibilização causada pelo estresse relacionado ao TEPT deixa uma vulnerabilidade residual que facilita a ocorrência de episódios de humor (106). Futuros estudos longitudinais, entretanto, são necessários para avaliar o impacto da comorbidade com TEPT e confirmar tais resultados. Nosso estudo também demonstrou que pacientes com TEPT tem um pior funcionamento psicossocial. Esse fenômeno pode estar relacionado tanto à própria base fisiopatológica do TEPT, mas também ao número aumentado de episódios maníacos que pacientes com TB e TEPT apresentam, uma vez que pacientes bipolares com múltiplos episódios apresentam pior funcionamento (107). Adicionalmente, uma importante questão clínica emerge nesse contexto: o uso de antidepressivos em pacientes com TEPT que possuem TB. Prazosin e intervenções psicossociais seriam alternativas (108).

Por fim, obtemos sucesso na construção de um modelo objetivo que pudesse estratificar a probabilidade de um paciente com transtorno de humor apresentar um

padrão suicida. O principal resultado desse estudo é a produção de um instrumento clínico que é capaz de dar um escore entre 0 e 100 para cada paciente. Quanto mais próximo do 100 maior a probabilidade daquele paciente ter um padrão suicida. Esse achado poderá tornar mais objetiva a avaliação de pacientes com relação ao suicídio, bem como tornar mais rápida a instituição de medidas preventivas contra o suicídio. No entanto, esse estudo serve como uma *proof-of-concept* e estudos futuros devem avaliar esse modelo em amostras maiores e representativas a partir de vários centros.

O uso de técnicas de *machine learning* só recentemente começou a despontar na literatura científica da psiquiatria (95). Sua capacidade de integrar múltiplas variáveis de diferentes campos de pesquisa (genética, clínica, marcadores periféricos, neuroimagem, neurocognição, etc) pode mudar o curso da psiquiatria na busca por uma medicina mais personalizada. Inúmeros fatores que individualmente não conseguem identificar, diagnosticar ou prever um padrão podem ser então integrados em uma ferramenta clínica poderosa. Desfechos como resposta ao tratamento (95), previsão de curso mais graves, bem como diagnóstico (96), identificação de subtipos diagnósticos (109) e padrões associado ao suicídio (110) são exemplos de variáveis que estão sendo estudadas com técnicas de *machine learning*. Com relação ao nosso estudos, embora tenhamos achado um boa acurácia e AUC, futuros estudos podem integrar dados de variáveis de marcadores biológicos para atingir uma acurácia ainda maior.

8 - Referências da tese

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9 – Anexo

9.1. Anexo 1

Comentário acerca do artigo: “Inflammatory markers in posttraumatic stress disorder: a meta-analysis and meta-regression study”

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Chronic Inflammation: Promising New Therapeutic Target for Posttraumatic Stress Disorder or Universal Outcome of Trauma?

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Increasing evidence linking Posttraumatic Stress Disorder (PTSD) with other adverse health outcomes, such as cardiovascular disease, has triggered the investigation of underlying biological signals that are responsible these co-morbidities. While the leading candidate mechanism for this relationship between PTSD and negative physical health outcomes is augmentation of inflammation, results over the last decade have been equivocal. In the current issue of *Lancet Psychiatry*, Passos and colleagues [1] undertake a meta-analysis of available published findings addressing whether increased inflammatory markers are present in individuals with PTSD compared to healthy control subjects. The meta-analysis was based on twenty published studies and performed several sophisticated analyses of the data; the results showed that levels of interleukin (IL)-6, IL-1 β , and interferon- γ are elevated in individuals with PTSD compared to controls.

The strong appeal of this line of research is in pointing to a solid set of putative biomarkers for PTSD whose anticipated utility is broad, ranging from aiding in diagnosis

and tracking treatment outcomes to providing targets for therapeutic interventions [2]. In the era of experimental medicine and Research Domain Criteria (RDoC) ushered in by the National Institutes of Mental Health [3], there is a great need for the discovery and validation of such targets. Accordingly, this meta-analysis showing increased inflammation in PTSD makes a significant contribution to the literature demonstrating that chronic psychopathology, including depression, is associated with a pro-inflammatory state [4]. Therein also lays the problem: the specificity of chronic inflammation to PTSD rather than mental illness in general. Furthermore, it is important to note that the selection of healthy volunteer controls as the comparison group to individuals with PTSD for the meta-analysis compromises the interpretation of the findings since another meta-analysis shows that trauma exposure itself is associated with increased levels of TNF- α , IL-1 β , IL-6, and C-reactive protein (CRP) [5]. Thus, increased inflammation may not be a marker of PTSD specifically as implicated by the meta-analysis, but rather, of adverse psychosocial experience that is known to increase risk for harmful outcomes.

In order to address some of these issues, Passos and colleagues carry out subgroup meta-analyses that indicate that TNF- α levels are increased in unmedicated PTSD, and TNF- α , IL-1 β , and IL-6 remained increased in the absence of co-morbid depression. Furthermore, the duration of illness was positively associated with pro-inflammatory markers. While these subgroup analyses address important questions regarding specificity for PTSD, the stringent inclusion criteria of the study limits the interpretation of the findings. The authors were not able to account for an array of potential confounders of the PTSD-inflammation relationship as such variables were not addressed in primary published reports. Factors such as smoking status, alcohol use, obesity, infection, and pulmonary and cardiovascular disease increase systemic inflammation and thus must be accounted for in future studies assessing the association between PTSD and inflammation. Similarly, it is critical that the effects of genetic diversity on individual differences in inflammation are also addressed in the future, as single nucleotide polymorphisms (SNPs) influence levels of inflammatory markers and

are associated with PTSD symptoms, as recently shown with CRP in a highly traumatized population [6].

Moreover, the current meta-analysis was based on cross-sectional studies, limiting the ability to assess a causal relationship between PTSD and inflammation. It is important that the relationship between inflammation and PTSD continues to be examined empirically with indepthindividual assessments, including intermediate phenotypes such as neuroimaging or psychophysiology. These neurobiological indices may add more precision, such as specific associations with hyperarousal symptoms [6], and dimensionality as advocated by RDoC. Prospective studies are critical for tracking changes in inflammatory markers before and after trauma and stressor exposure alongside the development of psychopathology (PTSD and depression) and other adverse health outcomes (cardiovascular disease, obesity, diabetes). This is highlighted by a recent report indicating that baseline levels of CRP pre-deployment are predictive of PTSD development post-deployment [7]. Such prospective biomarker studies will shed light on the causal relationship between inflammation and increased risk for these highly co-morbid conditions that account for high rates of morbidity and mortality worldwide [8]. The directionality of the relationship is also important from the treatment perspective, as reducing systemic inflammation is a viable option for decreasing the rate of negative health outcomes and increasing quality of life in individuals with PTSD. A similar anti-inflammatory treatment regimen has proved to be efficacious for treatment-resistant depression [9]. The promise of inflammatory markers is that in the near future, they may serve as such therapeutic targets for alleviating PTSD symptoms; the current meta-analysis by Passos and colleagues [1] may not get us all the way there, but does take an important step in the right direction.

Conflict of Interest: None

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9.2. Anexo 2

Além dos três trabalhos que compõem essa tese, outros artigos foram produzidos durante o período que compreendeu esse doutorado.

Artigos publicados:

1. Kapczinski F, **Passos IC**. Wet minds, dry minds, and the future of psychiatry as a science. *Acta Psychiatr Scand*. **2015** Jan;131(1):7-8. doi: 10.1111/acps.12357.
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7. **Passos IC**, Jansen K, Kapczinski F. Developmental staging models in bipolar disorder. *International Journal of Bipolar Disorders* 2015 Dec;3(1):33. Epub 2015 Jul 31. PubMed PMID: 26228989.

Artigos aceitos:

1. **Passos IC**, Kapczinski F. Immune activation as a mediator of the gut-brain axis in manic episodes. *Australian and New Zealand Journal of Psychiatry*. **2015**
2. Costa SC, **Passos IC**, Reus GZ, Soares J, Kapczinski F, Quevedo J. The comorbidity of Bipolar Disorder and Migraine: the role of inflammation and oxidative and nitrosative stress. *Current Molecular Medicine*. **2015**
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Artigos submetidos:

1. Mwangi B, Mon-Ju Wu, Bo Cao, **Passos IC**, Lavagnino L, Keser Z, Zunta-Soares GB, Hasan KM, Kapczinski F, Soares JC (2015) – Individualized Prediction and Clinical Staging of Bipolar Disorders using Neuroanatomical Biomarkers and Machine Learning – Submitted to *American Journal of Psychiatry*
2. Cao B, **Passos IC**, Mwangi B, Bauer IE, Zunta-Soares GB, Kapczinski F, Soares JCI. (2015) – Hippocampal volume and verbal memory performance in late-stage bipolar disorder – Submitted to *Acta Psychiatrica Scandinavica*
3. Vasoncelos-Moreno M, Bucker J, Burke KP, Czepielewski L, Santos BT, Fijtman A, **Passos IC**, Kunz M, Bonnin CM, Vieta E, Kapczinski F, Rosa AR, Kauer-Sant'Anna M (2015). Comparison of cognitive performance and psychosocial functioning in bipolar patients, siblings and healthy controls. Submitted to *Journal of Psychiatric Research*

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