UNIVERSIDADE FEDERAL DE MINAS GERAIS

Instituto de Ciências Biológicas

Programa de Pós-Graduação em Neurociências

AVALIAÇÃO DE FATORES NEUROTRÓFICOS E MARCADORES IMUNOLÓGICOS EM INDIVÍDUOS COM TRANSTORNO BIPOLAR

Izabela Guimarães Barbosa

Belo Horizonte
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Tese apresentada ao Programa de Pós–Graduação em Neurociências da Universidade Federal de Minas Gerais (UFMG), como pré-requisito para obtenção do Títulode Doutora em Neurociências.

Professor Orientador: Antônio Lúcio Teixeira Júnior

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Aluna: Izabela Guimarães Barbosa

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“Digam de mim o que quiserem (pois não ignoro como a Loucura é difamada todos os dias, mesmo pelos que são os mais loucos), sou eu, no entanto, somente eu, por minhas influências divinas, que espalho a alegria sobre os deuses e sobre os homens.”

“Mais loucos que todos os outros loucos juntos, os inventores das ciências e das artes sonharam com não sei qual reputação, que, no entanto, é a coisa mais quimérica do mundo, para recompensá-los de seus trabalhos e vigílias. Enfim, é a loucura que deveis as principais satisfações da vida...”

In: O Elogio da Loucura

Erasmo de Roterdã

RESUMO

Introdução: O transtorno bipolar (TB) é uma síndrome psiquiátrica de prevalência elevada, curso crônico, associada com grande morbidade. Evidências na literatura sugerem que alterações nos fatores neurotróficos e em parâmetros imunológicos tem um importante papel na fisiopatologia do TB.
Objetivo: O objetivo deste estudo é avaliar a concentração plasmática dos níveis de fatores neurotróficos (BDNF, NGF e GDNF) e parâmetros imunológicos (TNF-α, sTNFR1, sTNFR2, adiponectina, leptina, resistina, e as quimiocinas CCL2, CCL3, CCL11, CCL24, CXCL8 e CXCL10), assim como avaliar a frequência de populações leucocitárias e vias intracelulares ativadas por fatores neurotróficos e fatores do sistema imunológico de pacientes com o diagnóstico de transtorno bipolar em comparação a controles.
Métodos: Noventa e três pacientes com o diagnóstico de TB tipo I e 58 controles - pareados por idade, gênero e nível educacional - foram envolvidos no presente estudo. Todos os sujeitos foram avaliados pelo *Mini-International Neuropsychiatry Interview* e os pacientes foram avaliados pelas escalas de mania de Young e pela escala de depressão de Hamilton. Níveis plasmáticos de BDNF, NGF, GDNF, TNF-α e seus receptores solúveis (sTNFR1 and sTNFR2), adipocinas, diversas quimiocinas foram avaliados por ELISA. A frequência de populações leucocitárias e de ativação das vias NF-κB (p65) e MAPKs (ERK1/2 e p38) foi feita por técnica de FACS. A ativação das vias de MAPKs (ERK1/2 e p38) e NF-κB (p65), foi realizada através de ensaios de *Western blot* com extratos proteicos.
Resultados: Pacientes com o diagnóstico de TB apresentaram elevação nos níveis plasmáticos de GDNF e BDNF e diminuição nos níveis plasmáticos de NGF em comparação com controles. Em relação aos parâmetros imunológicos, pacientes com o diagnóstico de TB demonstraram aumento nos níveis plasmáticos de sTNFR1, adipocinas (principalmente adiponectina e leptina) e CCL11, CCL24 e CXCL10. Pacientes com o diagnóstico de TB apresentaram redução nos níveis plasmáticos de CXCL8 em comparação com controles. Pacientes com o diagnóstico de TB apresentaram aumento da frequência de monócitos (CD14+) e redução de frequência de linfócitos T regulatórios (CD4+CD25+FOXp3+). Além disso, os pacientes com o diagnóstico de TB apresentaram aumento da fosforilação de NF-κB/p65, ERK1/2 e p38.
Conclusões: O presente estudo fortalece a visão de que o TB é associado a alterações em fatores neurotróficos e parâmetros imunológicos.

Palavras-chave: transtorno bipolar; mania; eutimia; fatores neurotróficos; imunologia; mecanismos inflamatórios.

ABSTRACT

Introduction: Bipolar disorder (BD) is a prevalent and chronic illness associated with substantial morbidity. There is a growing body of evidence indicating that disturbance at neurotrophic factors and immunologic parameters play an important role in the pathophysiology of BD.

Objective: The aim of this study was to evaluate the plasma levels of different neurotrophic factors (BDNF, NGF and GDNF) and immunologic parameters (TNF-α, sTNFR1, sTNFR2, adiponectin, leptin, resistin, and the chemokines CCL2, CCL3, CCL11, CCL24, CXCL8 and CXCL10), and evaluate the frequency of leucocytes and patways activated by neurotrophic factors and immunologic parameters in BD patients in different mood state in comparison with controls.

Methods: 93 BD type I patients and 58 controls matched by age, gender and education level were enrolled in this study. All subjects were assessed by the Mini-International Neuropsychiatry Interview and the patients were also evaluated by the Young Mania Rating Scale and the Hamilton Depression Rating Scale. The plasma levels of BDNF, NGF, GDNF, TNF-α and its soluble receptors (sTNFR1 and sTNFR2), adipokines, and several chemokines were measured by ELISA. The frequency of leucocytes and activated pathways of NF-κB (p65) and MAPKs (ERK1/2 and p38) was measured by FACS. The activaction of MAPK pathway (ERK1/2 and p38) and NF-κB (p65) pathway was measured by *Western blot*with proteic extracts.

Results: BD patients presented higher plasma levels of GDNF and BDNF and lower plasma levels of NGF in comparison with controls. Regarding immunologic parameters, BD patients showed higher plasma levels of sTNFR1, adipokines (mainly adiponectin and leptin), and CCL11, CCL24, and CXCL10. BD patients presented lower plasma levels of CXCL8 in comparison with controls. BD patients presented increased frequency of monocytes (CD14+) and lower frequency levels of T regulatory lymphocytes (CD4+CD25+FOXp3+). Moreover, BD patients presented increased phosphorylation levels of NF-κB/p65, p38 and ERK1/2.

Conclusions: The present study reinforces the view that BD is associated with neurotrophic factors and immune parameters imbalance.

Key-words: Bipolar disorder; mania; euthymia; neurotrophic factor; immunology, inflammatory mechanisms.

LISTA DE ABREVIATURAS

BAF - Bateria de avaliação frontal

BDNF - Fator neurotrófico derivado do cérebro

CID-10 - Classificação estatística internacional de doenças e problemas relacionados à saúde – 10ª. Edição

CMSP - Células mononucleares do sangue periférico

COX-2 - Cicloxigenase-2

DSM-IV - Manual diagnóstico e estatístico de transtornos mentais – 4ª. Edição

ELISA - Ensaio imunoenzimático

FACS - Citometria de fluxo

GDNF - Fator neurotrófico derivado da glia

GSK3 - Glicogênio sintase quinase - 3

GWA - Estudo genômico de associação

HAMD - Escala de depressão de Hamilton

HPA - Hipotálamo-pituitária-adrenal

ICAM-1 - Molécula de adesão intracelular-1

IMC - Índice de massa corporal

IPSEMG - Instituto de previdência dos servidores do Estado de Minas Gerais

MAPK - Proteína quinase ativada por mitógenos

MEEM - Mini exame do estado mental

MINI Plus - Mini-International Neuropsychiatric Interview – versão plus

NF-κB - Fator nuclear kappa B

NGF - Fator de crescimento neuronal

iNOS - Óxido nítrico sintase induzida

NTs - Neurotrofinas

SNC - Sistema nervoso central

SOE - Sem outra especificação

sTNFR - Receptores solúveis de TNF

TAG - Transtorno de ansiedade generalizada

TB - Transtorno bipolar

Th - Células T citotóxicas

TNF-α - Fator-alfa de necrose tumoral

TOC - Transtorno obsessivo-compulsivo

TP - Transtorno de pânico

YOUNG - Escala de mania de Young

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SUMÁRIO

1. PREFÁCIO

A minha formação da residência de psiquiatria do Instituto de Previdência dos Servidores do Estado de Minas Gerais (IPSEMG) foi pautada pelo exercício da clínica psiquiátrica associada a constantes discussões de evidências científicas atualizadas. A oportunidade de discussões sobre o tratamento e a fisiopatologia dos transtornos psiquiátricos instigou-me a completar minha formação clínica comuma atividade de pesquisa. Era natural aaproximação com o Professor Dr. Fernando Silva Neves, meu preceptor durante a residência, que me auxiliou na construção de um projeto de pesquisa, entretanto o mesmo não poderia me orientar dado a sua não conclusão de seu doutoramento à época. O Professor Dr. Fernando Silva Neves me apresentou o admiradopesquisador, Professor Dr. Antônio Lúcio. A orientação do Professor Dr. Antônio Lúcio, baseada em seu extremo rigor, organização e perfeccionismo, potencializaram algumas características próprias a minha personalidade que permitiram a realização do trabalho.

O Professor Dr. Antônio Lúcio apresentou a linha depesquisa em que o mesmo demonstrava grande interesse de pesquisar, ainda não tão explorado em nosso meio, as alterações neuroimunoendócrinas e de fatores neurotróficos em doenças neuropsiquiátricas. A escolha do tema relacionado ao transtorno bipolar (TB) deveu-se a preferências pessoais, dado que eu vinha auxiliando no atendimento clínico e na expansão do ambulatório especializado em TB do IPSEMG. O grande fascínio pelo tema surgiu pela curiosidade da compreensão de mecanismos fisiopatológicos do transtorno bipolar e das mudanças de humor observadas em tais pacientes.

Muitas pessoas foram importantes na construção deste projeto. No laboratório de Imunofarmacologia do Instituto de Ciências Biológicas, as portas do laboratório foram abertas pelo prof. Dr. Mauro Teixeira que permitiu a realização das análises das diversas moléculas avaliadas no presente trabalho. O apoio, auxílio e constantes ensinamentos das Professoras Dra. Vanessa Amaral Mendonça e Dra Lirlândia Pires Sousa em técnicas laboratoriais aplicadas no presente trabalho. As diversas horas de dedicação, apoio, auxílio, o desenvolvimento do protocolo para o congelamento e descongelamento de células mononucleares de sangue periférico e realização de

técnicas de ELISA e de leitura de FACs da amiga e doutoranda Natália Pessoa Rocha. A receptividade da equipe do ambulatório de transtorno bipolar do IPSEMG em especial pelo apoio do Rodrigo Barreto Huguet. As discussões com o objetivo de pensar e criticar os resultados, ocorridas em sua maioria por trocas de emails, com o Professor Dr. Moisés Evandro Bauer. Os grupos de pesquisa em Neuropsiquiatria e Neuroimunologia lugar de analisar, pensar e criticar nossos resultados e incrementar os projetos. A receptividade encontrada no Programa de Pós-Graduação em Neurociências. A oportunidade de conhecer e trabalhar no laboratório do serviço de Psiquiatria Perinatal, Estresse e Imunologia do King's College de Londres, onde fui tão bem acolhida pelo Professor Dr. Carmine M. Pariante e toda a sua equipe, em especial a Dra. Patricia A. Zunszain e aos amigos Nilay Hepgul, Christoph Anacker e Naghmeh Nikkheslat.

A proposta do trabalho é apresentar os resultados da avaliação de fatores neurotróficos e marcadores imunológicos em pacientes com o diagnóstico de transtorno bipolar atendidos no serviço de psiquiatria do IPSEMG em comparação com controles da comunidade. Este estudo justifica-se pelo fato de o transtorno bipolar ser uma síndrome psiquiátrica de prevalência elevada, associada a altas taxas de recorrência, suicido, incapacidade funcional, gastos financeiros elevados ao sistema de saúde, cujos mecanismos fisiopatológicos ainda permanecem desconhecidos. Evidências científicas recentes têm demonstrado o envolvimento dos fatores neurotróficos e marcadores imunológicos nas regulações de neurotransmissores, plasticidade sináptica, expressão gênica, sobrevivência e morte neuronal como os principais fatores associados à fisiopatologia do TB (KAPCZINSKI et al, 2011).

Para a apresentação de seus elementos, a tese foi estruturada em três partes principais: introdução, que parte de uma breve revisão da literatura para apresentar relevância, justificativa, objetivos e métodos relativos ao tema pesquisado e que abordam diferentes aspectos e questões trabalhadas durante o processo de desenvolvimento da tese. A segunda parte trata dos resultados no formato de artigos científicos e dados adicionais ainda não publicados. A parte final é composta pela discussão dos achados à luz da literatura científica.

A revisão da literatura neste trabalho é inaugurada com o artigo 1, intitulado"***Circulating levels of brain-derived neurotrophic factor: correlation with***

mood, cognition and motor function", publicado na Biomarkers in Medicine em quefoi realizada uma revisão da literatura sobre o fator neurotrófico derivado do cérebro (BDNF), sua ação biológica enfocando principalmente seu papel em humor, cognição e funções motoras, assim como doenças neuropsiquiátricas. No artigo 2, intitulado *"**Imunologia do transtorno bipolar**"*, publicado no Jornal Brasileiro de Psiquiatria,em que foi realizada uma revisão sistemática da literatura com o objetivo de avaliar os trabalhos que investigaram alterações imunes no TB.

No artigo 3, intitulado "***Comorbidades clínicas e psiquiátricas em pacientes com transtorno bipolar do tipo I***", publicado no Jornal Brasileiro de Psiquiatria,foi realizadauma análisede características demográficas, comorbidades clínicas e psiquiátricas e presença de tentativas de suicídio na amostra de todos os pacientes com o diagnóstico de TB incluídos no presente trabalho. Os artigos 4, 5 e 6 tratam sobre a análise de níveis plasmáticos de fatores neurotróficos em pacientes com TB em comparação a controles. No artigo 4, "***Increased BNDF levels in BD patients in late stage***", submetido a Revista Brasileira de Psiquiatria, foi avaliado o BDNF, no Artigo 5, "***Impaired nerve growth factor homeostasis in patients with bipolar disorder***", publicado no The World Journal of Biological Psychiatry, foi realizada análise de fator de crescimento neuronal (NGF) e no artigo 6, "***Circulating levels of GDNF in bipolar disorder***", publicado na Neuroscience Letters foi realizada análise de fator neurotrófico derivado da glia (GDNF). Foi demonstrado que pacientes com o diagnóstico de TB apresentam redução dos níveis de NGF e aumento dos níveis de GDNF e BDNF.

Estudo prévio do nosso grupo demonstrou que pacientes com o diagnóstico de TB apresentamelevações dos níveis de fator de necrose tumoral alfa (TNF-α) e de seus receptores circulantes, refletindo um estado pró-inflamatório exacerbado associado ao TB. Demais moléculas relacionadas ao sistema imune em pacientes com o diagnóstico de TB nos artigos foram avaliadas nos 7, 8 e 9. O artigo 7, "***Increased levels of adipokines in bipolar disorder***", publicado no Journal of Psychiatric Research avalia os níveis plasmáticos de adipocinas, citocinas produzidos por tecido adiposo. O artigo 8, "***Chemokines in bipolar disorder: trait or state?***", recentemente aceito para publicação na European Archives of Psychiatry and Clinical Neuroscience, avalia os níveis plasmáticos de quimiocinas, moléculas associadas ao tráfico leucocitário. A avaliação da presença de comorbidade como o deficit cognitivo e suas interrelações com fatores neurotróficos e marcadores imunológicos é demonstrada no artigo 9 "***Executive***

dysfunction in euthymic bipolar disorder patients and association with plasma biomarkers", publicado no Journal of Affective Disorders.

Finalmente, nos Resultados Adicionais são apresentados resultados referentes a avaliação de frequência leucocitária e possíveis mecanismos intracelulares associados a fisiopatologia do TB.

O Diagrama 1 apresenta a estrutura da tese.

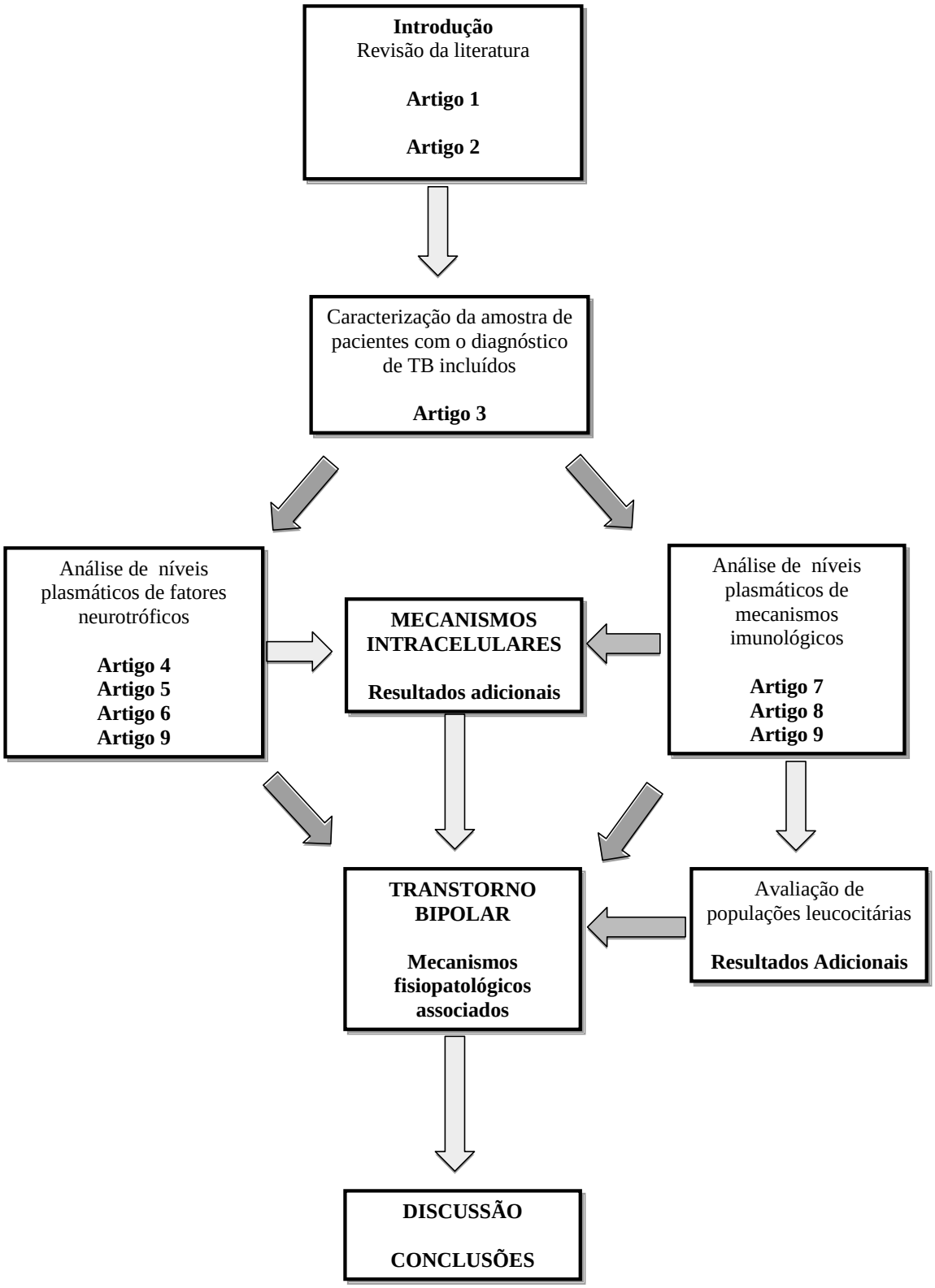

Figura 1. Diagrama da estrutura da tese.

2. REVISÃO DA LITERATURA

2.1. CLÍNICA DO TRANSTORNO BIPOLAR

O transtorno bipolar (TB) é uma síndrome psiquiátrica clássica e tem sido relatada em diversas culturas. Os termos mania e melancolia já eram descritos por volta de 400 a. C. por Hipócrates para caracterizar alterações do estado mental (SADOCK & SADOCK, 2007). Em 1899, Emil Kraepelin, reuniu, sob o termo de psicose maníaco-depressiva, um conjunto de formas clínicas caracterizadas por alteração primária do afeto, perturbações específicas do curso do pensamento e da psicomotricidade, e tendência à recidiva, adotando um ponto de vista unitárioaos estados melancólicos, a mania unitária e os quadros circulares (DOYLE, 1956). Em 1957, Karl Leonhard propôs a divisão em psicoses monopolares e bipolares. Pacientes com psicoses monopolares exibiam quadros como mania, melancolia e depressões unitárias. Psicoses bipolares seriam constituídas pela doença maníaco-depressiva e pelas psicoses ciclóides. A classificação atual do Manual Diagnóstico e Estatístico de Transtornos Mentais (DSM-IV) e da Revisão da Classificação Internacional de Doenças (CID 10) foram baseadas, em parte, nesses conceitos clássicos (DEL PORTO, 2004).

O quadro maníaco consiste em alteração primária do humor com sentimentos de alegria ou de cólera. Há aceleração do tempo psíquico e de atos motores. O pensamento revela uma disposição otimista e expansiva. A atividade verbal mostra-se intensa, rápida e, ao discurso, transparece fuga de ideias. Os processos associativos realizam-se velozmente e o limite entre as ideias mostra-se superficial e frouxo, predominando as associações por continuidade e por simples assonância. A atenção espontânea exagera-se em detrimento da voluntária. A distrabilidade é frequente. Podem ser encontradas ideias delirantes de supervalorização pessoal, temas de grandeza, intervenção ou reforma social (DOYLE, 1956). Os quadros de menor intensidade são denominados hipomania. Já a depressão pode ser definida como uma alteração primária do humor no sentido da tristeza ou da angústia. Observa-se dificuldade, e até mesmo inibição, dos processos psíquicos e da mobilidade. Podem associar-se ideiasdelirantes compatíveis ou não com o fundo afetivo (DOYLE, 1956). Eutimia é usualmente definida como remissão dos sintomas.

Os critérios diagnósticos, segundo DSM-IV TR para mania e depressão são apresentados na **Tabela 1** e **Tabela 2** abaixo:

Tabela 1. Critérios do DSM-IV-TR para definição de episódio maníaco

A) Um período distinto, durante o qual existe um humor anormal e persistentemente elevado, expansivo ou irritável. A duração é mínima de 1 semana ou qualquer duração se a hospitalização for necessária.
B) Durante o período de perturbação, três ou mais dos seguintes sintomas persistiram e estiveram presentes em grau significativo. No caso de humor irritável são necessários pelo menos quatro dos sintomas abaixo:
1) autoestima inflada ou grandiosidade.
2) necessidade de sono diminuída, exemplificado por sentir-se descansado com 3 horas de sono.
3) mais loquaz que o habitual ou pressão por falar.
4) fuga de ideias ou experiência subjetiva de que os pensamentos estão correndo.
5) distrabilidade, isto é, atenção desviada com excessiva facilidade por estímulos externos insignificantes ou irrelevantes.
6) aumento da atividade dirigida a objetivos, socialmente, no trabalho, na escola ou sexualmente. Pode ser considerada agitação psicomotora.
7) envolvimento excessivo em atividades prazerosas com um alto potencial para consequências dolorosas. Exemplificado por envolvimento em surtos incontidos de compras, indiscrições sexuais ou investimentos financeiros insensatos.
C) Os sintomas não satisfazem os critérios para um Episódio Misto.
D) A perturbação deve ser suficientemente severa para causar prejuízo acentuado no funcionamento ocupacional, nas atividades sociais, nos relacionamentos costumeiros ou para exigir a hospitalização. Pode ser marcado pela presença de características psicóticas.
E) Os sintomas não se devem aos efeitos fisiológicos diretos de uma substância (droga de abuso ou medicamento) ou de uma condição médica geral, exemplificado por hipertireoidismo.

Tabela 2. Critérios do DSM-IV-TR para definição de episódio depressivo

A) No mínimo cinco dos seguintes sintomas estiveram presentes durante o período mínimo de duas semanas e representaram uma alteração a partir do funcionamento anterior. Pelo menos um dos sintomas deve ser: (1) humor deprimido ou (2) perda do interesse ou prazer.
Nota: Não incluir sintomas nitidamente devidos a uma condição médica geral, alucinações ou delírios incongruentes com o humor.
1) humor deprimido na maior parte do dia, quase todos os dias. Indicado por relato subjetivo ou observação feita por outros.
2) acentuada diminuição do interesse ou prazer em todas ou quase todas as atividades, na maior parte do dia, quase todos os dias. Indicado por relato subjetivo ou observação feita por outros.
3) perda ou ganho significativo de peso sem estar em dieta (exemplificado por mais de 5% do peso corporal em 1 mês). Poder ser considerado diminuição ou aumento do apetite quase todos os dias.
4) insônia ou hipersonia quase todos os dias;
5) agitação ou retardo psicomotor quase todos os dias. Observáveis por outros, não meramente sensações subjetivas de inquietação ou de estar mais lento.
6) fadiga ou perda de energia quase todos os dias.
7) sentimento de inutilidade, culpa excessiva ou inadequada (pode ser delirante), quase todos os dias. Não meramente auto-recriminação ou culpa por estar doente.
8) capacidade diminuída de pensar ou concentrar-se, ou indecisão, quase todos os dias. Indicado por relato subjetivo ou observação feita por outros.
9) pensamentos de morte recorrente. Ideação suicida recorrente sem um plano específico. Tentativa de suicídio ou plano específico para cometer suicídio.
B) Os sintomas não satisfazem os critérios para um Episódio Misto.
C) Os sintomas causam sofrimento clinicamente significativo ou prejuízo no funcionamento social, ocupacional ou em outras áreas importantes da vida do indivíduo.
D) Os sintomas não se devem aos efeitos fisiológicos diretos de uma substância (droga de abuso ou medicamento) ou de uma condição médica geral, exemplificado por hipotireoidismo.
E) Os sintomas não são mais bem explicados por luto. Os sintomas persistem por mais de 2 meses ou são caracterizados por acentuado prejuízo funcional, preocupação mórbida com desvalia, ideação suicida, sintomas psicóticos ou retardo psicomotor.

Para caracterizar o diagnóstico de TB, pacientes devem apresentar pelo menos um episódio de mania ou hipomania ao longo da vida acompanhado por outro transtorno de humor (seja ele outro episódio de mania, hipomania, estado misto ou depressão). É importante ressaltar que tal quadro não seja secundário a uma doença clínica preexistente (ex: hipertireoidismo), abuso de substâncias (ex: anfetaminas) ou efeito de medicação (ex: corticosteróides). A síndrome na qual se evidencia pelo menos um episódio de mania é denominada TB tipo I. A síndrome na qual se observa exclusivamente episódios de hipomania é denominada TB tipo II. Os pacientes com TB tipo I e tipo II podem apresentar quadros depressivos durante o curso do transtorno (DSM-IV TR, 2004).

A presente tese irá versar sobre o TB do tipo I.

2.2. EPIDEMIOLOGIA DO TRANSTORNO BIPOLAR

O TB tipo I tem prevalência de cerca de 1% da população mundial e não há diferenças entre os gêneros masculino e feminino (KESSLER et al, 2007; BELMAKER, 2004). A idade média de início dos sintomas ocorre principalmente entre 15 e 19 anos (KUPFER et al, 2002), sendo que cerca de 50% dos pacientes apresentam inicialmente episódio depressivo (KUPFER et al, 2002).

A duração média de um episódio de humor em pacientes com TB tipo I é de cerca de treze semanas (SOLOMON et al, 2010). Em estudo prospectivo com tempo médio de acompanhamento de 12 anos, foi demonstrado que pacientes com TB tipo I apresentam alterações de humor em 46,6% do tempo (JUDD et al, 2003).Além disso, o risco de novos episódios de humor em pacientes com TB permanece relativamente alto após 40 anos de início da doença, indicando um risco de recrudescência dos sintomas até por volta de 70 anos (ANGST et al, 2003). Além de estar associado à grande morbidade o TB também é associado a grande mortalidade. A mortalidade por suicídio em paciente com o diagnóstico de TB é nove vezes maior que na população em geral (DUTTA et al, 2007). Uma recente meta-análise demonstrou que mais de 30% de pacientes com o diagnóstico de TB apresentam tentativas de suicídio ao longo da vida (NOVICK et al, 2010).

Assim, a expectativa de vida em pacientes com o diagnóstico de TB é menor em comparação a população em geral e apontam-se como as possíveis causas os elevados índices de suicídio e elevada prevalência de comorbidades. Entre 50 e 70% dos pacientes apresentam algum tipo de comorbidade psiquiátrica, com maior prevalência de transtornos ansiosos e de dependência e/ou abuso de substâncias (KRISHNAN et al, 2005).Uma tabela demonstrando frequência de comorbidades psiquiátricas em pacientes com TB na literatura nacional é mostrada a seguir na **Tabela 3**.

Tabela 3. Estudos brasileiros sobre frequência de comorbidades psiquiátricas no transtorno bipolar

Estudo	Amostra	TAG	TP	TOC	Dependência/ uso nocivo	
					Álcool	Demais psicotrópicos
Cardoso et al, 2008	N=186	---	---	---	28,5%	---
Nery-Fernandes et al, 2009	N = 61	12,9%	3,2%	1,6%	6,4%	1,6%
Neves et al, 2009	N = 239	44,4%	20,9%	---	28,0%	13,8%
da Silva Magalhães et al, 2009	N =230	---	---	---	29,0%	21,0%
da Silva Magalhães et al, 2010	N=259	---	---	12,4%	---	---

Abreviações: TAG, transtorno de ansiedade generalizada. TOC, transtorno obsessivo-compulsivo. TP: transtorno de pânico SOE, sem outra especificação.

Em relação às comorbidades médicas em geral, o TB é frequentemente associado a desordens metabólicas e endocrinológicas (particularmente obesidade e diabetes mellitus), doenças cardiovasculares e/ou hipertensão arterial sistêmica e declínio cognitivo (ALTAMURA et al, 2011; YATHAM et al, 2010; FAJUTRAO et al, 2009).

Portanto, apesar dos sistemas classificatórios atuais serem baseados em observações clínicas do paciente, o transtorno bipolar não deve ser considerado uma

doença que afeta “somente” o humor, mas uma condição multisistêmica que envolve o humor, cognição, alterações endocrinológicas e autonômicas (PHILLIPS & FRANK, 2006).

2.3. BASES NEUROBIOLÓGICAS

A fisiopatologia do transtorno bipolar ainda permanece desconhecida. Evidências recentes apontam que o transtorno bipolar surge a partir de uma complexa interação de diversos fatores, entre eles fatores genéticos, fatores ambientais, disfunção em diversos circuitos cerebrais, mecanismos associados à neuroplasticidade neuronal e disfunções neuroimunoendocrinológicas (SCHLOESSER et al, 2008).

Ademais, apesar do antigo conceito de Kraepilin de que o transtorno bipolar seria uma desordem do humor, sem alterações cognitivas e com o curso estável ao longo do tempo (BERRIOS, 1988), evidências recentes da literatura têm apontado o transtorno bipolar como uma condição de curso progressivo (BERK et al, 2011). Corroboram com a hipótese de condição neuroprogressiva do transtorno bipolar a observação de declínio cognitivo, aumento da prevalência de comorbidades médicas e mortalidade por condições cardiovascularesassociados com os diversos episódios de humor (BERK et al, 2011; KAPCZINSKI et al, 2010; POST, 2007).Os episódios de humor atuariam como “tóxicos” ao organismo e o componente neurobiológico associado seria a desregulação dos fatores neurotróficos e dos marcadores imunológicos nos pacientes com o diagnóstico de TB (BERK et al, 2011; KAPCZINSKI et al, 2010).

2.3.1 Genética

Estudos apontam que cerca de 50% dos pacientes têm em sua família algum membro acometido com a doença (BELMAKER, 2004, KUPFER et al, 2002). Estudos envolvendo gêmeos monozigóticos e dizigóticos apontam uma concordância entre 40 e 80% e entre 10 e 20%, respectivamente (BELMAKER et al, 2004). Reconhece-se, assim, a relevância da contribuição genética, uma vez descrita herdabilidade de até 84% (MERIKANGAS & YU, 2002). Evidências recentes têm mostrado que pacientes com o

TB apresentam uma expressão aberrante de genes relacionados à cascata inflamatória (PDE4B, IL1B, IL6, TNF, TNFAIP3, PTGS2, e PTX3), tráfico leucocitário (CCL2, CCL7, CCL20, CXCL2, CCR2, e CDC42), sobrevivência (BCL2A1 e EMP1) ea via da proteína quinase ativada por mitógenos (MAPK) (MAPK6, DUSP2, NAB2, e ATF3) (PADMOS et al, 2008). A literatura aponta que há genes comuns entre a esquizofrenia e o TB como o CACNA1C, ZNF804A, PBRM1 e NRGN, sugerindo mecanismos moleculares em comum para o desenvolvimento de psicose (LEE et al, 2012). Ressalta-se que apesar de esperanças de que novas técnicas de análise genética, tais como o estudo genômico de associação (GWA, ou genome–wide association study), pudessem esclarecer o componente genético associado ao TB, atualmente acredita-se que nem um único gene, nem a teoria poligênica por si só,poderão promover o conhecimento das bases biológicas associadas ao TB (LEE et al, 2012; GERSHON et al, 2011).

2.3.2 Neuroanatomia e Neuropatologia

Técnicas de neuroimagem estruturais e funcionais vêm sendo amplamente utilizadas para investigar a neurobiologia da TB. Assim, várias regiões cerebrais e circuitos neurais que medeiam as alterações de estado em transtornos do humor têm sido propostas com base nas descobertas da neuroimagem.

Revisões sistemáticas da literatura apontam como achados mais consistentes que pacientes com o diagnóstico de TB apresentam redução volumétrica do córtex pré-frontal, principalmente em região dorso-lateral, córtex orbitofrontal, região subgenual e regiões ventrais e dorsais do córtex anterior do cíngulo (KUPFERSCHMIDT & ZAKZANIS, 2011; LANGAN & MCDONALD, 2009; SAVITZ & DREVETS, 2009; KONARSKI et al, 2008). Ainda têm sido descritos um aumento relativo dos ventrículos (LANGAN & MCDONALD, 2009; MANJI et al, 2000), sem alteração no volume cerebral global (KONARSKI et al, 2008). Curiosamente, em relação ao volume da amigdala, estudos apontam que há uma variação de tamanho, alteração esta aparentemente relacionada com a idade. Pacientes com o diagnóstico de TB na infância e adolescência apresentam diminuição do volume da amígdala, enquanto pacientes com transtorno bipolar em idade adulta evidenciam aumento e hiperatividade desta estrutura (KUPFERSCHMIDT & ZAKZANIS, 2011; LANGAN & MCDONALD, 2009;

SAVITZ & DREVETS, 2009; KONARSKI et al, 2008). Os dados são limitados e/ou conflitantes em relação a alterações cerebelares, lobo temporal, hipocampo e tálamo (KONARSKI et al, 2008).

Uma vez que regiões como o hipocampo e o córtex pré-frontal estariam relacionadas principalmente com organização da memória de trabalho, integração de domínios cognitivos, regulação de estímulos inibitórios para regiões da amigdala e mecanismos de recompensa, regulação do afeto, regulação de respostas autonômicas ao estresse e regiões associadas ao processamento de impulsos e deficits atencionais, sintomas como mudanças de humor, labilidade emocional e distrabilidade também seriam resultantes das mesmas alterações anatômicas (SAVITZ & DREVETS, 2009).

Estudos *post-mortem* têm demonstrado que pacientes com o diagnóstico de TBapresentam uma redução do volume do núcleo neuronal. A alteração na densidade e morfologia neuronal parece ser específica por diferentes camadas celulares e tipos neuronais. Têm sido descritas reduções na densidade e no tamanho do corpo neuronal em neurônios piramidais glutamatérgicos nas regiões III e Vdo córtex dorso lateral pré-frontal, em camadas III-V de sub-regiões do cíngulo anterior e em células piramidais da região CA1 do hipocampo (revisto por SCHLOESSER et al., 2008).Sugerem-se, ainda, alterações na densidade e no número de células gliais (SANCHES et al, 2008; SCHLOESSER et al., 2008). É importante mencionar que resultados de estudos *post-mortem*em pacientes com o diagnóstico de TB não são convergentes, principalmente por variáveis que podem ser conflitantes, tais como abuso de substâncias, artefatos secundários a mortes violentas (suicídio) e, ainda, a dificuldade de se encontrar uma amostra de controles a tal grupo de pacientes.

2.4. FATORES NEUROTRÓFICOS

Fatores neurotróficos são uma família de proteínas essenciais para o desenvolvimento, diferenciação e sobrevivência neuronal (LU et al, 2005) e também exercem um importante papel na modulação da excitabilidade neuronal e transmissão sináptica (POO, 2001). Os fatores neurotróficos podem ser divididos em três grandes famílias: a família das neurotrofinas; a família dos sítios do fator neurotrófico das células de linhagem glia; e a família das citocinas neuropoiéticas. A distinção entre estas

três famílias é baseada nas estruturas moleculares e nas interações com seus receptores, nos padrões de expressão após lesão neuronal, e nos efeitos celulares (BOYD & GORDON, 2003).

A família das neurotrofinas é composta pelo fator neurotrófico derivado do cérebro (*brain derived neurotrophic factor* ou BDNF), fator de crescimento neuronal (*nerve growth factor* ou NGF), NT-3 e NT-4/5. Entre todos os fatores neurotróficos o BDNF é apontado como o principal fator relacionado com as desordens de humor e com o transtorno bipolar. O BDNF é o mais abundante fator neurotrófico no sistema nervoso central (SNC), sendo particularmente abundante na amigdala, hipocampo e córtex pré-frontal, áreas do cérebro que estão envolvidas com a regulação de emoção e diversos aspectos cognitivos como atenção, memória e função executiva (KOJIMA et al, 2001; LINDHOLM et al, 1996; GOSH et al, 1994; HYMAN et al, 1991; ALDERSON et al, 1990).

Em relação aos transtornos de humor, os níveis de BDNF tem se mostrado geralmente diminuídos em modelos animais de depressão e de mania em regiões hipocampais (VALVASSORI et al, 2011; JORNADA et al, 2010; FREY et al, 2006; DUMAN et al, 2006). A administração de medicamentos antidepressivos no hipocampo de modelos animais de depressão ocasiona o aumento de níveis de BDNF hipocampais, assim como a administração de BDNF no hipocampo de ratos tem resultado em efeitos antidepressivos (SHYRAIAMA et al, 2002). Por sua vez, o tratamento com estabilizadores de humor tem ocasionado elevação dos níveis de BDNF em modelos animais de mania (VALVASSORI et al, 2011; JORNADA et al, 2010; FREY et al, 2006). Em assonância com os achados dos dados experimentais, duas recentes meta-análises demonstraram que pacientes com o diagnóstico de TB apresentam redução dos níveis circulantes (plasma e soro) de BDNF em comparação com controles (FERNANDES et al, 2011; LIN, 2009). Quando uma sub-análise de acordo com os diferentesestados de humor, foi realizada em pacientes com o diagnóstico de TB, as meta-análises demonstraram que os pacientes com o diagnóstico de TB em episódios de mania e depressão apresentam redução dos níveis circulantes de BDNF em comparação com controles (FERNANDES et al, 2011; LIN, 2009), entretanto aqueles pacientes com diagnóstico de TB em eutimia não apresentam alterações dos níveis de BDNF em relação aos controles (FERNANDES et al, 2011; LIN, 2009). Estes resultados sugerem

que o BDNF pode ser considerado um biomarcador de estado ao invés de um marcador de traço de TB.

Os demais fatores neurotróficos são menos estudados no transtorno bipolar. Apesar de o NGF ter sido o primeiro a ser descoberto na década de cinquenta, não há estudos sobre sua avaliação em pacientes com o diagnóstico de TB. No SNC, o NGF possui um importante papel no desenvolvimento e na manutenção do sistema nervoso simpático e de neurônios sensoriais primários, assim como nas funções colinérgicas,e apresenta elevada produção no córtex, hipocampo, pituitária e medula espinhal(NIEWIADOMSKA et al, 2011; CUELLO et al, 2010; COUNTS & MUFSON, 2005). Devido ao fato de neurônios colinérgicos relacionarem-se com o córtex cerebral e hipocampo, reconhece-se seu papel na memória e atenção (NIEWIADOMSKA et al, 2011). O papel do NGF tem sido avaliado em transtornos neuropsiquiátricos classicamente relacionados com declínio cognitivo, tais como doença de Alzheimer, doença de Parkinson e esclerose múltipla (VALENZUELA et al, 2007; ZIEGENHORN et al, 2007; HELLWEG et al, 1998).

A família dos sítios do fator neurotrófico das células de linhagem glia apresenta como principal representante o fator neurotrófico derivado da glia (g*lial cell-derived neurotrophic factor* ou GDNF), considerado um dos mais potentes fatores neurotróficos de neurônios dopaminérgicos, sendo largamente encontrado nas diversas regiões cerebrais (AIRAKSINEN & SAARMA, 2002). No passado, acreditava-se que o GDNF tinha o papel de auxiliar no desenvolvimento e na manutenção do sistema nigroestriatal e sua relação com o sistema dopaminérgico foi implicada somente na fisiopatologia da doença de Parkinson. Evidências recentes, no entanto, sugerem que o GDNF exerce papel fundamental no desenvolvimento e manutenção de células gliais (DUCRAY et al, 2006), assim como na regulação de vias noradrenérgicas e GABAérgicas (SARABI et al, 2000). Ademais, o aumento na regulação de GDNF por astrócitos e por micróglias pode ser um mecanismo de proteção para conter a perda neuronal observada em diferentes tipos de doenças neuropsiquiátricas (MILLER, 2011; SAAVEDRA et al, 2008). Os estudos em pacientes com o diagnóstico de TB têm demonstrado resultados contraditórios em relação aos níveis circulantes de GDNF, tais como elevações (ROSA et al, 2006) e reduções dos níveis de GDNF (ZHANG et al, 2010; TAKEBAYASHI et al, 2006).

Uma revisão sobre a neurotrofina BDNF, abordando suas propriedades biológicas e suas interrelações com transtornos psiquiátricos é descrita no **Artigo 1**: “Circulating levels of brain-derived neurotrophic factor: correlation with mood, cognition and motor function”.

Artigo 1: Circulating levels of brain-derived neurotrophic factor: correlation with mood, cognition and motor function

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Circulating levels of brain-derived neurotrophic factor: correlation with mood, cognition and motor function

Brain-derived neurotrophic factor (BDNF) is the most widely distributed neurotrophin in the CNS, where it plays several pivotal roles in synaptic plasticity and neuronal survival. As a consequence, BDNF has become a key target in the physiopathology of several neurological and psychiatric diseases. Recent studies have consistently reported altered levels of BDNF in the circulation (i.e., serum or plasma) of patients with major depression, bipolar disorder, Alzheimer's disease, Huntington's disease and Parkinson's disease. Correlations between serum BDNF levels and affective, cognitive and motor symptoms have also been described. BDNF appears to be an unspecific biomarker of neuropsychiatric disorders characterized by neurodegenerative changes.

KEYWORDS: Alzheimer's disease • amyotrophic lateral sclerosis • bipolar disorder • brain-derived neurotrophic factor • depression • Huntington's disease • Parkinson's disease • plasma • serum

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Neurotrophins are a family of proteins that are essential for the development, differentiation and survival of neurons. In the 1950s, Rita Levi-Montalcini identified the first neurotrophin, the nerve growth-promoting factor, later renamed NGF, a molecule that could promote the growth of neurons of the sympathetic nervous system [1]. Since then, there has been significant evolution in the field and other neurotrophins have been described. Today the group is composed of NGF, brain-derived neurotrophic factor (BDNF), and neurotrophin-3, -4, -5 and -6. All neurotrophins present similar biochemical characteristics with variable domains, which determine the binding to specific receptors and lead to different biological effects [2].

Brain-derived neurotrophic factor is the most widely distributed neurotrophin in the CNS. A series of studies demonstrated that BDNF displays several functions, including regulation of axonal and dendritic growth and guidance, participation in neurotransmitter release, and long-term potentiation [3]. Therefore, BDNF has emerged as a major regulator of synaptic plasticity.

These broad functions in the CNS ascribed to BDNF make this peptide a key target in the physiopathology of several neurological and psychiatric diseases. In this article, we revise the putative role of BDNF in mood, cognitive and motor functions, and its potential use as a biomarker of neuropsychiatric disorders.

Neurobiology of BDNF

Human mature BDNF is a 13.5-kDa, 119-amino acid nonglycosylated polypeptide whose primary structure is conserved among humans, mice and rats. BDNF contains six cysteine residues that are believed to form three intrachain disulfide linkages. BDNF is more than 50% identical to NGF at the amino acid level. Cells known to express BDNF include neurons, astrocytes, Schwann cells, fibroblasts and, possibly, smooth muscle cells. Similar to other neurotrophins, BDNF protein complex is synthesized as a precursor protein, pro-BDNF. This protein weighs approximately 28 kDa and is cleaved by a proteolytic process to the mature form of BDNF [2–4].

After transcription, pro-BDNF is wrapped and packed by the trans-Golgi system in secretory vesicles. Vesicles can be spontaneously released or more often released after stimuli [5,6]. Therefore, pro-BDNF can be secreted by neurons in different sites of the CNS, such as the cerebral cortex, cerebellum, substantia nigra, amygdala and hypothamalus, or cleaved intracellularly by furin or proconvertases. Extracellular proteases such as plasmin and matrix metalloprotease-7 are also responsible for the conversion of pro-BDNF into a mature BDNF molecule [4].

Brain-derived neurotrophic factor is the most widely distributed neurotrophin in the CNS. Immunohistochemistry techniques demonstrated the presence of the protein and/or its related mRNA in cortical and hippocampal neurons. Pyramidal neurons in the primary visual cortex and other occipital areas, motor and somatosensory cortex, and insula and temporal cortex were immunoreactive to BDNF, suggesting that these cells are one of the most important sources of BDNF. The anatomical distribution of

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BDNF in the hippocampal formation supports the hypothesis that a large proportion of hippocampal BDNF is contained in the mossy fiber system, particularly in the region of dentate gyrus granule cells and hilar region of the dentate gyrus. BDNF protein is also abundant in the amygdala, basal ganglia, cerebellum and brainstem [7–13]. The presence of BDNF in these areas seems to be related to its relevant role in promoting the survival of many CNS neurons [7–13].

Studies in mice have successfully elucidated the physiological roles of BDNF. Complete suppression of the *BDNF* gene compromises the progress of the developing fetus beyond the embryonic stage [14]. The disruption of BDNF homeostasis in adults leads to several changes in neuronal function and, thus, to neurodegeneration. In animal models, changes in BDNF activity, either by the inhibition of the BDNF intracellular cascades or by genetic manipulation, interfere with several intracellular cascades that lead to a decrease in dendritic spines and arborization, and, finally, neuritic dystrophy and degeneration, and neuronal atrophy and reduced neurogenesis [15–22]. The behavioral correlates of these functional and structural changes are severe memory impairment and depressive behavior [22,23]. The reestablishment of normal BDNF activity can restore structural changes and behavior [24,25]. It is worth noting that BDNF itself does not seem to control mood; direct evidence that loss or reduction of BDNF signaling is sufficient to mediate depression-like behavior is lacking [26]. In fact, BDNF seems to play a role in the modulation of networks involved in mood regulation [27], such as the hippocampus and hypothalamus–pituitary–adrenal axis [28]. For example, increased levels of BDNF in the CNS of mouse models submitted to chronic stress is associated with an improved capacity to maintain neuroendocrine activity (measured by changes in the levels of cortisol) and, thus, with lower susceptibility to develop a depressive state [29].

Long-term potentiation (LTP) in the hippocampus is the most recognizable form of synaptic plasticity and underlies memory and learning. LTP presents two phases: one independent of genes transcription (early LTP) and the other requiring changes in gene expression and *de novo* protein synthesis (late LTP). The persistence of early LTP for hours or days promotes gene transcription and protein synthesis with long-lasting cellular changes that characterizes late LTP. BDNF seems to play a role in both phases of LTP. Deletion of BDNF in mice or blocking the binding of BDNF to tyrosine kinase receptor B (TrkB) disrupted the normal induction of early LTP, while the administration of BDNF rescued the process [30]. BDNF also seems to stimulate the synthesis and the rapid dendritic trafficking of mRNA encoded by the immediate early response gene, *Arc*, whose protein mediates late LTP [31–33]. The infusion of recombinant BDNF in the CA1 region of the dorsal hippocampus restored memory deficits caused by local inhibition of protein synthesis in rats [34].

Most of BDNF's neuronal effects are mediated through the high-affinity TrkB (Figure 1). Binding of BDNF to TrkB leads to receptor dimerization and transautophosphorylation of specific tyrosine residues, creating docking sites for signaling cascades. This event initiates several complex intracellular signaling cascades, including Ras/MAPK, PI3K/Akt and phospholipase C, which lead to neuronal differentiation and survival [35].

Brain-derived neurotrophic factor can also interact with p75 neurotrophin receptor, leading to the activation of a different set of transduction cascades, such as NF-κB and c-Jun N-terminal kinase-p53-Bax (Figure 1). The p75 neurotrophin receptor presents an extracellular domain with cysteine-rich motifs, a single transmembrane domain and a cytoplasmic 'death' domain [35]. Following the activation of the 'death domain', proapoptotic signaling develops with an increase in Rac and c-Jun N-terminal kinase-p53-Bax [3]. While activation of the TrkB receptor supports neuronal survival, axonal and dendritic growth, the activation of p75 determines neuronal apoptosis, and axonal and dendritic degeneration. Therefore, the balance between the activation of TrkB and p75 receptors is important for changes in synaptic structure and critical for synaptic plasticity [36].

Brain-derived neurotrophic factor effects vary according to the stage of human development. In the early fetal stage, BDNF is relevant for formation and maturation of neurons in general. In adulthood, BDNF plays a critical role in synaptic plasticity processes, such as LTP involved in episodic memory consolidation [37]. BDNF production increases until the age of 40 years, decreasing thereafter [38]. Several factors, including physical activity, use of certain drugs or hormones, and inflammatory and neurodegenerative diseases, may influence BDNF production [28,39–41].

The gene that encodes BDNF is located on the short arm of chromosome 11 and presents a complex genomic structure with at least eight promoter regions for transcription [42]. These

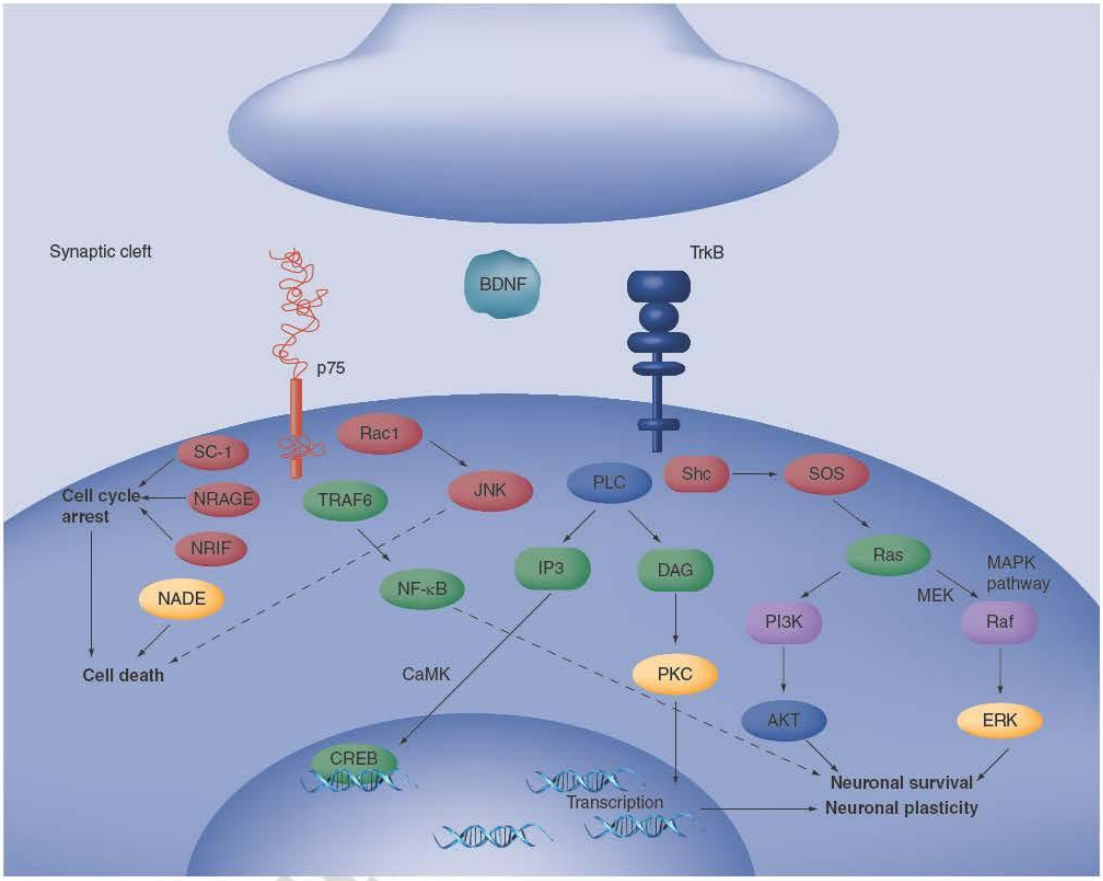

Figure 1. Brain-derived neurotrophic factor binds to TrkB and p75 receptors, activating signaling cascades with opposing effects. The interaction between BDNF and the TrkB receptor mediates differentiation and survival signaling through the activation of three main intracellular signaling pathways: the Ras-MAPK signaling cascade, which promotes neuronal differentiation and growth through MEK and ERK; the PI3K cascade, which promotes neuronal growth and survival through Akt; and PLC with the generation of IP3 and DAG, which activates, PKC and CaMK, respectively. This signaling cascade is followed by the activation of transcription factors, such as CREB, which are involved in synaptic plasticity. The p75 receptor activates NF-κB and JNK, and modulates RhoA activity. These responses are mediated through adaptor proteins including NADE and NRAGE.
BDNF: Brain-derived neurotrophic factor; CaMK: Ca^{2+}/calmodulin-dependent protein kinase; DAG: Diacylglycerol; JNK: c-Jun N-terminal kinase-p53-Bax; NADE: Neurotrophin-associated cell death executor; NRAGE: Neurotrophin-receptor-interacting MAGE homolog; NRIF: Neurotrophin receptor interacting factor; PLC: Phospholipase C; SC: Schwann cell factor; SOS: Son of sevenless; Trk: Tyrosine receptor kinase.

promoter regions are differently distributed across brain regions, cell types and even neuronal structures [42]. The biological significance of eight distinct BNDF mRNAs is uncertain and intriguing as the BDNF protein transcript is identical. It might determine specific patterns of *BDNF* gene expression in different areas of the CNS [28].

The most widely studied polymorphism of the *BDNF* gene is the substitution of guanine by adenosine at nucleotide 196, resulting in a change from valine to methionine at codon 66 (Val66Met). The frequency of this polymorphism in a Caucasian sample is approximately 19–25% [43]. This modification does not promote alteration in neuronal structure, but is related to a decrease in neuronal BDNF concentration, changes in intracellular traffic and secretion of mature BDNF and, as a consequence, a final reduction of BDNF levels by approximately 50% [44,45]. Reduction in volume of the hippocampus has been associated with the Val66Met polymorphism, even in healthy subjects [43,44].

The recognition of the major role of BDNF in brain development and plasticity has triggered several researchers to investigate the changes of BDNF in neuropsychiatric disorders.

Methodological issues in the measurement of BDNF

Most studies involving human neuropsychiatric disorders have investigated BDNF polymorphisms and/or BDNF circulating levels (i.e., serum, plasma or whole blood levels). A relevant issue regarding these latter studies is whether circulating levels of BDNF correlate with the CNS concentration of the molecule. As the concentration of BDNF in the cerebrospinal fluid (CSF) is very low, CSF seems to be a less useful source for investigating BDNF in neuropsychiatric disorders [46].

Under physiological conditions it is uncertain whether BDNF crosses the blood–brain barrier [47,48]. However, BDNF appears to cross the blood–brain barrier in pathological conditions [49–51]. For example, peripheral administration of BDNF is effective in promoting regeneration of the spinal cord after traumatic injury [52,53]. Regardless of this controversy, evidence from experimental studies suggest that peripheral levels of BDNF are correlated with BDNF levels in the CNS [54–56]. Studies assessing this correlation in humans are not available currently.

In blood, BDNF is stored in platelets and may be released upon stimulation [54,57]. Platelets do not produce BDNF, being responsible only for its storage. BDNF in plasma is detected in the pg/ml range, while BDNF in serum is measured in the ng/ml range. The difference is attributable to platelet degranulation and BDNF release during clotting. This fact raises the question of whether measuring serum, plasma or whole blood is comparable.

One study compared BDNF levels in human plasma versus serum. It concluded that platelets are, by far, the predominant source of BDNF in the serum, and that in the absence of platelet lysis, plasma levels of BDNF may be negligible [57]. Therefore, serum may be more appropriate than plasma to study the circulating levels of BDNF. There are convincing results that indicate that there is neither a significant difference between whole blood and serum BDNF levels, nor between thrombocyte count and whole blood levels of BDNF [58]. However, BDNF concentrations tend to decrease in serum stored for more than 1 year, a problem that does not appear to happen with whole blood. It is also of note that Val66Met polymorphisms do not seem to alter BDNF concentrations in blood [58].

A series of commercially available ELISA kits for BDNF measurement in human samples do not show significant cross-reactivity with other neurotrophins. They may provide accurate and reproducible BDNF measurements.

BDNF & mood disorders

Mood disorders are probably the most prevalent, recurrent and disabling mental illness worldwide. There are two main forms of mood disorders. Major depressive disorder is marked by recurrent episodes of depressed mood and/or anedonia along with cognitive, vegetative and behavioral changes. The lifetime incidence of major depression is approximately 12% in men and 20% in women [59]. Bipolar disorder (BD) is also a severe and disabling chronic disease. The hallmark of BD is mania or hypomania, although the majority of these patients also suffer from depressive episodes. The lifetime incidence of BD is around 1% and, even under treatment, many of these individuals remain vulnerable for relapses, psychiatric and clinical comorbidities and cognitive impairment [60].

The physiopathology of mood disorders includes a complex interaction of multiple susceptibility genes and environmental stressors that possibly affect CNS plasticity. As BDNF seems to be relevant in neuroplastic phenomena by which the brain perceives, adapts and responds to external and internal stimuli, it is a putative molecule in the pathogenesis of mood disorders [61].

A number of genetic loci and candidate genes have been implicated in the pathogenesis of mood disorders, including the *BDNF* polymorphism [62,63]. Recent meta-analyses concluded that the Val66Met polymorphism does not associate with either major depressive disorder [64,65] or with antidepressant response [66]. Although initial studies have demonstrated an association between BD with the *BDNF* polymorphism, a recent meta-analysis involving 3143 BD patients and 6347 healthy controls did not confirm this association [67,68]. Other studies have investigated the association between response to stress, the Val66Met polymorphism and two short alleles of serotonin-transporter-linked promoter region 5, a functional polymorphism in the promoter region of the serotonin transporter gene [69]. Depression severity in maltreated children was predicted from the interaction between this polymorphism in serotonin-transporter-linked

promoter region 5 and the *BDNF* Met allele [69]; however, these findings do not seem to be applicable for adolescents [70]. Interestingly, Gatt *et al.* observed that individuals carrying the *BDNF* Met allele who had been exposed to early life stress presented reduced gray matter in the hippocampus [71]. In this study, the size of the hippocampus correlated with reduced lateral prefrontal cortex volume and higher depression scores.

A significant decrease in the levels of BDNF has been observed in the hippocampus of several animal models of depression, including immobilization stress, forced swim stress, conditional foot shock, learned helplessness and social defeat stress (reviewed in [26]). The administration of antidepressant drugs increases hippocampal BDNF levels and the infusion of BDNF into rat hippocampus results in antidepressant-like effects [72]. In accordance with these animal studies, a decreased level of BDNF is seen in the hippocampus and prefrontal cortex of patients with major depression, but higher levels were observed in those who were taking antidepressants at the time of death [73,74].

A series of studies assessing circulating levels of BDNF in mood disorders has also been performed. Two recent meta-analyses confirmed that levels of BDNF in plasma or serum are decreased in major depression, supporting the potential role of this neurotrophin in disease pathogenesis [75,76]. Despite no significant differences in effect sizes, the reduction of BDNF levels seems to be larger in female patients [75].

A total of 15 studies comprising 489 depressed patients and 483 controls assessed BDNF in serum, while only five studies involving 161 depressed patients and 211 controls measured BDNF in plasma. A total of 13 out of 15 studies using serum samples and three out of five studies with plasma reported significant differences between patients and controls [75]. One study found discordant results when performing simultaneous BDNF assessment in serum and plasma. While BDNF content was reduced in serum from depressed patients, concentration in plasma was similar in patients and controls [75]. These authors also described a wider interindividual variability in plasma levels of BDNF in comparison with serum levels, proposing that standardized protocols for plasma collection and subsequent BDNF dosage must be developed [75]. These data, along with the methodological issues discussed previously, reinforce the view that serum is more appropriate for the investigation of circulating levels of BDNF. How serum BDNF levels may reflect the expression in the brain is still a matter of debate as BDNF can be synthesized by different cell types in the CNS and periphery, including active macrophages and lymphocytes.

Recently, we studied BDNF serum levels in geriatric depression [77]. As described for adult patients, elderly patients with depression presented lower serum levels of BDNF than age-matched controls [77]. Patients with late-onset depression had the lowest BDNF level when compared with patients with early-onset depression and healthy controls. This may reinforce the hypothesis that early- and late-onset geriatric depression may be, to some extent, biologically distinct. BDNF levels did not correlate with age, depression duration or cognitive performance assessed by the Mini Mental State Examination (MMSE) and the cognitive and self-contained part of the Cambridge Examination for Mental Disorders of the Elderly (CAMCOG). However, we found a significant negative correlation between BDNF levels and the severity of depressive symptoms, measured by the Hamilton Rating Scale for Depression [77].

Additional studies have also reported that serum levels of BDNF may be negatively correlated with the severity of depressive symptoms assessed by the Hamilton Rating Scale for Depression [78,79] or the Montgomery–Asberg Depression Rating Scale [80]. Furthermore, BDNF levels tend to increase following successful antidepressant treatment [78,81]. Taken together, serum levels of BDNF seem to be an interesting biomarker of depressive episodes.

Brain-derived neurotrophic factor also seems to play a central role in the pathogenesis of BD [82]. A recent meta-analysis, including ten studies, and comprising of 363 BD patients and 273 controls, found that BD patients had lower circulating levels of BDNF than healthy controls. However, when analyzing these studies based on the mood status, statistical significance only occurred when comparing BD patients in mania or depression with controls, not in euthymia [83]. These results suggest a role for blood BDNF level as a state-dependent biomarker of BD patients [83]. Furthermore, the severity of BD depression was negatively correlated with the serum levels of BDNF [84,85]. It is debatable whether there is any correlation between the severity of mania and BDNF levels [84,86,87].

In contrast with these previous results, we have recently found increased plasma levels of BDNF in BD patients [88]. Besides methodological issues, one possible explanation for this contradiction

could be that the BD sample in our study was comprised mainly of patients with longer disease duration (i.e., with more than 10 years of illness). By contrast, the majority of previous studies only enrolled recently diagnosed subjects. Therefore, BDNF levels may vary during the course of BD. This assumption is in-line with evidence from other neuropsychiatric disorders. For example, elevated serum levels of BDNF were described in long-term schizophrenic patients [89].

Data derived from animal [90,91] and human studies [92–94] demonstrated that treatment with lithium and valproate, traditional mood stabilizing agents, increased blood levels of BDNF. Conversely, antidepressants do not seem to change BDNF levels in BD, in contrast with the reported levels in major depression. This may reinforce differences in the physiopathology of major depression and BD [95].

Acute and chronic stress plays a relevant role in the development, progression and recurrence of mood disorders [96–99]. Data from animal studies indicated that neonatal stressors induce an acute decrease in BDNF levels in the hippocampus. Repeated neonatal stressors resulted in a long-term decrease in BDNF levels in the frontal cortex, which persisted during adulthood [98]. Another recent study demonstrated that chronic treatment with antidepressants increased BDNF mRNA expression in the dorsal hippocampus and prevented anhedonic behavior in rats submitted to chronic stress [100]. BDNF may underlie mechanisms by which environmental factors influence brain development and plasticity. In accordance with these experimental data, Grassi-Oliveira and colleagues observed that childhood maltreatment is associated with depression and lower serum levels of BDNF in adulthood [101]. Three or more stressful events were associated with decreased levels of whole blood BDNF in women [102]. Traumatic life events were also associated with higher psychiatric comorbidity, severity of depressive symptoms and lower serum levels of BDNF in BD [103].

Mood disorders are well-known risk factors for cognitive impairment. Reduced BDNF levels in patients with mood disorders are associated with hippocampal atrophy and activation of several deleterious cascades, determining heightened proinflammatory status, increased oxidative stress and hypothalamic–pituitary–adrenal axis dysfunction. The episodic and recurrent nature of mood disorders may give rise to a kindling process marked by BDNF decline in each additional episode and self-perpetuating activation of the aforementioned cascades [104–108]. These features are related, to some extent, to a myriad of neurodegenerative processes in these patients. Therefore, chronic reduced BDNF neurotrophic support may be a link between the lifetime history of mood disorders, and neurodegeneration and their related cognitive impairment.

BDNF, cognitive impairment & Alzheimer's disease

In recent years, great effort has focussed on understanding the relationship between the BDNF system and the core physiopathological features of Alzheimer's disease (AD), such as increased production of amyloid-β $(A\beta)_{42}$ peptide and Tau protein hyperphosphorylation [109,110].

The amyloid precursor protein (APP) is metabolized by two distinct pathways: the amyloidogenic pathway, comprised of the β-secretase and γ-secretase enzymes, and responsible for the production of the $A\beta_{42}$ peptide; and the nonamyloidogenic pathways, comprised of the α-secretase and γ-secretase enzymes [111]. BDNF regulates the processing of APP by stimulating the nonamyloidogenic processing pathway [112,113]. This may yield two beneficial effects:

- A reduction in β-amyloid peptide production;
- The release of the secreted form of APP (α-APP), which has neurotrophic and neuroprotective effects *per se* [114–116].

In primary neuronal cultures, pretreatment with BDNF protects against the cytotoxic effects of $A\beta_{42}$ [117]. The administration of BDNF is also able to restore changes in neurons pretreated with $A\beta_{42}$ [118]. Sublethal doses of $A\beta_{42}$ downregulate BDNF expression in cultured cortical neurons and impair BDNF intracellular trafficking [118,119]. Another important effect of $A\beta_{42}$ in cultured neurons is the impairment of BDNF-induced *Arc* expression [31–33]. This is an immediate early response gene that is rapidly activated by BDNF and whose product, Arc protein, is believed to be an important mediator of BDNF effects in synaptic plasticity and LTP formation [120].

In opposition to the negative effects of $A\beta_{42}$ on neuronal BDNF expression, BDNF is upregulated in astrocytes exposed to $A\beta_{42}$. The increased production of BDNF by astrocytes rescued neuritic degeneration in differentiated human neuroblastoma cells [121]. It seems that $A\beta_{42}$ has a distinct effect on the expression and production of BDNF depending on the CNS cellular lineage. The increased expression of BDNF in astrocytes may be regarded as a compensatory event to maintain adequate neurotrophic support to neurons in the initial stages of AD pathology.

Studies in animal models of AD largely support these findings from cellular models suggesting a pivotal role for BDNF in the physiopathology of AD. In transgenic mouse models of AD, a correlation between decreased neurotrophic support and cerebral amyloid burden has been described [122]. Exogenous $A\beta_{42}$ determines neurodegenerative changes, such as lower synaptic density, formation of dystrophic neurites, neuronal shrinkage and regional brain atrophy, especially in the hippocampus, which can be reversed by administration of BDNF [123]. As observed in cultured astrocytes, the upregulation of BDNF mRNA expression and protein synthesis is also found in microglia and astrocytes in the vicinity of amyloid plaques in a transgenic mouse model of AD [124]. These findings corroborate the hypothesis that an increase in BDNF levels may be a compensatory mechanism to the amyloid-induced toxicity in the early stages of AD.

In-line with these experimental data, a growing body of evidence implicates BDNF in the pathophysiological changes that ultimately lead to the clinical expression of AD in humans. Post-mortem studies have demonstrated a significant reduction in BDNF mRNA expression and protein levels in several brain regions of AD patients [125–127]. The presence of BDNF polymorphisms, in particular Val66Met, was associated with worse global cognition, memory and executive performance [128,129] and increased risk of AD [130,131]. The Val66Met polymorphism was associated with reduced volumes in hippocampus, parahippocampal gyrus, amygdala and dorsolateral prefrontal cortex [99,132–136]; however, negative findings have also been reported [137,138].

Decreased serum concentration of BDNF has been consistently described in AD patients [93,139–141]. Serum BDNF levels were significantly lower in AD patients than those in matched patients with vascular dementia and controls, but did not correlate with age or scores in MMSE or Functional Assessment Staging (FAST) [46]. Reduced BDNF levels have also been reported in patients with amnestic mild cognitive impairment (aMCI) [142], a condition recognized as a prodromal stage of AD [143]. Indeed, patients with aMCI have an increased risk of progressing to clinical AD upon follow-up and a great number of patients clinically classified as aMCI share very similar neurobiological features with AD patients [143–149].

A recent study published by our group analyzed serum BDNF levels in a large cohort of elderly subjects with no evidence of cognitive impairment and patients with aMCI who were followed-up for up to 4 years [150]. We observed decreased BDNF levels in patients with MCI and AD compared with controls. These results remained significant after controlling for confounding factors such as age, years of education and Apo E genotype [150]. The follow-up analyses showed that serum BDNF levels were not predictive of progression to AD or cognitive deterioration in MCI. However, the presence of the Met allele (Val66Met polymorphism) was a significant predictor of cognitive deterioration in these patients. In addition, we found a significant negative correlation between the Short Cognitive Test score and BDNF levels in the whole sample. There were no statistically significant correlations between BDNF levels and demographic, clinical and a series of neuropsychological variables, including the MMSE, the Clock Drawing Test, the Rivermead Behavioral Memory Test, the Fuld Object Memory Evaluation, and the Trail Making Test A and B [150]. In opposition to these findings, other investigators have also reported increased BDNF levels in patients with MCI and early AD [151]. These latter findings are in accordance with the hypothesis that this may be a compensatory mechanism and additional studies are required to clarify the dynamics of BDNF changes in the prodromal and early stages of AD.

In healthy elderly subjects, reduced serum BDNF levels were also associated with worse cognitive performance [152]. Even when there are no cognitive symptoms, lower CSF BDNF levels may be a predictor of future cognitive, especially memory, decline in the elderly [153]. Reduced CSF BDNF level has been appointed as an independent predictor of cognitive decline and progression of aMCI to AD and significantly correlates with hallmarks of AD pathology (i.e., with CSF levels phosphorylated Tau protein) [Forlenza OV, Diniz BS, Teixeira AL, Unpublished Data] [154]. These findings suggest that serum changes in BDNF levels are not only present in AD, but may also indicate a progressive shift toward a neurodegenerative state in the healthy elderly and, in the last instance, to the progression from aMCI to AD. Longitudinal studies must be performed to confirm the role of serum BDNF as a biomarker of progressive cognitive decline and AD. Furthermore, a better comprehension of the role of BDNF levels in the pathophysiology of AD may open new frameworks for the development of disease-modifying strategies for this disorder. Recent clinical trials have demonstrated that lithium and a cholinesterase inhibitor (donepezil) are able to increase serum BDNF levels in

patients with early AD [155]. Increments in BDNF levels may contribute to the potential disease-modifying effects of these drugs observed in AD and MCI patients [140,156].

Despite the importance of changes in the BDNF cascades for predicting the risk of developing cognitive impairment and neurodegenerative disorders, such as AD, other pathways must be taken into account. Increased proinflammatory activity [157,158], abnormal oxidative stress and mitochondrial dysfunction [159], decreased hippocampal neurogenesis [160,161], glycogen synthase kinase-3β hyperactivity [162,163] and stimulation of apoptotic cascades are also associated with the AD-related neurodegenerative changes induced by reduced neurotrophic support. In addition, the activity of these biological cascades influence or are influenced by BDNF and share similar intracellular regulatory pathways. Therefore, changes in one system may have a significant impact on the others. These pathological changes may have additive and/or multiplicative deleterious effects with a self-perpetuating nature. The net result of these pathological changes may be the stimulation of several neurodegenerative cascades that lead to the development of AD. It is plausible to hypothesize that, in complex neurodegenerative diseases, protective and deleterious cascades are in a delicate homeostatic balance that is tightly controlled and, thus, neurodegeneration arises from an imbalance between these cascades [164].

BDNF & motor impairment

Studies have documented evidence of altered expression of BDNF in different neurodegenerative diseases in which the motor system is mainly affected, such as Parkinson's disease (PD), Huntington's disease (HD), Sydenham's chorea and dystonia [165–171]. In fact, neurotrophic factors have played a role in the motor system since its early development. For example, BDNF has been demonstrated to be required for the establishment of an adequate number of dopaminergic neurons in the substantia nigra [172].

Parkinson's disease is a chronic neurodegenerative disease characterized by a progressive and irreversible loss of the dopaminergic neurons of the substantia nigra pars compacta. The etiology of PD is unknown, but the neurodegenerative process may result from an insufficient supply of neurotrophic factors [169].

Indeed, overexpression of α-synuclein decreases the expression of BDNF through disruption of several signal pathways, including nuclear factors of activated T cells, CREB and PKC [173]. In experimental models, inhibition of *BDNF* expression by the infusion of antisense oligonucleotide indicates the loss of dopaminergic neurons in the substantia nigra pars compacta [174]. In addition, dopamine output is decreased in the striatum of heterozygous *BDNF* mutant mice in comparison with wild-type controls, leading to impaired behavioral responses associated with disruption of the nigrostriatal pathway [175].

In a 6-OH-dopamine rat model, intrastriatal grafts of fibroblasts genetically engineered to produce BDNF partially prevented the loss of nerve terminals and completely prevented the loss of cell bodies of the nigrostriatal dopaminergic pathway [176]. Similarly, cell-mediated delivery of BDNF increases dopamine levels in the 1-methyl-4-phenylpyridinium-positive rat model of PD [177] and nigral infusion of BDNF can partially reverse the reduction in dopamine levels in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mice model of PD [178]. As neurotoxins did not decrease the levels of BDNF in the substantia nigra, these results suggest that an upregulation of BDNF synthesis by pharmacological means may be a viable therapy to slow down the progress of PD and other neurodegenerative diseases [179]. PD treatment itself may increase *BDNF* levels, as demonstrated by Okazawa *et al.* who found that levodopa increases the expression of BDNF mRNA in the striatum of mice [180]. The increased levels of BDNF could also be responsible for the potential neuroprotective effect of several drugs such as selegiline and (-)deprenyl, inhibitors of type B monoamine oxidase [181,182], pramipexol, a dopaminergic agonist [183], echinacoside, a polyphenol extracted from herbs [184], D-264, a novel D3 receptor-preferring agonist [185], and omega-3 polyunsaturated fatty acids [186].

In humans, post-mortem studies demonstrated lower concentrations of BDNF [186] and decreased expression of the *BDNF* gene in the substantia nigra of PD patients, especially in the ventro-lateral tier [167,168]. CSF levels of BDNF are also decreased in PD patients compared with controls, although BDNF levels in CSF do not differ between PD and AD patients [187]. Recently, we reported decreased serum levels of BDNF in PD patients when compared with age- and gender-matched controls [169]. Interestingly, BDNF correlated positively with a longer time span of the disease, as well as with the severity of PD symptoms, more advanced stages of the disease and worse performance in activities of

daily living as assessed by the Unified PD Rating Scale (UPDRS), the Modified Hoehn and Yahr Staging Scale and the Modified Schwab and England Activities of Daily Living Scale, respectively. In addition, higher BDNF levels also correlated with poor balance as assessed by the Berg Balance Scale, more time spent at the Timed Up & Go Test, reduced speed of gait and shorter distance walked during the Six-Minute Walk Test [169]. These preliminary results are encouraging and warrant further investigation on the role of BDNF as a useful biomarker related to PD severity.

It is worth considering the apparent paradox in the finding of increased BDNF levels among PD patients who had a longer history of disease and exhibited more severe symptoms, which, in turn, caused them to have a poorer motor performance in a series of clinical tests. We have suggested that lower BDNF levels in early stages of the disease may be associated with underlying pathogenic mechanisms in PD. Higher BDNF levels with PD progression may be a compensatory mechanism in the advanced stages of the disease or a long-term effect of some antiparkinsonian drugs [169]. A similar conclusion was provided by one study that found increased CSF levels of BDNF in PD patients in comparison with healthy controls [188]. These findings may have implications for research in therapeutic trials. For example, clinical trials with glial cell line-derived neurotrophic factor, which performs better than BDNF in protecting nigrostriatal dopaminergic neurons, proved to be disappointing when treating patients with PD [189]. We speculate that a critical period of time may exist in which the intervention with neurotrophic factors would be more effective.

The evidence for a role of BDNF in PD also comes from genetic studies that have demonstrated an association between *BDNF* gene polymorphisms and PD [190]. Furthermore, it has been demonstrated that two missense pathogenic mutations (A30P and A53T) of the α-synuclein gene that are associated with early-onset familial PD are linked to the loss of BDNF production in glial cells [191].

Brain-derived neurotrophic factor might also be involved in other neurodegenerative diseases affecting the motor system. The relevance of BDNF for neuronal survival is most evident in HD. HD is a genetic disease caused by a CAG triplet repeat expansion in the *HTT* gene, which encodes huntingtin. This mutation results in the production of a protein bearing a polyglutamine expansion in its N-terminus, which leads to widespread brain neurodegenerative changes that preferentially affect the medium spiny neurons of the striatum and the cerebral cortex [192]. It is already known that the mutated huntingtin protein decreases the transcriptional activity of the *BDNF* promoters. This reduces the transcription of the *BDNF* gene, decreasing protein production in the cerebral cortex, which is critical for the survival of striatal neurons, especially the striatal enkephalinergic neurons [193].

An early and progressive loss of BDNF has been demonstrated in cellular and mouse models of HD. Post-mortem studies have observed reduced levels of BDNF and BDNF mRNA in the cortex, caudate and putamen of HD patients [193,194]. Low serum levels of BDNF have been described in HD patients in comparison with matched healthy controls [195]. Lower BDNF serum levels were associated with a longer CAG repeat length and a longer duration of illness. There was also a negative correlation between the severity of illness assessed by the Unified Hungtington's Disease Rating Scale and serum BDNF levels. In fact, BDNF levels in serum are already decreased in presymptomatic subjects and associate with the early brain degenerative changes (i.e., cortical atrophy) [196]. These results confirm the effect of the *HTT* mutation in BDNF production and suggest a potential role of BDNF as a useful biomarker related to the patient's clinical phenotype [197].

Several studies have also assessed the possible neuroprotective role of BDNF in HD. In a culture of rat striatal cells transfected with mutant huntingtin, BDNF provided protection against neuronal death [198]. Furthermore, BDNF increased the expression of encephalin as well as the number of encephalin-expressing striatal neurons in mice overexpressing exon 1 of human mutant *HTT* [199]. The possibility of clinical improvement after the delivery of BDNF has been evidenced in other animal models [200], although a number of issues, such as dosage and the method of delivery, remain to be established [201].

Amyotrophic lateral sclerosis (ALS) is characterized by progressive muscle weakness and atrophy throughout the body as both upper and lower motor neurons degenerate. Studies have not observed a difference in serum or CSF levels of BDNF [202,203], or in BDNF mRNA expression in muscle biopsies of ALS patients [204]. By contrast, a selective increase in BDNF mRNA and protein in the muscle of ALS patients may occur, which is more pronounced in the early course of disease [205]. BDNF, along with other growth

factors, also exhibits robust neuroprotective effects on motor neurons in ALS models [206]. Based on these experimental data, recombinant human BDNF was administered by intrathecal infusion to ALS patients, but failed to demonstrate any significant clinical effect [207,208]. A trial of recombinant BDNF treatment for ALS was suspended in France owing to deterioration of the clinical course of subjects in the active treatment group [209]. In another trial, Beck *et al.* also observed that sympathetic function measures (i.e., sudomotor function and blood pressure response to handgrip) significantly declined during treatment with BDNF [208]. These data suggest that motor neurons may respond differently to neurotrophic factors.

Similarly, while protective for several neurodegenerative diseases, vigorous physical activity can also be a risk factor for motor neuron diseases [210]. Physical activity increases BDNF levels in the brain [211] and improves behavioral and cognitive deficits in animal models of AD, PD and HD [212–214]. In humans, physical activity has also demonstrated beneficial effects in cognition of people at high risk of cognitive decline [215]. In PD, moderate to vigorous activity is associated with a reduction in PD risk, although it is unknown whether such an association reflects a decreased baseline activity related to preclinical signs of the disease [216]. As the Met allele of the *BDNF* gene is associated with lower activity-dependent secretion of BDNF, physical activity would be less protective in people with this allele [217,218]. Age also seems to affect the secretion of BDNF associated with physical activity. Older mice experienced a larger increase in BDNF levels following exercise when compared with younger mice, suggesting that physical activity results in a proportionally lower increase in BDNF in the young [219].

Conclusion

In the adult brain, BDNF plays a pivotal role in synaptic plasticity and neuronal survival, and reductions in its levels have been implicated in the pathogenesis of several neurological and psychiatric disorders. This is largely supported by the reduced BDNF protein levels and mRNA expression in the CNS and circulation (i.e., serum or plasma) of these patients.

Serum BDNF levels are reduced in depression, bipolar disorder, AD and PD. In some studies, a correlation has been described between serum levels of BDNF and the severity of mood, cognitive and motor symptoms according to the disease. In addition, the increase in the circulating levels of BDNF after successful treatment of these conditions suggests that the partial or complete recovery of adequate neurotrophic support underlies clinical improvement.

Despite the strong evidence linking changes in BDNF and neuropsychiatric disorders, there are several points that remain unclear. First, it remains to be elucidated whether circulating BDNF levels reflect its expression in the CNS, as it can be synthesized by different cell types in the periphery, such as endothelial and mononuclear cells. It is important to clarify whether other signaling proteins of the BDNF cascade are also disrupted in neuropsychiatric disorders as well as the pattern of interactions of BDNF with other relevant pathophysiological mechanisms of these disorders, such as neuroinflammation, increased oxidative stress, deregulation of calcium metabolism and decreased neurogenesis. Furthermore, it seems that other trophobiological functions of BDNF have just begun to be understood. Besides its neurotrophic function, BDNF has immunotrophic and metabotrophic effects that are probably implicated in the pathogenesis of neuropsychiatric, inflammatory and cardiometabolic disorders [220]. For example, it has been demonstrated that BDNF can improve glucose and lipid metabolism [221], control energy homeostasis and feeding behavior [221,222], induce robust angiogenesis in CNS endothelia [223] and influence some antigen-specific immune responses [224]. Neurotrophins are likely to play a critical role in the bidirectional signaling mechanisms between the neural network and structural, immune and metabolic cells [220–227]. Thus, BDNF levels have been found altered in several diseases, including obesity [221,222], diabetes [225], allergic inflammatory skin disease [227] and asthma [228]. Finally, it is important to ascertain whether changes in the BDNF system are a primary or downstream event in the pathophysiology of these disorders.

Collectively, the evidence so far suggests that a low level of BDNF is an unspecific biomarker of several neuropsychiatric disorders characterized by neurodegenerative changes. Alternatively, BDNF may be regarded as a surrogate marker of brain-based pathological processes associated with the disruption of neurotrophic cascades.

Future perspective

Our knowledge of the different roles and dynamics of changes in the BDNF system in many neurological and psychiatric disorders is still in its infancy. A better understanding of the physiological mechanisms of BDNF action, along with its changes in specific neuropsychiatric disorders,

may aid the development of new and more specific therapeutic strategies. Long-term longitudinal studies in large clinical and epidemiological cohorts are required to ascertain the role of BDNF in different stages of some disorders, such as preclinical versus prodromal stages of AD, or as a trait or state marker of major mood disorders. These studies would help to clarify some of the neurobiological mechanisms underlying the link between mood and neurodegenerative disorders.

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Executive summary

- Brain-derived neurotrophic factor (BDNF) plays pivotal roles in synaptic plasticity and neuronal survival in the CNS.
- It is uncertain whether there is a clear correlation between brain and circulating (i.e., serum or plasma) levels of BDNF.
- Serum appears to be a more appropriate source for investigating circulating levels of BDNF than plasma.
- Serum levels of BDNF are decreased in major depression and may be negatively correlated with the severity of depressive symptoms, but tend to increase following successful antidepressant treatment.
- Serum levels of BDNF are decreased in mood episodes of bipolar disorder.
- Serum levels of BDNF are reduced in Alzheimer's disease and mild cognitive impairment, a condition recognized as a prodromal stage of Alzheimer's disease.
- Changes in BDNF levels may indicate a progressive shift toward a neurodegenerative state in the healthy elderly and to the progression from mild cognitive impairment to Alzheimer's disease.
- A decrease in serum levels of BDNF has been observed in Parkinson's disease and correlates with a series of motor parameters.

Bibliography

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1 Cowan WM: Viktor Hamburger and Rita Levi-Montalcini: the path to the discovery of nerve growth factor. *Ann. Rev. Neurosci.* 24, 551–600 (2001).

2 Hu Y, Russek SJ: BDNF and the diseased nervous system: a delicate balance between adaptive and pathological processes of gene regulation. *J. Neurochem.* 105(1), 1–17 (2008).

3 Chao MV: Neurotrophins and their receptors: a converge point for many signaling pathways. *Nat. Rev. Neurosci.* 4(4), 199–209 (2003).

4 Lee R, Kermani P, Teng KK, Hempstead BL: Regulation of the survival secreted proneurotrophins. *Science* 294(5548), 1945–1948 (2001).

5 Chen ZY, Jing D, Bath KG *et al.*: Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science* 314(5796), 140–143 (2006).

6 Woo NH, Teng HK, Siao CJ *et al.*: Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. *Nat. Nerosci.* 8(8), 1069–1077 (2005).

7 Murer MG, Yan Q, Raisman-Vorazi R: Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease. *Prog. Neurobiol.* 63(1), 71–124 (2001).

8 Ghosh A, Carnahan J, Greenberg ME: Requirement for BDNF in activity-dependent survival of cortical neurons. *Science* 263, 1618–1623 (1994).

9 Lindholm D, Carroll P, Tzimagiogis G, Thoenen H: Autocrineparacrine regulation of hippocampal neuron survival by IGF-1 and the neurotrophins BDNF, NT-3 and NT-4. *Eur. J. Neurosci.* 8, 1452–1460 (1996).

10 Alderson RF, Alterman AL, Barde YA, Lindsay RM: Brain-derived neurotrophic factor increases survival and differentiated functions of rat septal cholinergic neurons in culture. *Neuron* 5, 297–306 (1990).

▪ **Describes the role of brain-derived neurotrophic factor (BDNF) in neuronal survival and differentiation.**

11 Hyman C, Hofer M, Barde YA *et al.*: BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature* 350, 230–232 (1991).

12 Rumajogee P, Vergé D, Haunon N *et al.*: Adaption of the serotoninergic neuronal phenotype in the absent of 5-HT autoreceptors or the 5-HT transporter: involvement of BDNF and cAMP. *Eur. J. Neurosci.* 19, 937–944 (2004).

13 Kojima M, Takei N, Numakawa T *et al.*: Biological characterization and optical imaging of brain-derived neurotrophic factor-green fluorescent protein suggest an activity-dependent local release of brain-derived neurotrophic factor in neurites of cultured hippocampal neurons. *J. Neurosci. Res.* 61(1), 1–10 (2001).

14 Ernorfs P, Lee KF, Jaenisch R: Mice lacking brain-derived neurotrophic factor develop with sensory deficits. *Nature* 368(6467), 147–150 (1994).

15 Jones KR, Fariñas I, Backus C, Reichardt LF: Target disruption of the *BDNF* gene perturbs brain and sensory neuron development but not motor development. *Cell* 76(6), 989–999 (1994).

16 Linnarsson S, Björklund A, Ernfors P: Learning deficit in BDNF mutant mice. *Eur. J. Neurosci.* 9(12), 2581–2587 (1997).

17 Lee J, Duan W, Mattson MP: Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediated, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. *J. Neurochem.* 82, 1367–1375 (2002).

18 Lyons WE, Mamounas LA, Ricaurte GA *et al.*: Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotoninergic abnormalities. *Proc. Natl Acad. Sci. USA* 96, 15239–15244 (1999).

19 Ferrer I, Marvin C, Rey MJ *et al.*: BDNF and full-length and truncated TrkB expression in Alzheimer disease. Implications in therapeutic strategies. *J. Neuropathol. Exp. Neurol.* 58(7), 729–739 (1999).

20 Batchelor PE, Liberatore GT, Porritt MJ, Donnan GA, Howells DW: Inhibition of brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor expression reduces dopaminergic sprouting in the injured striatum. *Eur. J. Neurosci.* 12(10), 3462–3468 (2000).

21 Chigr F, Rachidi F, Segura S *et al.*: Neurogenesis inhibition in the dorsal vagal complex by chronic immobilization stress in the adult rat. *Neuroscience* 158(2), 524–536 (2009).

22 Taliaz D, Stall N, Dar DE, Zangen A: Knockdown of brain-derived neurotrophic factor in specific brain sites precipitates behaviors associated with depression and reduces neurogenesis. *Mol. Psychiatry* 15(1), 80–92 (2010).

23 Heldt SA, Stanek L, Chhatwal JP, Ressler KJ: Hippocampus-specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories. *Mol. Psychiatry* 12(7), 656–670 (2007).

24 Gourley SL, Kiraly DD, Howell JL, Olausson P, Taylor JR: Acute hippocampal brain-derived neurotrophic factor restores motivational and forced swim performance after corticosterone. *Biol. Psychiatry* 64(10), 884–890 (2008).

25 Marais L, Stein DJ, Daniels WM: Exercise increases BDNF levels in the striatum and decreases depressive-like behavior in chronically stressed rats. *Metab. Brain Dis.* 24(4), 587–597 (2009).

26 Duman RS, Monteggia LM: A neurotrophic model for stress-related mood disorders. *Biol. Psychiatry* 59 (12), 116–127 (2006).

27 Castrén E, Võikar V, Rantamäki T: Role of neurotrophic factors in depression. *Curr. Opin. Pharmacol.* 7(1), 18–21 (2007).

28 Tapia-Arancibia L, Rage F, Givalois L, Arancibia S: Physiology of BDNF: focus on hypothalamic function. *Front. Neuroendocrinol.* 25 (2), 77–107 (2004)

29 Cirulli F, Berry A, Bonsignore LT *et al.*: Early life influences on emotional reactivity: evidence that social enrichment has greater effects than handling on anxiety-like behaviors, neuroendocrine response to stress and central BDNF levels. *Neurosci. Biobehav. Rev.* 34(6), 808–820 (2010).

30 Minichello L: TrkB signaling pathways in LTP and learning. *Nat. Rev. Neurosci.* 10(12), 850–860 (2009).

31 Chen TJ, Wang DC, Chen SS: Amyloid-β interrupts the PI3K–Akt–mTOR signaling pathway that could be involved in brain-derived neurotrophic factor-induced Arc expression in rat cortical neurons. *J. Neurosci. Res.* 87(10), 2297–2307 (2009).

32 Wang DC, Chen SS, Lee YC, Chen TJ: Amyloid-β at sublethal level impairs BDNF-induced arc expression in cortical neurons. *Neurosci. Lett.* 398(1–2), 78–82 (2006).

33 Echeverria V, Berman DE, Arancio O: Oligomers of β-amyloid peptide inhibit BDNF-induced Arc expression in cultured cortical neurons. *Curr. Alzheimer Res.* 4(5), 518–521 (2007).

34 Bekinschtein P, Cammarota M, Katche C: BDNF is essential to promote persistence of long-term memory storage. *Proc. Natl Acad. Sci. USA* 105(7), 2711–2716 (2008).

35 Reichardt LF: Neurotrophin-regulated signaling pathways. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 361(1473), 1545–1564 (2006).

36 Lu B, Pang PT, Woo NH: The yin and yang of neurotrophin action. *Nat. Rev. Neurosci.* 6(8), 603–614 (2005).

37 Post RM: Role of BDNF in bipolar and unipolar disorder: clinical and theoretical implications. *J. Psychiatr. Res.* 41(12), 979–990 (2007).

** **Outstanding review of preclinical and clinical data on the role of BDNF in mood disorders.**

38 Katoh-Semba R, Wakako R, Komori T *et al.*: Age-related changes in BDNF protein levels in human serum: differences between autism cases and normal controls. *Int. J. Dev. Neurosci.* 25(6), 367–372 (2007).

39 Marini AM, Jiang X, Wu X *et al.*: Precondition and neurotrophins: a model for brain adaptation to seizures, ischemia and other stressful stimuli. *Amino Acids* 32, 299–304 (2007).

40 Kumamaru E, Numakawa T, Adachi N *et al.*: Glucocorticoid prevents brain-derived neurotrophic factor-mediated maturation of synaptic function in developing hippocampal neurons through reduction in activity of mitogen-actived protein kinase. *Mol. Endocrinol.* 22(3), 546–558 (2008).

41 Beglioumini S, Casarosa E, Pluchino N *et al.*: Influence endogenous and exogenous sex hormones on brain-derived neurotrophic factor. *Hum. Reprod.* 22(4), 995–1002 (2007).

42 Pattabiraman PP, Tropea D, Chiaruttini C, Tongiorgi E, Cattaneo A, Domenici L: Neuronal activity regulates the developmental expression and subcellular localization of cortical BDNF mRNA isoforms *in vivo*. *Mol. Cell Neurosci.* 28(3), 556–570 (2005).

43 Bueller JA, Aftab M, Sen S, Gomez-Hassan D, Burmeister M, Zubieta JK: *BDNF* Val66Met allele is associated with reduced hippocampal volume in healthy subjects. *Biol. Psychiatry* 59(9), 812–815 (2006).

44 Egan MF, Kojima M, Callicott JH *et al.*: The *BDNF* Val66Met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* 112(2), 257–269 (2003).

45 Martinowich K, Manji H, Lu B: New insights into BDNF functions in depression and anxiety. *Nat. Neurosci.* 10(9), 1089–1093 (2007).

46 Yasutake C, Kuroda K, Yanagawa T, Okamura T, Yoneda H: Serum BDNF, TNF-α and IL-1β levels in dementia patients: comparison between Alzheimer's disease and vascular dementia. *Eur. Arch. Psychiatry Clin. Neurosci.* 256(7), 402–406 (2006).

47 Pan W, Banks WA, Fasold MB, Bluth J, Kastin AJ: Transport of brain-derived neurotrophic factor across the blood–brain barrier. *Neuropharmacology* 37, 1553–1561 (1998).

48 Pardridge WM, Kang YS, Buciak JL: Transport of human recombinant brain-derived neurotrophic factor (BDNF) through the rat blood–brain barrier *in vivo* using vector-mediated peptide drugs delivery. *Pharm. Res.* 11(5), 738–746 (1994).

49 Di Lazzaro V, Profice P, Pilato F *et al.*: BDNF plasma levels in acute stroke. *Neurosci. Lett.* 422(2), 128–130 (2007).

50 Sharma HS, Johanson CE: Intracerebroventricularly administered neurotrophins attenuate blood cerebrospinal fluid barrier breakdown and brain pathology following whole-body hyperthermia: an experimental study in the rat using biochemical and morphological approaches. *Ann. NY Acad. Sci.* 1122, 112–129 (2007).

51 Zhou JP, Feng ZG, Yuan BL *et al.*: Transduced PTD-BDNF fusion protein protects against β-amyloid peptide-induced learning and memory deficits in mice. *Brain Res.* 1191, 12–19 (2008).

52 Song XY, Li F, Zhang FH, Zhong FH, Zhou XF: Peripherally-derived BDNF promotes regeneration of ascending sensory neurons after spinal cord injury. *PLoS ONE* 3(3), e1707 (2008).

53 Sharma HS: A select combination of neurotrophins enhances neuroprotection and functional recovery following spinal cord injury. *Ann. NY Sci.* 1122, 95–111 (2007).

54 Karege F, Schwald M, Cisse M: Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets. *Neurosci. Lett.* 328, 261–264 (2002).

55 Sartorius A, Hellweg R, Litzke J *et al.*: Correlations and discrepancies between serum and brain tissue levels of neurotrophins after electroconvulsive treatment in rats. *Pharmacopsychiatry* 42, 270–276 (2009).

56 Klein AB, Williamson R, Santini MA *et al.*: Blood BDNF concentrtations reflect brain-tissue BDNF levels across species. *Int. J. Neuripsychopharmacol.* 4, 1–7 (2010).

57 Radka SF, Holst PA, Fritsche M, Altar CA: Presence of brain-derived neurotrophic factor in brain and human and rat but not mouse serum detected by a sensitive and specific immunoassay. *Brain Res.* 709, 122–130 (1996).

58 Trajkovska V, Marcussen AB, Vinberg M, Hartvig P, Aznar S, Knudsen GM: Measurements of brain-derived neurotrophic factor: methodological aspects and demographical data. *Brain Res. Bull.* 73, 143–149 (2007).

▪ **Provides interesting data on methodological issues of *BDNF* measurements in blood.**

59 Belmaker RH, Agam G: Major depressive disorder. *N. Engl. J. Med.* 358(1), 55–68 (2008).

60 Belmaker RH: Bipolar disorder. *N. Engl. J. Med.* 351(5), 476–486 (2004).

61 Zarate CA Jr, Singh J, Manji HK: Cellular plasticity cascades: targets for the development of novels therapeutics for bipolar disorder. *Biol. Psychiatry* 59, 1006–1020 (2006).

62 Serretti A, Mandelli L: The genetics of bipolar disorder: genome 'hot regions', genes, new potential candidates and future directions. *Mol. Psychiatry* 13(8), 742–771 (2008).

63 Levinson DF: The genetics of depression: a review. *Biol. Psychiatry* 60(2), 84–92 (2006).

64 Chen L, Lawlor DA, Lewis SJ *et al.*: Genetic association study of BDNF in depression: finding from two cohort studies and a meta-analysis. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* 147B(6), 814–821 (2008).

65 Verhagen M, van der Meiji A, van Deurzen PA *et al.*: Meta-analysis of the *BDNF* Val66Met polymorphism in major depressive disorder: effects of gender and ethnicity. *Mol. Psychiatry* 15(3), 260–271 (2010).

66 Domschke K, Lawford B, Laje G *et al.*: Brain-derived neurotrophic factor (BDNF) gene: no major impact on antidepressant treatment response. *Int. J. Neuropsychopharmacol.* 13(1), 93–101 (2010).

67 Neves-Pereia M, Mundo E, Muglia P, King N, Macciardi F, Kennedy JL: The brain-derived neurotrophic factor gene confers susceptibility to bipolar disorder: evidence from a a family-based association study. *Am. J. Hum. Genet.* 71, 651–655 (2002).

68 Kanazawa T, Glatt SJ, Kia-Keating B, Yoneda H, Tsuang MT: Meta-analysis reveals no association of the Val66Met polymorphism of brain-derived neurotrophic factor with either schizophrenia or bipolar disorder. *Psychiatr. Genet.* 17(3), 165–170 (2007).

69 Kaufman J, Yang BZ, Dougla-Palumberi H: Brain-derived neurotrophic factor-5-*HTTLPR* gene interactions and environmental modifiers of depression in children. *Biol. Psychiatry* 59, 673–680 (2006).

70 Nederhof E, Bouma EM, Oldehinkel AJ, Ormel J: Interaction between childhood adversity, brain-derived neurotrophic factor val/met and serotonin transporter promoter polymorphism on depression: the TRAILS study. *Biol. Psychiatry* 68(2), 209–212 (2010).

71 Gatt JM, Nemeroff CB, Dobson-Stone C *et al.*: Interactions between BDNF Val66Met polymorphism and early life stress predict brain and arousal pathways to syndromal depression and anxiety. *Mol. Psychiatry* 14(7), 681–695 (2009).

72 Shirayama Y, Chen AC, Nakagawa S, Russel DS, Duman RS: Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *J. Neurosci.* 22(8), 3251–3261 (2002).

73 Chen B, Dowlatshahi D, MacQueen GM, Wang JF, Young LT: Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Biol. Psychiatry* 50(4), 260–265 (2001).

74 Karege F, Vaudan G, Schwald M, Perroud N, La Harpe R: Neurotrophin levels in postmortem brains of suicide victims and the effects of antemortem diagnosis and psychotropic drugs. *Brain Res. Mol. Brain Res.* 136, 29–37 (2005).

75 Bocchio-Chiavetto L, Bagnardi V, Zanardini R *et al.*: Serum and plasma BDNF levels in major depression: a replication study and meta-analyses.*World J. Biol. Psychiatry* 11(6), 763–773 (2010).

▪▪ **A meta-analysis of the literature that highlights differences on the evaluation of BDNF levels in serum and plasma.**

76 Sen S, Duman R, Sanacora G: Serum brain-derived neurotrophic factor depression and antidepressant medications: meta-analyses and implications. *Biol. Psychiatry* 64, 527–532 (2008).

77 Diniz BS, Teixeira AL, Talib LL, Mendonça VA, Gattaz WF, Forlenza OV: Serum brain-derived neurotrophic factor level is reduced in antidepressant-free patients with late-life depression. *World J. Biol. Psychiatry* 11(3), 550–555 (2010).

78 Gonul AS, Akdeniz F, Taneli F, Donat O, Eker C, Vahip S: Effect of treatment on serum brain-derived neurotrophic factor levels in depressed patients. *Eur. Arch. Psychiatry Clin. Neurosci.* 255, 381–386 (2005).

79 Shimizu E, Hashimoto K, Okamura N *et al.*: Alterations of serum brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. *Biol. Psychiatry* 54, 70–75 (2003).

80 Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry JM: Decreased serum brain-derived neurotrophic factor levels in major depressed patients. *Psychiatry Res.* 109, 143–148 (2002).

81 Aydemir O, Deveci A, Taneli F: The effect of chronic antidepressant treatment on serum brain-derived neurotrophic factor levels in depressed patients: a preliminar study. *Prog. Neuropychopharmacol. Biol. Psychiatry* 29, 261–265 (2005).

82 Kapczinski F, Frey BN, Kauer-Sant'Anna M, Grassi-Oliveira R: Brain-derived neurotrophic factor and neuroplasticity in bipolar disorder. *Expert Rev. Neurother.* 8(7), 1101–1113 (2008).

▪ **Focuses on the involvement of BDNF in the physiopathology of bipolar disorders.**

83 Lin PY: State-dependent decrease in levels of brain-derived neurotrophic factor in bipolar disorder: a meta-analytic study. *Neurosci. Lett.* 466(3), 139–143 (2009).

84 Cunha AB, Frey BN, Andreazza AC *et al.*: Serum brain-derived neurotrophic factor is decreased in bipolar disorder during depressive and manic episodes. *Neurosci. Lett.* 398(3), 215–219 (2006).

85 Fernandes BS, Gama CS, Kauer-Sant'Anna M, Lobato MI, Belmonte-de-Abreu P, Kapczinski F: Serum brain-derived neurotrophic factor in bipolar and unipolar depression: a potential adjunctive tool for differential diagnostic. *J. Psychiatr. Res.* 43(15), 1200–1204 (2009).

86 Machado-Vieira R, Dietrich MO, Leke R *et al.*: Decreased plasma brain derived neurotrophic factor levels in unmedicated bipolar patients during manic episode. *Biol. Psychiatry* 61 (2), 142–144 (2007).

87 Tramontina JF, Andreazza AC, Kauer-Sant'Anna M *et al.*: Brain-derived neurotrophic factor serum levels before and after treatment for acute mania. *Neurosci. Lett.* 452, 111–113 (2009).

88 Barbosa IG, Huguet RB, Mendonça VA *et al.*: Increased plasma levels of brain-derived neurotrophic factor in patients with long-term bipolar disorder. *Neurosci. Lett.* 457(2), 95–98 (2010).

89 Reis HJ, Nicolato R, Barbosa IG, Teixeira do Prado PH, Romano-Siva MA, Teixeira AL: Increased serum levels of brain-derived neurotrophic factor in chronic institutionalized patients with schizophrenia. *Neurosci. Lett.* 439(2), 157–159 (2008).

90 Frey BN, Andreazza AC, Ceresér KM *et al.*: Effects of mood stabilizers on hippocampus BDNF levels in an animal model of mania. *Life Sci.* 79(3), 281–286 (2006).

91 Jornada LK, Moretti M, Valvassori SS *et al.*: Effects of mood stabilizers on hippocampus and amygdala BDNF levels in an animal model of mania induced by ouabain. *J. Psychiatr. Res.* 44(8), 506–510 (2010).

92 Yoshimura R, Nakano Y, Hori H, Ikenouchi A, Ueda N, Nakamura J: Effect of risperidone on plasma catecholamine metabolites and brain-derived neurotrophic factor in patients with bipolar disorders. *Hum. Psychopharmacol. Clin. Exp.* 21, 433–438 (2006).

93 Einat H, Yuan P, Gould TD *et al.*: The role of the extracellular signal-regulated kinase signaling pathway in mood modulation. *J. Neurosci.* 33, 7311–7316 (2003).

94 Pillai A, Terry AV Jr, Mahadik SP: Differential effects of long-term treatment with typical and atypical antipsychotics on NGF and BDNF levels in rat striatum and hippocampus. *Schizophr. Res.* 82, 95–106 (2006).

95 Chen B, Dowlatshahi D, MacQueen GM, Wang JF, Young LT: Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Biol. Psychiatry* 50(4), 260–265 (2001).

96 Roceri M, Hendriks W, Racagni G, Ellenbroek BA, Riva MA: Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in rat hippocampus. *Mol. Psychiatry* 7(6), 609–616 (2002).

97 Roceri M, Cirulli F, Pessina C, Peretto P, Racagni G, Riva MA: Postnatal repeated maternal deprivation produces age-dependent changes of brain-derived neurotrophic factor expression in selected rat brain regions. *Biol. Psychiatry* 55(7), 708–714 (2004).

98 Post RM: Kindling and sensitization as model for affective episode recurrence, cyclicity, and tolerance phenomena. *Neurosci. Biobehav. Rev.* 31(6), 858–873 (2007).

99 Heim C, Nemeroff CB: The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol. Psychiatry* 49, 1023–1039 (2001).

100 Larsen MH, Mikkelsen JD, Hay-Schmidt A, Sandi C: Regulation of brain-derived neurotrophic factor (BDNF) in the chronic unpredictable stress rat model and the effects of chronic antidepressant treatment. *J. Psychiatr. Res.* 44(13), 808–816 (2010).

101 Grassi-Oliveira R, Stein LM, Lopes RP, Teixeira AL, Bauer ME: Low plasma brain-derived neurotrophic factor and childhood physical neglect are associated with verbal memory impairment in major depression – a preliminary report. *Biol. Psychiatry* 64(4), 281–285 (2008).

102 Trajkovska V, Vinberg M, Aznar S, Knudsen GM, Kessing LV: Whole blood BDNF levels in healthy twins discordant for affective disorder: association to life events and neuroticism. *J. Affect. Disord.* 108(1–2), 165–169 (2008).

103 Kauer-Sant'Anna M, Tramontina J, Andreazza AC *et al.*: Traumatic life events in bipolar disorder: impact on BDNF levels and psychopathology. *Bipolar Disord.* 9(Suppl. 1), 128–135 (2007).

104 Post RM: Transduction of psychosocial stress into the neurobiology of recurrent affective disorder. *Am. J. Psychiatry* 149(8), 999–1010 (1992).

105 Maes M, Yirmyia R, Noraberg J *et al.*: The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. *Metab. Brain Dis.* 24(1), 27–53 (2009).

106 Kapczinski F, Vieta E, Andreazza AC *et al.*: Allostatic load in bipolar disorder: implications for pathophysiology and treatment. *Neurosci. Biobehav. Rev.* 32(4), 675–692 (2008).

107 Wolkowitz OM, Epel ES, Reus VI, Mellon Synthia H: Depression gets old fast: do stress and depression accelerate cell aging? *Depress. Anxiety* 27(4), 327–338 (2010).

108 Kapczinski F, Dal-Pizzol F, Teixeira AL *et al.*: A systemic toxicity index developed to assess peripheral changes in mood episodes. *Mol. Psychiatry* 15(8), 784–786 (2010).

109 Hardy J: The genetic causes of neurodegenerative diseases. *J. Alzheimers Dis.* 3(1), 109–116 (2001).

110 Tapia-Arancibia L, Aliaga E, Silhol M, Arancibia S: New insights into brain BDNF function in normal aging and Alzheimer disease. *Brain Res. Rev.* 59(1), 201–220 (2008).

111 Mattson MP: Pathways towards and away from Alzheimer's disease. *Nature* 430(7000), 631–639 (2004).

112 Fu W, Lu C, Mattson MP: Telomerase mediates the cell survival-promoting actions of brain-derived neurotrophic factor and secreted amyloid precursor protein in developing hippocampal neurons. *J. Neurosci.* 22(24), 10710–10719 (2002).

113 Rohe M, Synowitz M, Glass R, Paul SM, Nykjaer A, Willnow TE: Brain-derived neurotrophic factor reduces amyloidogenic processing through control of *SORLA* gene expression. *J. Neurosci.* 29(49), 15472–15478 (2009).

114 Scheuner, D, Eckman C, Jensen M *et al.*: Secreted amyloid β-protein similar to that in senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 *APP* mutations linked to familial Alzheimer's disease. *Nat. Med.* 2, 864–870 (1996).

115 Nishitomi K, Sakaguchi G, Horikoshi Y *et al.*: BACE1 inhibition reduces endogenous Aβ and alters APP processing in wild-type mice. *J. Neurochem.* 99(6), 1555–1563 (2006).

116 Thornton E, Vink R, Blumbergs PC, Van Den Heuvel C: Soluble amyloid precursor protein-α reduces neuronal injury and improves functional outcome following diffuse traumatic brain injury in rats. *Brain Res.* 1094(1), 38–46 (2006).

117 Arancibia S, Silhol M, Moulivre F *et al.*: Protective effect of BDNF against β-amyloid induced neurotoxicity *in vitro* and *in vivo* in rats. *Neurobiol. Dis.* 31(3), 316–326 (2008).

118 Aliaga E, Silhol M, Bonneau N, Maurice T, Arancibia S, Tapia-Arancibia L: Dual response of BDNF to sublethal concentrations of β-amyloid peptides in cultured cortical neurons. *Neurobiol. Dis.* 37(1), 208–217 (2010).

119 Poon WW, Blurton-Jones M, Tu CH *et al.*: β-amyloid impairs axonal BDNF retrograde trafficking. *Neurobiol. Aging* (2009) (Epub ahead of print).

120 Bramham CR, Worley PF, Moore MJ, Guzowski JF: The immediate early gene arc/arg3.1: regulation, mechanisms, and function. *J. Neurosci.* 28(46), 11760–11767 (2008).

121 Kimura N, Takahashi M, Tashiro T, Terao K: Amyloid-β up-regulates brain-derived neurotrophic factor production from astrocytes: rescue from amyloid-β-related neuritic degeneration. *J. Neurosci. Res.* 84(4), 782–789 (2006).

122 Peng S, Garzon DJ, Marchese M *et al.*: Decreased brain-derived neurotrophic factor depends on amyloid aggregation state in transgenic mouse models of Alzheimer's disease. *J. Neurosci.* 29(29), 9321–9329 (2009).

123 Nagahara AH, Merrill DA, Coppola G *et al.*: Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease. *Nat. Med.* 15(3), 331–337 (2009).

124 Burbach GJ, Hellweg R, Haas CA *et al.*: Induction of brain-derived neurotrophic factor in plaque-associated glial cells of aged APP23 transgenic mice. *J. Neurosci.* 24(10), 2421–2430 (2004).

125 Li Y, Rowland C, Tacey K *et al.*: The *BDNF* Val66Met polymorphism is not associated with late onset Alzheimer's disease in three case-control samples. *Mol. Psychiatry.* 10(9), 809–810 (2005).

126 Connor B, Young D, Yan Q, Faull RL, Synek B, Dragunow M: Brain-derived neurotrophic factor is reduced in Alzheimer's disease. *Brain Res. Mol. Brain Res.* 49(1–2), 71–81 (1997).

127 Peng S, Wuu J, Mufson EJ, Fahnestock M: Precursor form of brain-derived neurotrophic factor and mature brain-derived neurotrophic factor are decreased in the preclinical stages of Alzheimer's disease. *J. Neurochem.* 93(6), 1412–1421 (2005).

128 Miyajima F, Ollier W, Mayes A *et al.*: Brain-derived neurotrophic factor polymorphism Val66Met influences cognitive abilities in the elderly. *Genes Brain Behav.* 7(4), 411–417 (2008).

129 Schofield PR, Williams LM, Paul RH *et al.*: Disturbances in selective information processing associated with the *BDNF* Val66Met polymorphism: evidence from cognition, the P300 and fronto–hippocampal systems. *Biol. Psychol.* 80(2), 176–188 (2009).

130 Tsai SJ, Hong CJ, Liu HC, Liu TY, Hsu LE, Lin CH: Association analysis of brain-derived neurotrophic factor Val66Met polymorphisms with Alzheimer's disease and age of onset. *Neuropsychobiology* 49(1), 10–12 (2004).

131 Tsai SJ, Hong CJ, Liu HC, Liu TY, Liou YJ: The brain-derived neurotrophic factor gene as a possible susceptibility candidate for Alzheimer's disease in a Chinese population. *Dement. Geriatr. Cogn. Disord.* 21(3), 139–143 (2006).

132 Nemoto K, Ohnishi T, Mori T *et al.*: The Val66Met polymorphism of the brain-derived neurotrophic factor gene affects age-related brain morphology. *Neurosci. Lett.* 397(1–2), 25–29 (2006).

133 Sambataro K, Ohnishi T, Mori T *et al.*: BDNF modulates normal human hippocampal ageing. *Mol. Psychiatry* 15(2), 116–118 (2010).

134 Sublette ME, Baca-Garcia E, Parsey RV *et al.*: Effect of BDNF Val66Met polymorphism on age-related amygdala volume changes in healthy subjects. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 32(7), 1652–1655 (2008).

135 Toro R, Chupin M, Garnero L *et al.*: Brain volumes and Val66Met polymorphism of the *BDNF* gene: local or global effects? *Brain Struct. Funct.* 213(6), 501–509 (2009).

136 Chepenik LG, Fredericks C, Papademetris X *et al.*: Effects of the brain-derived neurotrophic growth factor Val66Met variation on hippocampus morphology in bipolar disorder. *Neuropsychopharmacology* 34(4), 944–951 (2009).

137 Benjamin S, McQuoid DR, Potter GG *et al.*: The brain-derived neurotrophic factor Val66Met polymorphism, hippocampal volume, and cognitive function in geriatric depression. *Am. J. Geriatr. Psychiatry* 18(4), 323–331 (2010).

138 Jessen F, Schuhmacher A, von Widdern O *et al.*: No association of the Val66Met polymorphism of the brain-derived neurotrophic factor with hippocampal volume in major depression. *Psychiatr. Genet.* 19(2), 99–101 (2009).

139 Laske C, Stransky E, Leyhe T *et al.*: BDNF serum and CSF concentrations in Alzheimer's disease, normal pressure hydrocephalus and healthy controls. *J. Psychiatr. Res.* 41(5), 387–394 (2007).

140 Forlenza OV, Diniz BS, Teixeira AL *et al.*: Effect of brain-derived neurotrophic factor Val66Met polymorphism and serum levels on the progression of mild cognitive impairment. *World J. Biol. Psychiatry* 11(6), 774–780 (2010).

141 Lee JG, Shin BS, You YS *et al.*: Decreased serum brain-derived neurotrophic factor levels in elderly Korean with dementia. *Psychiatry Invest.* 6(4), 299–305 (2009).

142 Winblad B, Palmer K, Kivipelto M *et al.*: Mild cognitive impairment – beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. *J. Intern. Med.* 256(3), 240–246 (2004).

143 Dubois B, Feldman HH, Jacova C *et al.*: Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. *Lancet Neurol.* 6(8), 734–746 (2007).

144 Petersen RC: Mild cognitive impairment as a diagnostic entity. *J. Intern. Med.* 256(3), 183–194 (2004).

145 Petersen RC, Parisi JE, Dickson DW *et al.*: Neuropathologic features of amnestic mild cognitive impairment. *Arch. Neurol.* 63(5), 665–672 (2006).

146 Diniz BS, Pinto Jr JA, Forlenza OV: Do CSF total tau, phosphorylated tau, and β-amyloid$_{42}$ help to predict progression of mild cognitive impairment to Alzheimer's disease? A systematic review and meta-analysis of the literature. *World J. Biol. Psychiatry* 9(3), 172–182 (2008).

147 Busatto GF, Diniz BS, Zanetti MV: Voxel-based morphometry in Alzheimer's disease. *Expert Rev. Neurother.* 8(11), 1691–1702 (2008).

148 Price LP, Morris JC: Tangles and plaques in nondemented aging and "preclinical". Alzheimer's disease. *Ann. Neurol.* 45(3), 358–368 (1999).

149 Jack CR Jr, Knopman DS, Jagust WJ *et al.*: Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol.* 9(1), 119–128 (2010).

150 Laske C, Stransky E, Leyhe T *et al.*: Stage-dependent BDNF serum concentrations in Alzheimer's disease. *J. Neural. Transm.* 113(9), 1217–1224 (2006).

151 Angelucci F, Spalletta G, di Iulio F *et al.*: Alzheimer's disease (AD) and Mild Cognitive Impairment (MCI) patients are characterized by increased BDNF serum levels. *Curr. Alzheimer Res.* 7(1), 15–20 (2010).

152 Gunstad J, Benitez A, Smith J *et al.*: Serum brain-derived neurotrophic factor is associated with cognitive function in healthy older adults. *J. Geriatr. Psychiatry Neurol.* 21(3), 166–170 (2008).

153 Li G, Peskind ER, Millard SP *et al.*: Cerebrospinal fluid concentration of brain-derived neurotrophic factor and cognitive function in non-demented subjects. *PLoS ONE* 4(5), e5424 (2009).

154 Diniz BS, Teixeira AL, Talib L, Gattaz WF, Forlenza OV: Interleukin-1β serum level is increased in antidepressant-free elderly depressed patients. *Am. J. Geriatr. Psychiatry* 18(2), 172–176 (2010).

155 Leyhe T, Eschweiler GW, Stransky E *et al.*: Increase of BDNF serum concentration in lithium treated patients with early Alzheimer's disease. *J. Alzheimers Dis.* 16(3), 649–656 (2009).

156 Diniz BS, Pinto JA Jr, Gonzaga ML, Guimarães FM, Gattaz WF, Forlenza OV: To treat or not to treat? A meta-analysis of the use of cholinesterase inhibitors in mild cognitive impairment for delaying progression to Alzheimer's disease. *Eur. Arch. Psychiatry Clin. Neurosci.* 259(4), 248–256 (2009).

157 Forlenza OV, Diniz BS, Talib LL *et al.*: Increased serum IL-1β level in Alzheimer's disease and mild cognitive impairment. *Dement. Geriatr. Cogn. Disord.* 28(6), 507–512 (2009).

158 Mrak RE: Neuropathology and the neuroinflammation idea. *J. Alzheimers Dis.* 18(3), 473–481 (2009).

159 Swerdlow RH, Khan SM: The Alzheimer's disease mitochondrial cascade hypothesis: an update. *Exp. Neurol.* 218(2), 308–315 (2009).

160 Shruster A, Melamed E, Offen D: Neurogenesis in the aged and neurodegenerative brain. *Apoptosis* (2010) (Epub ahead of print).

161 Chen Q, Nakajima A, Choi SH *et al.*: Adult neurogenesis is functionally associated with AD-like neurodegeneration. *Neurobiol. Dis.* 29(2), 316–326 (2008).

162 Hooper C, Killick R, Lovestone S: The GSK3 hypothesis of Alzheimer's disease. *J. Neurochem.* 104(6), 1433–1439 (2008).

163 Forlenza OV, Torres CA, Talib LL *et al.*: Increased platelet GSK3β activity in patients with mild cognitive impairment and Alzheimer's disease. *J. Psychiatr. Res.* (2010) (Epub ahead of print).

164 Swerdlow RH: Is aging part of Alzheimer's disease, or is Alzheimer's disease part of aging? *Neurobiol. Aging* 28(10), 1465–1480 (2007).

165 Ciammola A, Sassone J, Cannella M *et al.*: Low brain derived neurotrophic factor (BDNF) levels in serum of Huntington's disease patients. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 144B, 574–577 (2007).

166 Mogi M, Togaria A, Kondo T *et al.*: Brain-derived growth factor and nerve growth factor concentrations are decreased in the substantia nigra in Parkinson's disease. *Neurosci. Lett.* 270, 45–48 (1999).

167 Howells DW, Porritt MJ, Wong JYF *et al.*: Reduced BDNF mRNA expression in the Parkinson's disease substantia nigra. *Exp. Neurol.* 166, 127–135 (2000).

168 Parain K, Murer MG, Quiao Y *et al.*: Reduced expression of brain-derived neurotrophic factor protein in Parkinson's disease substantia nigra. *Neuroreport* 10, 557–561 (1999).

169 Scalzo P, Kümmer A, Bretas TL, Cardoso F, Teixeira AL: Serum levels of brain-derived neurotrophic factor correlate with motor impairment in Parkinson's disease. *J. Neurol.* 257(4), 540–545 (2010).

▪ **Provides the first evidence of correlation between serum levels of BDNF and motor parameters in Parkinson's disease.**

170 Cramer SC, Sampat A, Haske-Palomino M, Nguyen S, Procaccio V, Hermanowicz N: Increased prevalence of Val(66)Met BDNF genotype among subjects with cervical dystonia. *Neurosci. Lett.* 468(1), 42–45 (2010).

171 Teixeira AL, Bretas TL, Kummer A *et al.*: Serum levels of brain-derived neurotrophic factor in Sydenham's chorea. *Neurol. Sci.* 31(3), 399–401 (2010).

172 Baquet ZC, Bickford PC, Jones KR: Brain-derived neurotrophic factor is required for the establishment of the proper number of dopaminergic neurons in the substantia nigra pars compacta. *J. Neurosci.* 25, 6251–6259 (2005).

173 Yuan Y, Sun J, Zhao M, Hu J, Wang X, Du G, Chen NH: Overexpression of α-synuclein down-regulates BDNF expression. *Cell Mol. Neurobiol.* 30(6), 939–946 (2010).

174 Porritt MJ, Batchelor PE, Howells DW: Inhibiting BDNF expression by antisense oligonucleotide infusion causes loss of nigral dopaminergic neurons. *Exp. Neurol.* 192, 226–234 (2005).

175 Dluzen DE, Anderson LI, McDermott JL, Kucera J, Walro JM: Striatal dopamine output is compromised within +/- BDNF mice. *Synapse* 43, 112–117 (2002).

176 Levivier M, Przedborski S, Bencsics C, Kang UJ: Intrastriatal implantation of fibroblasts genetically engineered to produce brain-derived neurotrophic factor prevents degeneration of dopaminergic neurons in a rat model of Parkinson's disease. *J. Neurosci.* 15, 7810–7820 (1995).

177 Isacson O, Deacon TW, Pakzaban P, Galpern WR, Dinsmore J, Burns LH: Transplanted xenogeneic neural cells in neurodegenerative disease models exhibit remarkable axonal target specificity and distinct growth patterns of glial and axonal fibres. *Nat. Med.* 1, 1189–1194 (1995).

178 Hung HC, Lee EH: The mesolimbic dopaminergic pathway is more resistant than the nigrostriatal dopaminergic pathway to MPTP and MPP^+ toxicity: role of *BDNF* gene expression. *Brain Res. Mol. Brain Res.* 41, 14–26 (1996).

179 Mocchetti I, Bachis A, Nosheny RL, Tanda G: Brain derived neurotrophic factor expression in the substantia nigra does not change after lesions of dopaminergic neurons. *Neurotox. Res.* 12, 135–143 (2007).

180 Okazawa H, Murata M, Watanabe M, Kamei M, Kanazawa I: Dopaminergic stimulation up-regulates the *in vivo* expression of brain-derived neurotrophic factor in the striatum. *FEBS Lett.* 313, 138–142 (1992).

181 Gyárfás T, Knuuttila J, Lindholm P, Rantamäki T, Castrén E: Regulation of brain-derived neurotrophic factor (BDNF) and cerebral dopamine neurotrophic factor (CDNF) by antiparkinsonian drug therapy *in vivo*. *Cell. Mol. Neurobiol.* 30(3), 361–368 (2010).

182 Naoi M, Maruyama W: Functional mechanism of neuroprotection by inhibitors of type B monoamine oxidase in Parkinson's disease. *Expert Rev. Neurother.* 9(8), 1233–1250 (2009).

183 Li C, Guo Y, Xie W, Li X, Janokovic J, Le W: Neuroprotection of pramipexole in UPS impairment induced animal model of Parkinson's disease. *Neurochem. Res.* 35(10), 1546–1556 (2010).

184 Zhao Q, Gao J, Li W, Cai D: Neurotrophic and neurorescue effects of echinacoside in the subacute MPTP mouse model of Parkinson's disease. *Brain Res.* 1346, 224–236 (2010).

185 Li C, Biswas S, Li X, Dutta AK, Le W: Novel D3 dopamine receptor-preferring agonist D-264: evidence of neuroprotective property in Parkinson's disease animal models induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and lactacystin. *J. Neurosci. Res.* 88(11), 2513–2523 (2010).

186 Bousquet M, Gibrat C, Saint-Pierre M, Julien C, Calon F, Cicchetti F: Modulation of brain-derived neurotrophic factor as a potential neuroprotective mechanism of action of omega-3 fatty acids in a parkinsonian animal model. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 33(8), 1401–1408 (2009).

187 Zhang J, Sokal I, Peskind ER *et al.*: CSF multianalyte profile distinguishes Alzheimer and Parkinson diseases. *Am. J. Clin. Pathol.* 129(4), 526–529 (2008).

188 Salehi Z, Mashayekhi F: Brain-derived neurotrophic factor in the cerebrospinal fluid of patients with Parkinson's disease. *J. Clin. Neurosci.* 16, 90–93 (2009).

189 Yasuhara T, Shingo T, Date I: Glial cell line-derived neurotrophic factor (GDNF) therapy for Parkinson's disease. *Acta Med. Okayama*. 61, 51–56 (2007).

190 Zintzaras E, Hadjigeorgiou GM: The role of G196A polymorphism in the brain-derived neurotrophic factor gene in the cause of Parkinson's disease: a meta-analysis. *J. Hum. Genet.* 50, 560–566 (2005).

191 Kohno R, Sawada H, Kawamoto Y, Uemura K, Shibasaki H, Shimohama S: BDNF is induced by wild-type α-synuclein but not by the two mutants, A30P or A53T, in glioma cell line. *Biochem. Biophys. Res. Commun.* 318, 113–118 (2004).

192 Reiner A, Albin RL, Anderson KD, D'Amato CJ, Penney JB, Young AB: Differential loss of striatal projection neurons in Huntington disease. *Proc. Natl Acad. Sci. USA* 85, 5733–5737 (1988).

193 Zuccato C, Ciammola A, Rigamonti D *et al.*: Loss of huntingtin-mediated *BDNF* gene transcription in Huntington's disease. *Science* 293, 493–498 (2001).

194 Zuccato C, Marullo M, Conforti P, MacDonald ME, Tartari M, Cattaneo E: Systematic assessment of BDNF and its receptor levels in human cortices affected by Huntington's disease. *Brain Pathol.* 18, 225–238 (2008).

195 Ferrer I, Goutan E, Marín C, Rey MJ, Ribalta T: Brain-derived neurotrophic factor in Huntington disease. *Brain Res.* 866, 257–261 (2000).

196 Squitieri F, Cannella M, Simonelli M *et al.*: Distinct brain volume changes correlating with clinical stage, disease progression rate, mutation size, and age at onset prediction as early biomarkers of brain atrophy in Huntington's disease. *CNS Neurosci. Ther.* 15(1), 1–11 (2009).

197 Ciammola A, Sassone J, Cannella M *et al.*: Low brain-derived neurotrophic factor (BDNF) levels in serum of Huntington's disease patients. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* 144B(4), 574–577 (2007).

198 Zala D, Benchoua A, Brouillet E *et al.*: Progressive and selective striatal degeneration in primary neuronal cultures using lentiviral vector coding for a mutant huntingtin fragment. *Neurobiol. Dis.* 20, 785–798 (2005).

199 Canals JM, Pineda JR, Torres-Peraza JF *et al.*: Brain-derived neurotrophic factor regulates the onset and severity of motor dysfunction associated with encephalinergic neuronal degeneration in Huntington's disease. *J. Neurosci.* 24, 7727–7739 (2004).

200 Zuccato C, Liber D, Ramos C *et al.*: Progressive loss of BDNF in a mouse model of Huntington's disease and rescue by BDNF delivery. *Pharmacol. Res.* 52, 133–139 (2005).

201 Zuccato C, Cattaneo E: Brain-derived neurotrophic factor in neurodegenerative diseases. *Nat. Rev. Neurol.* 5, 311–322 (2009).

202 Ilzecka J, Stelmasiak Z: BDNF is not altered in the serum and CSF of amyotrophic. *Neurol. Sci.* 22(6), 473–474 (2002).

203 Grundstrom E, Lindholm D, Johansson A, Blennow K, Askmark H: GDNF but not BDNF is increased in cerebrospinal fluid in amyotrophic lateral sclerosis. *Neuroreport* 11, 1781–1783 (2000).

204 Grundstrom E, Askmark H, Lindeberg J, Nygren I, Ebendal T, Aquilonius SM: Increased expression of glial cell line derived neurotrophic factor mRNA in muscle biopsies from patients with amyotrophic lateral sclerosis. *J. Neurol. Sci.* 162, 169–173 (1999).

205 Küst BM, Copray JC, Brouwer N, Troost D, Boddeke HW: Elevated levels of neurotrophins in human biceps brachii tissue of amyotrophic lateral sclerosis. *Exp. Neurol.* 177(2), 419–427 (2002).

206 Lunn JS, Hefferan MP, Marsala M, Feldman EL: Stem cells: comprehensive treatments for amyotrophic lateral sclerosis in conjunction with growth factor delivery. *Growth Factors* 27(3), 133–140 (2009).

207 Ochs G, Penn RD, York M *et al.*: A Phase I/II trial of recombinant methionyl human brain derived neurotrophic factor administered by intrathecal infusion to patients with amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Other Motor Neuron Disord.* 1, 201–206 (2000).

208 Beck M, Flachenecker P, Magnus T *et al.*: Autonomic dysfunction in ALS: a preliminary study on the effects of intrathecal BDNF. *Amyotroph. Lateral Scler. Other Motor Neuron Disord.* 6(2), 100–103 (2005).

209 Mojsilovic-Petrovic J, Jeong GB, Crocker A *et al.*: Protecting motor neurons from toxic insult by antagonism of adenosine A2a and Trk receptors. *J. Neurosci.* 26(36), 9250–9263 (2006), erratum in *J. Neurosci.* 26(40), 10079 (2006).

210 Harwood CA, McDermott CJ, Shaw PJ: Physical activity as an exogenous risk factor in motor neuron disease (MND): a review of the evidence. *Amyotroph. Lateral Scler.* 10(4), 191–204 (2009).

211 Seifert T, Brassard P, Wissenberg M *et al.*: Endurance training enhances BDNF release from the human brain. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 298(2), R372–R377 (2010).

212 Nichol K, Deeny SP, Seif J, Camaclang K, Cotman CW: Exercise improves cognition and hippocampal plasticity in APOE ε4 mice. *Alzheimers Dement.* 5(4), 287–294 (2009).

213 Bezard E, Dovero S, Belin D *et al.*: Enriched environment confers resistance to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and cocaine: involvement of dopamine transporter and trophic factors. *J. Neurosci.* 23(35), 10999–11007 (2003).

214 Pang TY, Stam NC, Nithianantharajah J, Howard ML, Hannan AJ: Differential effects of voluntary physical exercise on behavioral and brain-derived neurotrophic factor expression deficits in Huntington's disease transgenic mice. *Neuroscience* 141(2), 569–584 (2006).

215 Baker LD, Frank LL, Foster-Schubert K *et al.*: Effects of aerobic exercise on mild cognitive impairment: a controlled trial. *Arch. Neurol.* 67(1), 71–79 (2010).

216 Thacker EL, Chen H, Patel AV *et al.*: Recreational physical activity and risk of Parkinson's disease. *Mov. Disord.* 23(1), 69–74 (2008).

217 Kim JM, Stewart R, Bae KY *et al.*: Role of BDNF Val66Met polymorphism on the association between physical activity and incident dementia. *Neurobiol. Aging* (2010) (Epub ahead of print).

218 Egan MF, Kojima M, Callicott JH *et al.*: The BDNF Val66Met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* 112(2), 257–269 (2003).

219 Adlard PA, Perreau VM, Cotman CW: The exercise-induced expression of BDNF within the hippocampus varies across the life-span. *Neurobiol. Aging* 26, 511–520 (2005).

220 Chaldakov GN, Tonchev AB, Aloe L: NGF and BDNF: from nerves to adipose tissue, from neurokines to metabokines. *Riv. Psichiatr.* 44(2), 79–87 (2009).

221 Chaldakov GN, Fiore M, Hristova MG, Aloe L: Metabotrophic potential of neurotrophins: implication in obesity and related diseases? *Med. Sci. Monit.* 9(10), HY19–HY21 (2003).

222 Golden E, Emiliano A, Maudsley S *et al.*: Circulating brain-derived neurotrophic factor and indices of metabolic and cardiovascular health: data from the Baltimore Longitudinal Study of Aging. *PLoS One* 5(4), e10099 (2010).

223 Kim H, Li Q, Hempstead BL, Madri JA: Paracrine and autocrine functions of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in brain-derived endothelial cells. *J. Biol. Chem.* 279(32), 33538–33546 (2004).

224 Javeri S, Rodi M, Tary-Lehmann M, Lehmann PV, Addicks K, Kuerten S: Involvement of brain-derived neurotrophic factor (BDNF) in MP4-induced autoimmune encephalomyelitis. *Clin. Immunol.* 137(2), 181–189 (2010).

225 Fujinami A, Ohta K, Obayashi H *et al.*: Serum brain-derived neurotrophic factor in patients with Type 2 diabetes mellitus: relationship to glucose metabolism and biomarkers of insulin resistance. *Clin. Biochem.* 41(10–11), 812–817 (2008).

226 Scuri M, Samsell L, Piedimonte G: The role of neurotrophins in inflammation and allergy. *Inflamm. Allergy Drug Targets* 9(3), 173–180 (2010).

227 Raap U, Kapp A: Neurotrophins in healthy and diseased skin. *G. Ital. Dermatol. Venereol.* 145(2), 205–211 (2010).

228 Müller GC, Pitrez PM, Teixeira AL *et al.*: Plasma brain-derived neurotrophic factor levels are associated with clinical severity in school age children with asthma. *Clin. Exp. Allergy.* (2010) (Epub ahead of print).

2.5. MARCADORES IMUNOLÓGICOS

Marcantes avanços nos conhecimentos da psiconeuroimunologia têm sido observados desde a década de oitenta. Desde esta época inúmeras pesquisas têm apontado sobre o papel de processos imunológicos em diversas doenças psiquiátricas. Evidências apontam que mecanismos imunológicos interagiriam bidirecionalmente com o sistema nervoso central. A base para esta comunicação é a liberação de citocinas, um grupo de proteínas com funções autócrinas, parácrinas e hormonais que agem em células-alvo e possuem múltiplas ações. As citocinas são produzidas por células do sistema imune, como macrófagos, células T ativadas, células B, assim como células do sistema nervoso central, como astrócitos, entre outras.

Apesar dos inúmeros estudos elucidando a interrelação entre as citocinas e as doenças psiquiátricas, não é totalmente esclarecido se a ativação de vias inflamatórias no sistema nervoso central (CNS) origina-se na periferia e/ou se o estresse ou outro processo ainda não identificado induziria respostas inflamatórias diretamente dentro do cérebro. De qualquer forma, é reconhecido o papel das citocinas no SNC e na fisiopatologia de distúrbios psiquiátricos e dentre os possíveis mecanismos sugeridos apontam-se: (i) citocinas podem influenciar na síntese, liberação e recaptação de neurotransmissores e monoaminas (tais como a serotonina, norepinefrina, dopamina e glutamato) em regiões do cérebro essenciais para a regulação da emoção, incluindo o sistema límbico (amígdala, núcleo accumbens e hipocampo), bem como a regulação da função psicomotora e de recompensa; (ii) citocinas podem influenciar no eixo hipotálamo-pituitária-adrenal (HPA) por meio de feedback negativo e / ou a perturbação na função dos receptores de glucocorticóides; (iii) citocinas podem afetar a plasticidade neuronal e diminuir a expressão hipocampal de BDNF (MILLER et al., 2009).

Uma revisão sistemática realizada em 2009 sobre os marcadores imunológicos em pacientes com o diagnóstico de TB é abordada no **Artigo 2**: “Imunologia do transtorno bipolar”.

Artigo 2: Imunologia do transtorno bipolar

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ARTIGO DE REVISÃO

Imunologia do transtorno bipolar

Immunology of bipolar disorder

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RESUMO

Objetivo: Pesquisas recentes têm implicado fatores imunes na patogênese de diversos transtornos neuropsiquiátricos. O objetivo do presente trabalho é revisar os trabalhos que investigaram a associação entre transtorno bipolar e alterações em parâmetros imunes. **Métodos:** Artigos que incluíam as palavras-chave: "bipolar disorder", "mania", "immunology", "cytokines", "chemokines", "interleukins", "interferon" e "tumor necrosis factor" foram selecionados em uma revisão sistemática da literatura. As bases de dados avaliadas foram MedLine e Scopus, entre os anos de 1980 e 2008. **Resultados:** Foram identificados 28 trabalhos que estudaram alterações imunes em pacientes com transtorno bipolar. Seis artigos investigaram genes relacionados à resposta imune; cinco, autoanticorpos; quatro, populações leucocitárias; 13, citocinas e/ou moléculas relacionadas à resposta imune e seis, leucócitos de pacientes *in vitro*. **Conclusões:** Embora haja evidências na literatura correlacionando o transtorno bipolar a alterações imunes, os dados não são conclusivos. O transtorno bipolar parece estar associado a níveis mais elevados de autoanticorpos circulantes, assim como à tendência à ativação imune com produção de citocinas pró-inflamatórias e redução de parâmetros anti-inflamatórios.

Palavras-chave
Transtorno bipolar, imunologia, citocinas, quimiocinas, interleucinas, interferon e fator de necrose tumoral-α.

ABSTRACT

Objective: *Emerging research has implicated immune factors in the pathogenesis of a variety of neuropsychiatric disorders. The objective of the present paper is to review the studies that investigated the association between bipolar disorder and immune parameters.* ***Methods:*** *Papers that included the keywords "bipolar to disorder", "mania", "immunology", "cytokines", "chemokines", "interleukins", "interferon" and "tumor necrosis factor" were selected in a systematic review of the literature. The evaluated databases were MedLine and Scopus in the period between 1980 and 2008.* ***Results:*** *Twenty eight works were found. Six studies investigated immune response-related genes; five, auto-antibodies; four, leukocyte population; 13, cytokines and/or immune-related molecules; six, leukocytes in vitro.* ***Conclusions:*** *Although there is evidence in the literature correlating affective disorders with immune parameters, the results are still inconclusive. Bipolar disorder seems to be associated with increased levels of auto-antibodies as well as with a trend for increased immune activation with production of pro-inflammatory cytokines and reduction of the anti-inflammatory parameters.*

Keywords
Bipolar disorder, immunology, cytokines, chemokines, interleukins, interferon and tumor necrosis factor-α.

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INTRODUÇÃO

O transtorno bipolar (TB) caracteriza-se clinicamente pela alternância entre estados de humor depressivo e de humor maníaco (tipo I) ou hipomaníaco (tipo II). O TB tipo I tem prevalência de cerca de 1% da população[1], enquanto o TB tipo II afeta cerca de 5% da população[2].

As alterações de humor ocorrem em cerca de dois terços do tempo de vida do paciente, conferindo grande morbidade e impacto socioeconômico associados à doença[3]. Cerca de 50% a 70% dos pacientes cursam com algum tipo de comorbidade psiquiátrica[4], enquanto cerca de 50% dos pacientes apresentam comorbidade clínica[5].

A fisiopatologia do TB é ainda pouco compreendida. Reconhece-se a relevância da contribuição genética, sendo descrita herdabilidade de até 85%[6]. Entre os genes candidatos, destacam-se aqueles relacionados a sistemas de neurotransmissores, sobretudo serotonina (SLC6A4 e TPH2), dopamina (DRD4 e SLC6A3) e glutamato (DAOA e DTNBP1), e de crescimento neuronal (BDNF)[7,8]. Contudo, os modelos focados em um único neurotransmissor não conseguem explicar a heterogeneidade da apresentação e do curso clínico do transtorno, sugerindo que a inter-relação entre múltiplos sistemas poderia estar comprometida nesses pacientes.

Mais recentemente, vem sendo estudado o papel das alterações do sistema imune, principalmente citocinas, na patogênese de transtornos psiquiátricos, como depressão maior[9-11], transtorno obsessivo-compulsivo[12] e esquizofrenia[13,14]. Citocinas são peptídeos produzidos e liberados por células imunes com potencial para interferir no metabolismo de sistemas de neurotransmissores, nas atividades neuroendócrina e neuronal, na regulação do crescimento e da proliferação das células da glia[15]. O objetivo do presente trabalho é revisar os trabalhos que investigaram possíveis alterações imunes no TB.

MÉTODOS

A partir das bases de dados MedLine e Scopus, foram buscados artigos em língua inglesa, espanhola, portuguesa e francesa, publicados no período de 1990 e outubro de 2008, com as seguintes palavras-chave: "bipolar disorder", "mania", "immunology", "cytokines", "chemokines", "interleukines", "interferon" e "tumor necrosis factor".

RESULTADOS

Foram identificados 28 trabalhos que estudaram alterações imunes em TB. Seis estudos investigaram genes relacionados à resposta imune em pacientes bipolares (Tabela 1).

Cinco estudos avaliaram autoanticorpos no TB (Tabela 2). Quatro deles mensuraram anticorpos antitireoidianos e apenas um observou elevação desses autoanticorpos em pacientes bipolares. Hornig *et al.*[23] investigaram, além de anticorpos antitireoidianos, anticorpos antinucleares (ANA), antidsDNA e anticardiolipina, não encontrando diferenças em relação a controles. O estudo de Padmos *et al.*[26] avaliou a expressão de anticorpos ligados à gastrite crônica autoimune (H/K-ATPase) e *diabetes mellitus* tipo I (GAD 65), encontrando maior expressão destes em pacientes bipolares.

Treze estudos avaliaram citocinas e/ou moléculas relacionadas à resposta imune em soro ou plasma de pacientes (Tabela 3). Quatro trabalhos realizaram dosagens séricas e/ou plasmática de TNF-α enquanto três evidenciaram elevação nos níveis séricos em relação aos controles[33-35]. Seis estudos investigaram os níveis circulantes de interleucina (IL)-6 e três mostraram alterações em relação a controles assintomáticos[27,34,35]. Somente um estudo avaliou dosagem sérica de IL-8 em pacientes em fases de mania e depressão, encontrando níveis mais elevados em relação a controles[33]. Um estudo avaliou dosagens plasmáticas de TGF-1β em pacientes bipolares e observou que pacientes em fases de mania, sem o uso de medicamentos há pelo menos 4 semanas, evidenciavam elevações nos níveis plasmáticos em relação aos controles e que, após atingirem a eutimia e em uso de medicamentos, os níveis plasmáticos não diferiam dos controles[32]. Resultados conflitantes podem ser observados em relação a dosagens séricas e/ou plasmáticas das citocinas IL-2, IL-4, IL-10, IL-12 e interferon gama (IFNγ).

Em relação a receptores solúveis de citocinas, cinco trabalhos avaliaram os níveis de receptores solúveis de IL-2. Três encontraram níveis elevados em pacientes bipolares em quadros de mania[27,29,31]. Quatro estudos investigaram níveis de receptores solúveis de IL-6 e somente um observou alteração em relação aos controles[27].

Quatro trabalhos estudaram diferentes populações leucocitárias no TB; dois encontraram alterações em relação a controles (Tabela 4). Um estudou observou diferenças em parâmetros celulares entre o estado de mania e o estado misto[38].

Seis estudos avaliaram as células mononucleares de sangue periférico isolados de pacientes bipolares *in vitro* (Tabela 5). Na ausência de estímulos, as células de pacientes não apresentaram alterações nos níveis de IL-1, IL-2, IL-6, IL-10 e INFγ em relação aos controles, independentemente da medicação ou fase da doença[40,43]. Seis estudos avaliaram os meios de culturas de células sob estimulação. Dados discordantes têm sido evidenciados em relação à produção de IL-2, IL-4, IL-6, IL-10, TNF-α, INFγ.

Tabela 1. Polimorfismos de genes ligados à resposta imune no transtorno bipolar (TB)

	Pacientes/ controles	População estudada	Especificação do TB	Polimorfismo estudado	Resultado
Pae *et al.*[16]	89/125	Coreana	ND	-308G/A do gene do TNF-α	- 308A p > c
Papiol *et al.*[17]	88/176	Espanhola	ND	-511C/T do gene do IL-1β alelo A2 do antagonista do receptor IL-1	-511 C p > c p > c
Kim *et al.*[18]	83/297	Coreana	ND	Gene IL-1RA	p = c
Roh *et al.*[19]	183/350	Coreana	TB I (145) II (38)	-2518 G/A do gene do MCP-1	p = c
Czerski *et al.*[20]	361/351	Polonesa	ND	-308G/A do gene TNF-α	-308 G p > c
Padmos *et al.*[21]*	42/25		TB I (35) II (7)	PDE4B IL-1B IL-6 TNF TNFAIP3 PTGS2 PTX3 HSPA1A CCL2 CCL7 CCL20 CXCL2 CCR2 CDC42 CX3CR1 BCL2A1 EMP1 MAPK6 DUSP2 NAB2 ATF3	p > c p > c p > c p > c p > c p > c p > c p = c p > c p > c p > c p > c p > c p > c p > c p = c p > c p > c p > c p > c

ND: não disponível; p: paciente; c: controle.

* Empregou a técnica de "*microarray*" que possibilita a investigação simultânea de vários polimorfismos.

Tabela 2. Autoanticorpos no transtorno bipolar (TB)

	Pacientes/controles	Especificação do TAB	Comorbidade psiquiátrica	Comorbidade clínica	Medicação em uso	Influência da medicação	Autoanticorpo	Resultado
Rapaport *et al.*[22]	26/34	TB I (16) II (10)	ND	ND	ND	ND	Anticardiolipina Antimicrossomal Antitireoglobulina	p = c p = c p = c
Hornig *et al.*[23]	103/22	TB I (79) II (24)	ND	ND	Lítio	Sem influência	ANA AntidsDNA Antitireoglobulina Anticardiolipina	p = c p = c p = c p = c
Kupka *et al.*[24]	226/252	ND	ND	ND	Lítio	Sem influência	AntiTPO	p > c
Baethge *et al.*[25]	64/100	ND	ND	ND	Lítio, hormônio tireoidiano	Sem influência	AntiTPO TgAb TrAb	p = c p = c p = c
Padmos *et al.*[26]	239/220	TB I, II, SOE, cicladores rápidos	ND	ND	Lítio, carbamazepina, valproato, antidepressivos e antipsicóticos	Sem influência	H/K-ATPase GAD 65 A GAD 67 A	p > c p > c p = c

ND: não disponível; SOE: sem outras especificações; p: paciente; c: controle; GAD: ácido glutâmico descarboxilase.

Tabela 3. Estudos que detectaram parâmetros imunes *in vivo*

	Número de pacientes/controles	Tempo sem uso de medicamentos	Especificação TB na entrada do estudo	Comorbidade	Parâmetros imunes e condições	Medicação para tratamento e tempo	Especificação TB após TTO	Parâmetros imunes após tratamento
Rapaport[22]	26/34	Pacientes medicados e não medicados	TB em eutimia TB I (16) TB II (10)	ND	SL-2R (sem alteração)	ND	ND	ND
Maes *et al.*[27]	10/21	7 (7-60) dias	TB I Mania	ND	IL-6 (sem alteração) ↑SL-6R ↑ SL-2R	Valproato 14 dias	Eutimia	IL-6 (sem alteração) ↑SL-6R ↑ SL-2R
Rapaport *et al.*[28]	17/18	2 semanas	Ciclatores rápidos Hipomania (2) Depressão (6) Eutimia (9) TB I (3) TB II (14)	Sem comorbidades clínicas ou psiquiátricas	IL-4, IL-6, IL-10 e INFγ (indetectáveis) IL-2 (sem alteração) SL-6R e SL-2R (sem alterações)	Carbonato de lítio (0,82 mEq/L) 30 dias	Eutimia	IL-4, IL-6, IL-10 e INFγ (indetectável) IL-2* (sem alteração) SL-6R e SL-2R (sem alterações)
Tsai *et al.*[29]	31/31	Em uso: carbamazepina	TB I Mania	ND	SL-6R sem alteração ↑ SL-2R	Introdução de lítio	Eutimia	↓ SL-2R
Kim *et al.*[30]	25/85	> 4 meses	TB I Mania	Sem comorbidades psiquiátricas relacionadas a abuso de álcool e substâncias, doença autoimunes	IL-12 (sem alteração)	Carbonato de lítio e/ou valproato e/ou antipsicóticos 8 semanas	Eutimia	IL-12 (sem alteração)
Breunis *et al.*[31]	64/66	Em uso de medicações	TB I e II	ND	↑ SL-2R em bipolares principalmente em mania	ND	ND	ND
Kim *et al.*[32]	70/96	> 4 meses	TB I Mania	Sem doenças autoimunes, infecções, abuso de álcool e outras substâncias	↑ IL-4 ↑ INFγ ↓ TGF-1β	Carbonato de lítio e/ou valproato e /ou antipsicóticos 8 semanas	Eutimia	↑ IL-4 ↑ INFγ ↑ TGF 1β
O´Brien *et al.*[33]	21/21	Em uso de medicações: antipsicóticos, antidepressivo estabilizador do humor	Mania (9) Depressão (12) Não houve especificação TB I ou II	Sem comorbidades clínicas, psiquiátricas ou uso de anti-inflamatórios	Mania:↑ IL-8 ↑TNF-α ↑IL-6 IL-10 (sem alteração) Depressão: ↑ IL-8 ↑ TNF-α IL-10 e IL-6 (sem alterações) SL-6R (sem alterações)	ND	ND	ND
Ortiz-Domínguez *et al.*[34]	20/33	> 3 semanas	TB I Mania (10) Depressão (10)	Sem comorbidades clínicas, psiquiátricas ou uso de anti-inflamatórios	Mania: ↑ TNF-α ↑ IL-4 ↓IL-2 ↓ IL-1β Depressão: ↑ TN-α ↑ IL-6 ↓IL-2, ↓ IL-4, IL-1β (sem alterações)	ND	ND	ND
Hung *et al.*[35]	15/14		Depressão sem especificação TAB I ou II	ND	TNF-α, IL-6 (sem alterações)	ND	ND	ND
Kauer-Sant'Anna *et al.*[36]	60/60	Em uso de medicações: antipsicóticos, antidepressivo estabilizador do humor	TAB I em eutimia diagnóstico < 3 anos diagnóstico > 10 anos	ND	Diagnóstico < 3 anos ↑ TNF-α ↑ IL-6 ↑IL-10 diagnóstico > 10 anos ↑ TNF-α ↑ IL-6, IL-10 sem alterações	ND	ND	ND

ND: não disponível; IL: interleucina; TNF: fator de necrose tumoral; INF: interferon; ↑: elevado; ↓: diminuído; SL-R: receptor solúvel.

Tabela 4. Alterações de populações leucocitárias em pacientes bipolares

	Pacientes/controles	Especificação de TB	Alteração leucocitária	Correlação	Medicação em uso	Influência da medicação
Rapaport *et al.*[22]	26/34	Eutimia TB I (16) II (10)	$CD3^+$ $CD4^+$ $CD\ 8^+$ $CD\ 16^+$ $CD19^+$ $CD\ 25^+$ $HLA\text{-}DR^+$ $CD19^+\ HLA^+$ $CD19^+\ CD5^+$ $CD29^+\ CD4^+$ CD4 CD8	p = c	ND	ND
Sourlingas *et al.*[37]	12/7	Mania/hipomania (3) Depressão (3) Eutimia (7)	Leucócitos totais	p < c	Lítio, tricíclicos, antipsicóticos	ND
Cassidy *et al.*[38]	174/0	TB I Mania (155) Misto (19)	Leucócitos totais Neutrófilos Linfócitos Monócitos	ms > m ms > m ms = m ms > m	Sem medicação > 2 semanas	ND
Breunis *et al.*[21]	64/29	TB I TB II	$CD3^+\ MHCII^+$ $CD3^+\ CD25^+$ $CD3^+\ CD71^+$ $CD3^+\ CD69^+$ Células B Células NK	p = c p > c p > c p = c p > c p = c	ND	ND

ND: não disponível; p: paciente; c: controle; ms: misto; m: mania.

DISCUSSÃO

Trata-se do primeiro estudo sistemático de alterações imunes no TB. Em conjunto, os resultados sugerem que os pacientes bipolares, independentemente da fase da doença, têm alterações em diferentes parâmetros estudados.

Os pacientes bipolares tenderam a apresentar maiores níveis de autoanticorpos circulantes com provável associação com doenças autoimunes. Em relação às citocinas, os pacientes bipolares parecem exibir um perfil pró-inflamatório, independentemente da fase da doença, com diminuição de citocinas e/ou moléculas anti-inflamatórias. Há trabalhos que sugerem que quadros de mania estejam marcados por perfil ainda mais pró-inflamatório. Cabe ressaltar que perfil anti-inflamatório *in vivo* e *in vitro* é observado classicamente em pacientes com quadros de depressão unipolar[11].

As alterações imunológicas em pacientes bipolares parecem ser evidenciadas também em seus familiares. Hillegers *et al.*[44] realizaram um estudo longitudinal com 140 filhos de pais com o diagnóstico de TB. No momento da entrada no estudo, os filhos tinham idade entre 12 e 21 anos e foram acompanhados por 55 meses e avaliados por 3 vezes durante esse período. Foram observadas elevações nos níveis de anticorpos antiTPO (tiroperoxidase) em filhos de pacientes bipolares, principalmente no sexo feminino. Os jovens que apresentavam elevações nos níveis de antiTPO não mostravam aumento na frequência de transtornos de humor ou demais transtornos psiquiátricos até o momento da avaliação. Padmos *et al.*[21] realizaram um estudo em monócitos de pacientes com o diagnóstico de TB e seus filhos e encontraram expressão alterada de RNA mensageiros de genes relacionados a inflamação, tráfego celular, sobrevivência e via mitógeno-proteína-cinase ativada. Os filhos de pacientes bipolares, que exibiam algum transtorno de humor durante a pesquisa, apresentavam maior expressão desses genes em comparação aos filhos não afetados.

Embora haja evidências na literatura correlacionando o TB a alterações imunológicas, os dados são ainda inconsistentes. Vários fatores provavelmente contribuem para a indefinição acerca do envolvimento de fatores imunes no TB, como o volume limitado de pesquisas realizadas, especialmente investigando pacientes bipolares nas três fases da doença, o que poderia demonstrar um perfil imunológico distinto em cada uma delas, e o pequeno número de pacientes incluídos nos estudos. Outro possível fator limitante seria a ausência de informação sobre as comorbidades clínicas e psiquiátricas dos pacientes que poderiam interferir em parâmetros imunes como, por exemplo, *diabetes mellitus*[45], doenças coronarianas[46], obesidade[47], tabagismo[48], esclerose múltipla[49] e transtorno obsessivo-compulsivo[12]. Dessa forma, os próximos estudos deverão ser mais criteriosos no recrutamento das populações com TB.

Uma grande discussão tem sido enfocada no papel da interferência da medicação nos fatores imunes. Segundo revisão da literatura, os estudos que avaliaram esses fatores antes do uso de medicamentos não apresentaram, em sua maioria, alterações dos parâmetros imunes, sugerindo que as alterações imunológicas seriam intrínsecas ao TB[27,28,30-32,39,40-42]. Entretanto, Padmos *et al.*[21] demonstraram que o uso de lítio, carbamazepina, valproato de sódio e

Tabela 5. Estudos que detectaram parâmetros imunes *in vitro*

	Número de pacientes/controles	Tempo sem uso de medicamentos	Especificação TB na entrada do estudo	Comorbidade	Parâmetros imunes e condições	Medicação para tratamento e tempo	Especificação TB após TTO	Parâmetros imunes após tratamento
Rapaport[22]	26/34	Pacientes medicados e não medicados	TB em eutimia TB I (16) TB II (10)	ND	Cultura com estímulo IL-2 (sem alteração)	ND	ND	ND
Su *et al.*[39]	20/15	Pacientes medicados e não medicados	TB I Mania	Sem comorbidades psiquiátricas, abuso de álcool e substâncias, doença autoimunes ou infecciosa	Cultura com estímulo ↑ INF-γ IL-10 (sem alteração)	Estabilizadores do humor e antipsicótico	TB I Eutimia	Cultura com estímulo ↑ INF-γ IL-10 (sem alteração)
Boufidou *et al.*[40]	40/20	Em uso de lítio (20) Sem uso de lítio (20)	TB em eutimia TB I (27) TB II (13)	Sem doença autoimune, alergia ou infecção	Cultura sem estímulo INF-γ, IL-1, IL-2, IL-6, IL-10 (sem alterações) Cultura com estímulo ↓ INF, ↓ IL-1, ↓ IL-2*, ↓IL-6, ↓IL-10	ND	ND	Nd
Liu *et al.*[41]	52/45	Pacientes medicados e não medicados	TB I Mania	ND	PBMC com estímulo ↓ INF-γ, ↑ IL-1 RA IL-2, IL-4, IL-10 (sem alterações)	Estabilizadores do humor e antipsicótico	TB I Eutimia	PBMC com estímulo ↓ INF-γ, ↑ IL-1 RA, ↑IL-2 IL-4, IL-10 (sem alterações)
Kim *et al.*[42]	37/74	> 4 meses	TB I Mania	Sem comorbidades psiquiátricas, abuso de álcool e substâncias, doença autoimunes	Cultura com estímulo ↑ TNF-α ↑ IL-6 ↑IL-4 IL-2 e INF-γ (sem alterações)	Estabilizadores do humor e antipsicóticos 6 semanas	TB I Eutimia	Cultura com estímulo ↑ TNF-α ↑ IL-6 ↑IL-4 IL-2 e INF-γ (sem alterações)
Knijff *et al.*[43]	80/59	Em uso de lítio (59) Sem uso de lítio (21)	TB I (61) II (19) Mania (11) Eutimia (50) Depressão (15)	ND	Cultura sem estímulo IL-1, IL-6 (sem alterações) Cultura com estímulo IL-1, IL-6 (sem alterações)** ↑IL-1*** ↓ IL-6***	ND	ND	ND

ND: não disponível; IL: interleucina; TNF: fator de necrose tumoral; INF: interferon; ↑: elevado; ↓: diminuído; SL–R: receptor solúvel.

* Pacientes não responsivos a lítio não apresentaram diferenças estatísticas com controles.

** Pacientes em uso de lítio.

*** Pacientes não estavam em uso de lítio.

antipsicóticos poderia ser responsável pela indução de uma menor expressão de alguns genes (PDE4B, *IL-1*B, IL6, TNF, TNFAIP3, PTGS2, PTX3, CCL20, CXCL2, BCL2A1 e DUSP2). Além disso, Rapaport *et al.*[28] apontaram uma possível interferência do uso de lítio na dosagem sérica dos receptores solúveis de IL-2 e IL-6 ao avaliarem pacientes bipolares cicladores rápidos em eutimia, comparando-os a controles saudáveis. Os pesquisadores observaram que os pacientes cicladores rápidos apresentavam uma tendência a níveis mais altos de receptores solúveis de IL-2 e IL-6, quando comparados a controles livres de medicação. Após a introdução de lítio, os níveis de receptores solúveis em pacientes bipolares diminuíam, enquanto aumentavam nos controles medicados com lítio. Rapaport e Manji[50] avaliaram o papel do lítio na produção *in vitro* de citocinas por leucócitos de pacientes bipolares sem comorbidades clínicas ou psiquiátricas. Observaram que o lítio induzia aumento na produção de IL-4 e IL-10 e diminuição nas citocinas pró-inflamatórias IL-6 e INFγ. Mais recentemente, foi confirmado que o tratamento com lítio *in vivo* ou *in vitro* restaurava o perfil de produção de IL-1β e IL-6 dos pacientes com TB[43]. Dessa maneira, os próximos estudos deverão controlar o uso dos estabilizadores do humor nas análises das citocinas.

CONCLUSÃO

Embora haja crescentes evidências na literatura correlacionando o TB a alterações imunes, os dados não são ainda conclusivos. Os pacientes bipolares tendem a exibir níveis mais elevados de autoanticorpos circulantes e perfil pró-inflamatório de citocinas, independentemente da fase da doença ou do uso de medicação, quando comparados a controles saudáveis. Isso que poderia sugerir a participação de mecanismos imunes e/ou inflamatórios na fisiopatologia do TB. Seguindo essa linha de evidência, é interessante destacar recentes estudos investigando drogas anti-inflamatórias como nova estratégia terapêutica para pacientes bipolares[51].

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REFERÊNCIAS

1. Belmaker RH. Bipolar disorder. N Engl J Med. 2004;351(5):476-86.
2. Benazzi F. Bipolar II disorder: epidemiology, diagnosis and management. CNS Drugs. 2007;21(9):27-40.
3. Judd LL, Schettler PJ, Akiskal HS, Maser J, Coryell W, Solomon D, et al. Long-term symptomatic status of bipolar I vs. bipolar II disorders. Int J Neuropsychopharmacol. 2003;6(2):127-37.
4. Krishnan KR. Psychiatric and medical comorbidities of bipolar disorder. Psychosom Med. 2005;67(1):1-8.
5. Beyer J, Kuchibhatla M, Gersing K, Krishnan KR. Medical comorbidity in a bipolar outpatient clinical population. Neuropsychopharmacology. 2005;30(2):401-4.
6. Merikangas K, Yu K. Genetic epidemiology of bipolar disorder. Clin Neurosci Res. 2002; 2:127-41.
7. Carter CJ. Multiple genes and factors associated with bipolar disorder converge on growth factor and stress activated kinase pathways controlling translation initiation: implications for oligodendrocyte viability. Neurochem Int. 2007;50(3):461-90.
8. Serretti A, Mandelli L. The genetics of bipolar disorder: genome 'hot regions', genes, new potential candidates and future directions. Mol Psychiatry. 2008;13(8):742-71.
9. Simon NM, McNamarab K, Chowa CW, Maserb RS, Papakostas GI, Pollacka MH, et al. A detailed examination of cytokine abnormalities in major depressive disorder. Eur Neuropsychopharmacol. 2008;18:230-3.
10. Sutcigil L, Oktenli C, Musabak U, Bozkurt A, Cansever A, Uzun O, et al. Pro- and anti-inflammatory cytokine balance in major depression: effect of sertraline therapy. Clin Dev Immunol. 2007:763-96.
11. Teixeira AL, Bauer ME, Nicolato R, Reis HJ, Palotás A. Cytokines and the brain: beyond immune response. In: Fedorovich SV (ed.). Signal Transduction in Nervous Cells, India; 2008.
12. da Rocha FF, Correa H, Teixeira AL. Obsessive-compulsive disorder and immunology: a review. Prog Neuropsychopharmacol Biol Psychiatry. 2008;32(5):1139-46.
13. Coelho FM, Reis HJ, Nicolato R, Romano-Silva MA, Teixeira MM, Bauer ME, Teixeira AL. Increased serum levels of inflammatory markers in chronic institutionalized patients with schizophrenia. Neuroimmunomodulation. 2008;15(2):140-4.
14. Teixeira AL, Reis HJ, Nicolato R, Brito-Melo G, Correa H, Teixeira MM, et al. Increased serum levels of CCL11/eotaxin in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 2008;32(3):710-4.
15. Silverman MN, Pearce BD, Biron CA, Miller AH. Immune modulation of the hypothalamic-pituitary-adrenal (HPA) axis during viral infection. Viral Immunol. 2005;18(1):41-78.
16. Pae CU, Lee KU, Han H, Serretti A, Jun TY. Tumor necrosis factor alpha gene-G308A polymorphism associated with bipolar I disorder in the Korean population. Psychiatry Res. 2004;125(1):65-8.
17. Papiol S, Rosa A, Gutiérrez B, Martín B, Salgado P, Catalán R, et al. Interleukin-1 cluster is associated with genetic risk for schizophrenia and bipolar disorder. J Med Genet. 2004;41(3):219-23.
18. Kim SJ, Lee HJ, Koo HG, Kim JW, Song JY, Kim MK, et al. Impact of IL-1 receptor antagonist gene polymorphism on schizophrenia and bipolar disorder. Psychiatr Genet. 2004;14(3):165-7.
19. Roh MS, Lee KY, Joo EJ, Lee N, Kim YS. No association of the MCP-1 promoter A-2518G polymorphism with bipolar disorder in the Korean population. Neurosci Lett. 2007;427(1):1-5.
20. Czerski PM, Rybakowski F, Kapelski P, Rybakowski JK, Dmitrzak-Weglarz M, Leszczyńska-Rodziewicz A. Association of tumor necrosis factor -308G/A promoter polymorphism with schizophrenia and bipolar affective disorder in a Polish population. Neuropsychobiology. 2008;57(1-2):88-94.
21. Padmos RC, Hillegers MH, Knijff EM, Vonk R, Bouvy A, Staal FJ, et al. A discriminating messenger RNA signature for bipolar disorder formed by an aberrant expression of inflammatory genes in monocytes. Arch Gen Psychiatry. 2008;65(4):395-407.
22. Rapaport MH. Immune parameters in euthymic bipolar patients and normal volunteers. J Affect Disord. 1994;32:149-56.
23. Hornig M, Amsterdam JD, Kamoun M, Goodman DB. Autoantibody disturbances in affective disorders: a function of age and gender? J Affect Disord. 1999;55(1):29-37.
24. Kupka RW, Nolen WA, Post RM, McElroy SL, Altshuler LL, Denicoff KD, et al. High rate of autoimmune thyroiditis in bipolar disorder: lack of association with lithium exposure. Biol Psychiatry. 2002;51(4):305-11.

25. Baethge C, Blumentritt H, Berghöfer A, Bschor T, Glenn T, Adli M, et al. Long-term lithium treatment and thyroid antibodies: a controlled study. J Psychiatry Neurosci. 2005;30(6):423-7.

26. Padmos RC, Bekris L, Knijff EM, Tiemeier H, Kupka RW, Cohen D, et al. A high prevalence of organ-specific autoimmunity in patients with bipolar disorder. Biol Psychiatry. 2004;56(7):476-82.

27. Maes M, Bosmans E, Calabrese J, Smith R, Meltzer HY. Interleukin-2 and interleukin-6 in schizophrenia and mania: effects of neuroleptics and mood stabilizers. J Psychiatr Res. 1995;29(2):141-52.

28. Rapaport MH, Guylai L, Whybrow P. Immune parameters in rapid cycling bipolar patients before and after lithium treatment. J Psychiatr Res. 1999;33(4):335-40.

29. Tsai SY, Yang YY, Kuo CJ, Chen CC, Leu SJ. Effects of symptomatic severity on elevation of plasma soluble interleukin-2 receptor in bipolar mania. J Affect Disord. 2000;64(2-3):185-93.

30. Kim YK, Suh IB, Kim H, Han CS, Lim CS, Choi SH, et al. The plasma levels of interleukin-12 in schizophrenia, major depression, and bipolar mania: effects of psychotropic drugs. Mol Psychiatry. 2002;7(10):1107-14.

31. Breunis MN, Kupka RW, Nolen WA, Suppes T, Denicoff KD, Leverich GS, et al. High numbers of circulating activated T cells and raised levels of serum IL-2 receptor in bipolar disorder. Biol Psychiatry. 2003;53(2):157-65.

32. Kim YK, Myint AM, Lee BH, Han CS, Lee SW, Leonard BE, et al. T-helper types 1, 2, and 3 cytokine interactions in symptomatic manic patients. Psychiatry Res. 2004;129(3):267-72.

33. O'Brien SM, Scully P, Scott LV, Dinan TG. Cytokine profiles in bipolar affective disorder: focus on acutely ill patients. J Affect Disord. 2006;90(2-3):263-7.

34. Ortiz-Domínguez A, Hernández ME, Berlanga C, Gutiérrez-Mora D, Moreno J, Heinze G, et al. Immune variations in bipolar disorder: phasic differences. Bipolar Disord. 2007;9(6):596-602.

35. Hung YJ, Hsieh CH, Chen YJ, Pei D, Kuo SW, Shen DC, et al. Insulin sensitivity, proinflammatory markers and adiponectin in young males with different subtypes of depressive disorder. Clin Endocrinol (Oxf). 2007;67(5):784-9.

36. Kauer-Sant'anna M, Kapczinski F, Andreazza AC, Bond DJ, Lam RW, Trevor Young L, et al. Brain-derived neurotrophic factor and inflammatory markers in patients with early- vs. late-stage bipolar disorder. Int J Neuropsychopharmacol. 2008;4:1-12.

37. Sourlingas TG, Issidorides MR, Havaki S, Trikkas G, Sekeri-Pataryas KE. Peripheral blood lymphocytes of bipolar affective patients have a histone synthetic profile indicative of an active cell state. Prog Neuropsychopharmacol Biol Psychiatry. 1998;22(1):81-96.

38. Cassidy F, Wilson WH, Carroll BJ. Leukocytosis and hypoalbuminemia in mixed bipolar states: evidence for immune activation. Acta Psychiatr Scand. 2002;105(1):60-4.

39. Su KP, Leu SJ, Yang YY, Shen WW, Chou YM, Tsai SY. Reduced production of interferon-gamma but not interleukin-10 in bipolar mania and subsequent remission. J Affect Disord. 2002;71(1-3):205-9.

40. Boufidou F, Nikolaou C, Alevizos B, Liappas IA, Christodoulou GN. Cytokine production in bipolar affective disorder patients under lithium treatment. J Affect Disord. 2004;82(2):309-13.

41. Liu HC, Yang YY, Chou YM, Chen KP, Shen WW, Leu SJ. Immunologic variables in acute mania of bipolar disorder. J Neuroimmunol. 2004;150(1-2):116-22.

42. Kim YK, Jung HG, Myint AM, Kim H, Park SH. Imbalance between pro-inflammatory and anti-inflammatory cytokines in bipolar disorder. J Affect Disord. 2007;104(1-3):91-5.

43. Knijff EM, Breunis MN, Kupka RW, de Wit HJ, Ruwhof C, Akkerhuis GW, et al. An imbalance in the production of IL-1beta and IL-6 by monocytes of bipolar patients: restoration by lithium treatment. Bipolar Disord. 2007;9(7):743-53.

44. Hillegers MH, Reichart CG, Wals M, Verhulst FC, Ormel J, Nolen WA, et al. Signs of a higher prevalence of autoimmune thyroiditis in female offspring of bipolar parents. Eur Neuropsychopharmacol. 2007;17(6-7):394-9.

45. O'Connor JC, McCusker RH, Strle K, Johnson RW, Dantzer R, Kelley KW. Regulation of IGF-I function by proinflammatory cytokines: at the interface of immunology and endocrinology. Cell Immunol. 2008;252(1-2):91-110.

46. Kop WJ, Cohen N. Psychoneuroimmunological pathways involved in acute coronary syndromes. In: Ader R (ed.). Psychoneuroimmunology. New York: Elsevier Press; 2007. p. 921-44.

47. Guest CB, Gao Y, O'Connor JC, Freund GG. Obesity and Immunity. In: Ader R (ed.). Psychoneuroimmunology. New York: Elsevier Press; 2007. p. 933-1012.

48. Schleifer SJ. Psychoneuroimmunologic aspects of alcohol and substance abuse. In: Ader R (ed.). Psychoneuroimmunology. New York: Elsevier Press; 2007. p. 549-62

49. Moreira MA, Souza AL, Lana-Peixoto MA, Teixeira MM, Teixeira AL. Chemokines in the cerebrospinal fluid of patients with active and stable relapsing-remitting multiple sclerosis. Braz J Med Biol Res. 2006;39(4):441-5.

50. Rapaport MH, Manji HK. The effects of lithium on ex vivo cytokine production. Biol Psychiatry. 2001;50(3):217-24.

51. Nery FG, Monkul ES, Hatch JP, Fonseca M, Zunta-Soares GB, Frey BN, et al. Celecoxib as an adjunct in the treatment of depressive or mixed episodes of bipolar disorder: a double-blind, randomized, placebo-controlled study. Hum Psychopharmacol. 2008;23(2):87-94.

Assinala-se que desde 2009, um grande número de publicações no que concerne ao papel de marcadores imunológicos em pacientes com o diagnóstico de TB apresentou grandecrescimento, particularmente em relação ao papel do fator de necrose tumoral alfa (TNF-α). O TNF-α é considerado uma citocina pró-inflamatória prototípica e é produzida por macrófagos, linfócitos, neutrófilos e outras células do sistema imune, assim como células estruturais incluindo astrócitos e micróglias em resposta a uma injúria ou infecção (BRIETZKE & KAPCZINSKI, 2008). Diversos estudos têm demonstrado aumento da ativação do TNF-α em pacientes com o diagnóstico de TB, sugerindo um perfil pró-inflamatório associado (BARBOSA et al, 2011; HOPE et al, 2011; KAUER-SANT'ANNA et al, 2008; ORTIZ-DOMINGUES et al, 2007; O'BRIEN et al, 2006). O estado pró-inflamatório exacerbado em pacientes com o diagnóstico de TB é corroborado pelo aumento das demais citocinas pró-inflamatórias, como IL6, no soro e plasma (HOPE et al, 2011; BRIETZKE et al, 2009; KAUER-SANT'ANNA et al, 2008; ORTIZ-DOMINGUES et al, 2007; O'BRIEN et al, 2006) e aumento de IL1β no líquor (SÖDERLUND et al, 2011).

O estudo de células do sistema imunológico em pacientes com o diagnóstico de TB ainda é incipiente. Estudos apontam que pacientes com o diagnóstico de TB não apresentam alterações na contagem total de monócitos ou em sua expressão (DREXHAGE et al, 2011; TORRES et al, 2009), apesar de estudos *in vitro*, demonstrarem que monócitos de pacientes com o diagnóstico de TB apresentam exacerbação de sua atividade fagocítica de produção de IL6 (KNIJFF et al, 2007; MCADAMS & LEONARD, 1993).

Os linfócitos T ou células T são os principais efetores da chamada imunidade celular.Existem duasclasses principais de células T - as células T citotóxicas e as células T auxiliaresou *helper* (Th). Quando ativada por uma célula apresentadora de antígeno, umacélula T auxiliar pode diferenciar-se em três tipos distintos de células Tauxiliares efetoras, denominadas Th1, Th2 e Th17. As células Th1 auxiliam principalmente a ativação de macrófagos e células T citotóxicas; as células Th2 auxiliam na ativação das células B; enquanto as células Th17 que são responsáveis pela indução da inflamação tecidual e a proteção contra patógenos extracelulares. A célula T auxiliar efetora Th1 secreta o interferon gama (IFN-g) e o fator de necrosetumoral alfa (TNF-α) e ativa os macrófagos para fagocitarem antígenos. A célula T auxiliar efetora Th2 irá secretar IL-4, IL-5, IL-10, IL-13 defendendo oorganismo principalmente contra

patógenos extracelulares. A célula T auxiliar Th17 secreta principalmente citocinas pró-inflamatórias (IL-6, TNF-αe IL-1β) e quimiocinas (CXCL1, CXCL8, CCL2). Outro tipo de célula T que vem sendo recentemente estudado na resposta imunológica são as células T reguladoras (Tregs) que têm o propósito de controlar as respostas dos processos imunes. Ainda são escassos os estudos avaliando células T em pacientes com o diagnóstico de TB, apesar das evidências na literatura sugerirem alterações no perfil Th1/Th2 de tais pacientes (DREXHAGE et al, 2011; BRIETZKE et al, 2009; KIM et al, 2004).

2.6 MECANISMOS INTRACELULARES

Atualmente as pesquisas têm se voltado para o estudo de mecanismos complexos intracelulares associados à fisiopatologia do TB, particularmente o estudo de segundos mensageiros, mecanismos de regulação da expressão gênica, assim como da atuação intracelular de fatores neurotróficos e moléculas do sistema imunológico. Sugere-se que os efeitos agudos e crônicos dos estabilizadores de humor desencadeiam uma cascata de eventos intracelulares capazes de alterar a síntese protéicacom consequente reparação da plasticidade sináptica e restauração da transmissão nervosa (ZARATE et al, 2006; FREY et al, 2004; MANJI et al, 2003). Entretanto, a despeito de diversos alvos e mecanismos destes fármacos terem sido identificados, os efeitos exatos responsáveis pelas respostas terapêuticas ainda precisam ser elucidados. Uma possível via associada ao TB seria a via do fator nuclear kappa B (NF-κB), via ativada por moléculas pró-inflamatórias, em especial o TNF-α, e por fatores neurotróficos.

O NF-κB é um fator de transcrição considerado um mediador intracelular crítico da resposta inflamatória. NF-κBconstitui uma família de fatores de transcrição que contém as proteínas p65/*Rel*A, c-*Rel*, *Rel* B, p50/NF-κB1 e p52/NF-κB2 em várias combinações para formar o dímero transcricionalmente ativo, induzindo a ativação de vários genes envolvidos nas respostas inflamatória e imune. Em condições de repouso, NF-κB fica sequestrado no citoplasma ligado não covalentemente a proteínas inibitórias conhecidas como IκBs. Após a estimulação com agonistas apropriados, IκB é fosforilado e NF-κB é então liberado, translocando-se para o núcleo iniciando, assim, a expressão de diversos genes. Dentre estes, incluem-se os da molécula de adesão

intracelular-1 (ICAM-1), oxido nítrico sintase induzida (iNOS), cicloxigenase-2 (COX-2), citocinas: IL-1β, TNF-α e IL-6, e quimiocinas (CXCL8) (SOUSA et al, 2010; SOUSA et al, 2009; YE, 2001). As funções anti-apoptóticas do NF-κB podem ser explicadas em parte pela expressão de proteínas anti-apoptóticas. Entre as proteínas destaca-se a proteína inibidora de apoptose intracelular cAIP (FIGIEL, 2008).

Estudos avaliando a via do NF-κB em pacientes com o diagnóstico de TB ainda são escassos. Dois estudos*post mortem*mostraram uma maior expressão de mRNA e de proteínas da via do NF-κB (p50 e p65) no córtex frontal de pacientes com o diagnóstico de TB (RAO et al, 2010; SUN et al, 2001). Os autores sugeriram que a maior expressão de tais fatores poderia resultar em morte celular e contribuir para o déficit cognitivo observado em tais pacientes.

Outras vias celulares que são reguladas pela atividade inflamatória e pelos fatores neurotróficos e estão possivelmente ativadas no TB são: (1) as vias das proteínas quinases ativadas por mitógenos (MAPKs), que são proteínas sinalizadoras envolvidas em diferenciação celular, resposta a estresse, apoptose e inflamação (SOUSAet al, 2005) e um estudo prévio mostrou aumento da expressão de mRNAs para várias proteínas da via MAPK em monócitos de pacientes com o diagnóstico de TB (PADMOS et al, 2008); (2) a via da PI3K/Akt que é uma via envolvida na regulação de vários eventos celulares importantes como crescimento, proliferação e sobrevivência celular. Essa via medeia as ações anti-apoptóticas e pró-inflamatórias desencadeado por várias citocinas como, por exemplo, TNF-α, através da modulação de proteínas e fatores de transcrição que produzem sinais anti-apoptóticos (SOUSA et al, 2010; SOUSA et al, 2009; SONG et al, 2005). A via da PI3K/Akt ainda não foi estudada em pacientes com o diagnóstico de TB.

3. OBJETIVOS

3.1 Objetivo principal

Investigar a expressão de fatores neurotróficos e marcadores imunológicos em pacientes com o diagnóstico de transtorno bipolartipo I.

3.2 Objetivos secundários

Caracterizar clinicamente a população de pacientes com o diagnóstico de transtorno bipolar tipo I inclusos no presente trabalho.

Avaliar a possibilidade de fatores neurotróficos e marcadores imunológicos em pacientes com o diagnóstico de transtorno bipolar estarem especificamente relacionados com estado de humor ou traço.

Analisar a possível associação entre a presença de comorbidades, como sobrepeso e déficit cognitivo e fatores neurotróficos e marcadores imunológicos em pacientes com o diagnóstico de transtorno bipolar em comparação a controles.

Analisar a frequência de diferentes populações leucocitárias em células mononucleares do sangue periférico (CMSP) de pacientes com o diagnóstico de transtorno bipolar em comparação a controles.

Verificar a ativação das vias de NF-κB (p65 e IκB-α), MAPKs (ERK1/2, p38 e JNK) e PI3K/Akt, através de ensaios de *Western blot* e por FACS com extratos proteicos obtidos de CMSP de pacientes com o diagnóstico de transtorno bipolar em comparação a controles.

4. MÉTODOS

4.1. SUJEITOS DA PESQUISA

O presente estudo avaliou pacientes provenientes do serviço de psiquiatria do Instituto de Previdência dos Servidores do Estado de Minas Gerais (IPSEMG). Foram incluídos pacientes em tratamento na unidade de internação hospitalar, hospital dia, assim como ambulatório especializado em transtornos do humor. Os pacientes foram avaliados de forma consecutiva no período de abril de 2008 a setembro de 2010. O pesquisador responsável pelas avaliações psiquiátricas compareceu semanalmente aos serviços para a coleta de dados.

Os pacientes que preenchessem os critérios de diagnóstico de TB, e não se enquadrasse nos critérios de exclusão foram convidados a participarem do estudo. Os participantes foram entrevistados em sala disponível para consultas individuais. Cada avaliação durou entre uma hora e meia e duas horas e meia, dependendo do estado clínico do paciente. Foram realizadas avaliações conforme o número de pacientes disponíveis e o tempo de entrevista de cada participante. A avaliação psiquiátrica de todos os pacientes foi realizada pelo mesmo investigador. Após as avaliações psiquiátricas e clínica, os pacientes foram submetidos à punção venosa, resguardando-se todos os critérios de assepsia, procedimento realizado pela investigadora. O material coletado era levado ao Laboratório de Imunofarmacologia do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais sob a responsabilidade do orientador da pesquisa para o processamento objetivando a obtenção de plasma e congelamento a –80º C até o momento da análise.

Na anamnese semi-padronizada foram coletados dados sobre as características sócio-demográficas do participante (idade, sexo, escolaridade, estado civil, número de filhos, profissão), diagnóstico, tempo de diagnóstico, número e características de internações hospitalares, presença de tentativas de suicídio e histórico familiar. Sobre o tratamento, foram coletados dados sobre os medicamentos em uso e a dose. Foi obtido nos prontuários médicos o diagnóstico de comorbidades clínicas (tais como hipertensão

arterial sistêmica, diabetes mellitus, dislipidemia, problemas tireoidianos ou doenças autoimunes).

Os controles da pesquisa foram sujeitos entrevistados na comunidade, examinados por um único psiquiatra que não apresentassem quaisquer doenças psiquiátricas passada ou atual. Os controles foram submetidos à entrevista psiquiátrica estruturada (Mini-International Neuropsychiatric Interview, MINI-Plus) para a avaliação de doença psiquiátrica passada ou atual. Foram excluídos controles que apresentassem doenças psiquiátricas (exceto tabagismo) ou história familiar de esquizofrenia, TB, internações psiquiátricas ou tentativas de suicídio em parentes de primeiro e segundo graus.

Dados antropométricos foram obtidos no mesmo dia da avaliação clínica e coleta sanguínea. Todos os sujeitos foram pesados na mesma balança padronizada e regulada. O índice de massa corporal (IMC) foi calculado dividindo-se o peso em quilogramas pela altura elevada à segunda potência (IMC = kg/m^2).

Todos os sujeitos envolvidos na pesquisa assinaram o termo de consentimento livre esclarecido para a participação no presente trabalho. Os procedimentos realizados foram autorizados pelos Comitês de Ética e Pesquisa das Instituições do IPSEMG e da Universidade Federal de Minas Gerais.

4.2. CRITÉRIOS DE INCLUSÃO

- Idade maior que 18 anos;
- Assinar o Termo de Livre Esclarecimento para entrada no estudo;
- O critério de inclusão para os pacientes foi o diagnóstico de TB tipo I.

4.3. CRITÉRIOS DE EXCLUSÃO

- Uso atual (últimas 4 semanas) de antibióticos, anti-inflamatórios ou corticóide;
- Presença de doença autoimune em atividade nas últimas 8 semanas;
- Presença de demência definida segundo critérios clínicos do DSM-IV;
- Controles que apresentassem doenças psiquiátricas em entrevista estruturada (MINI-Plus) foram excluídos da pesquisa.

4.4. INSTRUMENTOS DE AVALIAÇÃO PSIQUIÁTRICA

Os sujeitos da pesquisa foram examinados por psiquiatra treinado na aplicação de entrevistas e escalas clínicas. Foram empregados os seguintes instrumentos:

Entrevista clínica estruturada (MINI-Plus) constitui uma entrevista diagnóstica breve (cerca de 30 minutos), utilizada para identificar ao longo da vida, na prática clínica e em pesquisas em psiquiatria, transtornos psiquiátricos do eixo I do DSM-IV-TR e da CID-10 (SHEEHAN et al, 1998), (AMORIM, 2000). O MINI-Plus explora sistematicamente 23 categorias diagnósticas do DSM-IV (AMORIM, 2000).

O Mini-Exame do Estado Mental (MEEM) foi utilizado a fim de se excluir comprometimento cognitivo global (BRUCKI et al, 2003; BERTOLUCCI et al, 1994). Bateria de Avaliação Frontal (BAF) foi utilizada para avaliar a presença de déficits frontais (BEATO et al, 2007; DUBOIS et al, 2000).

Aescala de Depressão de Hamilton (HAMD) (HAMILTON, 1960) foi utilizada para quantificar sintomas depressivos. Escala de mania de Young (YOUNG) foi utilizada para quantificar sintomas de mania (YOUNG et al, 1978).

4.5. AVALIAÇÃO DE FATORES NEUROTRÓFICOS E MARCADORES IMUNOLÓGICOS

No mesmo dia da avaliação clínica dos sujeitos, os mesmos foram submetidos à coleta de sangue periférico para a avaliação plasmática dos fatores neurotróficos e marcadores imunológicos.

4.5.1 Coleta do sangue periférico

De cada sujeito da pesquisa foram coletadas três amostras de 10 mL de sangue, utilizando-se heparina como anticoagulante. O plasma foi separado e estocado a –80 °C até o seu uso para análise de fatores neurotróficos e marcadores imunológicos através da técnica de imunoensaio ELISA (*Enzyme-Linked Immunoabsorbent Assay*).

4.5.2 Análise de fatores neurotróficos, marcadores inflamatórios e imunológicos

As concentrações de fatores neurotróficos e marcadores imunológicos dos sujeitos foram mensuradas pela técnica ELISA sanduíche.

A cada poço da placa de ELISA foram adicionados 100 μl de solução contendo anticorpo monoclonal contra osfatores neurotróficos e marcadores imunológicos que se pretendia mensurar diluído em tampão fosfato (PBS). As placas foram incubadas por pelo menos 12 horas à 4º C. Os anticorpos não aderidos nas placas foram descartados por inversão e lavagem em PBS–Tween. Em seguida, as placas foram bloqueadas com 200 μl/poço de uma solução contendo PBS–albumina bovina 1% durante 2 horas à temperatura ambiente. Após nova lavagem das placas, em cada poço, foi adicionado 100 μl da amostra ou padrão.

Para a avaliação de NGF, GDNF, TNF, leptina e resistina, as amostras de plasma não foram diluídas. Para a avaliação de BDNF, receptores solúveis de TNF (sTNFR1 e sTNFR2), adiponectinae quimiocinas, as amostras foram diluídas em uma proporção 1:10 em PBS-albumina bovina 0,1%. Para análise das quimiocinas, as amostras de plasma foram descongeladas e o excesso de proteínas foi removido por precipitação de ácido/sal, como é realizado rotineiramente no laboratório de imunofarmacologia do Instituto de Ciências Biológicas. Um volume igual de plasma e de ácido trifluoroacético 1,2%, 1,35 M NaCl foram misturados e deixados à temperatura ambiente por 10 min. As amostras foram então centrifugadas por 5 min a 3.000 g e os sobrenadantes foram ajustados para teor de sal (cloreto de sódio 0,14 M e 0,01 M de fosfato de sódio) e pH (7,4), para a determinação dos níveis de quimiocinas.

Após lavagem, anticorpos conjugados com biotina e diluídos em PBS-albumina bovina 0,1% foram adicionados, sendo as placas incubadas por duas horas à temperatura ambiente. Em seguida, após lavagem, estreptoavidina conjugada com peroxidase foi acrescentada nas placas, incubadas por 30 minutos à temperatura ambiente. Finalmente, após nova lavagem, o cromógeno 0-fenileno-diamina foi aplicado às placas, incubadas na ausência de luz. A reação foi interrompida com H_2SO_4 1M. A leitura da intensidade de marcação foi realizada em leitor de ELISA utilizando-se o comprimento de onda de 490 nM (SOFTmaxPro – versão 2.2.1).

4.5.3 Separação de células mononucleares do sangue periférico (CMSP)

Foram utilizados para este procedimento dois tubos de 10 mL de sangue fresco de cada sujeito da pesquisa utilizando-se heparina como anti-coagulante. O sangue fresco foi diluído em uma proporção 1:1 em PBS a temperatura ambiente. Cuidadosamente o sangue diluído foi despejado em uma camada de Ficoll a temperatura ambiente (Ficoll-PaquePlus, GE 400Healthcare Bio-Sciences AB, Uppsala, Sweden)na proporção de 2:1 (*isto é*, 20 ml de sangue diluído em 10 ml de Ficoll) em tubos de polipropileno de 50 mL. A solução foi centrifugada a temperatura ambiente a 405x g por 40 min. A camada contendo CMSP foi coletada e lavada duas vezes em PBS a 4º C. A viabilidade celular foi determinada pela coloração com azul de trypan e foram ressuspendidas a concentração de $1x10^7$ células em um meio de cultura 1.

Composição do meio de cultura 1: 90 % de RPMI-1640(Roswell Park Memorial Institute-1640), L-glutamina (Cultilab, Campinas, Brazil), 1% de penicilina (Ariston, Sao Paulo, Brazil) e 1% de gentamicina (Nova Farma, Anapolis, Brazil), 25 mM de buffer HEPES (4-(2-hydroxy-ethyl)-1-piperazine-ethane-sulfonic acid) (Sigma, St. Louis, MO, USA) e 10% de soro humano (Sigma, St. Louis, MO, USA).

As CMSP de um dos tubos de 10 mL foram congeladas no freezer -80º C para posterior análise por meio de Western Blot.

4.5.4 Congelamento de células mononucleares do sangue periférico (CMSP)

As CMSPs do outro tubo de 10 mL de sangue foram congeladas, seguindo o protocolo a seguir para posterior análise por meio de citometria de fluxo (FACS).Uma concetração de $1x10^6$ células foi determinada pela contagem em câmara de Neubauer após pela coloração com azul de trypan e separadas em um tubo de criopreservação (Nunc Brand Products, Denmark). Delicadamente, o meio de **cultura 2**gelado na proporção de 1:1 nas células resuspendidas foi adicionado, gota a gota. Os tubos de criospreservação foram adicionados ao container de congelamento (Mr. Frosty, Nalgene) e colocados no freezer -80º C por um período de 24-48hs. Após este período as célulasforam retiradas do container e permaneceram estocadas no freezer -80º C.

Composição do meio de cultura 2: 80% de meio de cultura 1 e 20% de DMSO (LabSynth, Diadema, SP).

4.5.5 Descongelamento de células mononucleares do sangue periférico (CMSP)

O tubo de criopreservação foi retirado do freezer -80º C e imerso em água a temperatura ambiente até o descongelamento. O conteúdo foi vertido em um tubo do tipo Falcon contendo 40 mL de meio RPMI-1640 (Roswell Park Memorial Institute-1640), L-glutamina (Cultilab, Campinas, Brazil) contendo 10% de soro humano (Sigma, St. Louis, MO, USA). A amostra foi centrifugada a 1200 rpm por 10 min, a 4ºC. O sobrenadante foi desprezado e o pellet foi resuspenso em 500 µL de meio RPMI-1640 (Roswell Park Memorial Institute-1640), L-glutamina (Cultilab, Campinas, Brazil) contendo 10% de soro humano (Sigma, St. Louis, MO, USA). A concetração celular foi determinada pela contagem em câmara de Neubauer após coloração com azul de trypan.

4.5.6 Análise de populações leucocitárias e das formas fosforiladas de proteínas das vias de NF-κB e MAPK por citometria de fluxo

As células mononucleares humanas (CMSP) foram imunomarcadas utilizando-se anticorpos associado à fluorocromo-contra CD3 (PerCP, BD Biosciences, San Jose, CA), CD4 (PE e/ou PE-Cy5, BD Biosciences, San Jose, CA), CD8 (PE-Cy7, BD Biosciences, San Jose, CA), CD14 (FITC, Invitrogen), CD19 (PerCP, BD Biosciences, San Jose, CA), CD25 (FITC, BD Biosciences, San Jose, CA), FoxP3 (PE, BD Biosciences, San Jose, CA), IL10 (APC, BD Biosciences, San Jose, CA) e controle de isotipo. As CMSP foram ressuspensos e plaqueadas em uma placa com 96 poços com o fundo em U na concentração de $1x10^4$células por poço. A cada poço foi adicionado o anticorpo de superfície na quantidade e concentração estipuladas na padronização. A placa foi incubada ao abrigo da luz a temperatura de 4º C por cerca de 30 minutos. Após o tempo de incubação foi adicionada a cada poço 150µL uma solução de PBS-BSA-Azida (50 mL de PBS (10X, pH 7,4), 450mL de água destilada, 0,5mL de Azida 1M (Sigma Aldrich) e 2,5g de BSA (Intalab, Diadema, SP). A placa foi centrifugada por 7 minutos, 1200 rpm a 4ºC e, após a centrifugação, o sobrenadante foi desprezado, por duas vezes. As células resuspensas 200µL de formaldeído a 2% (LabSynth, Diadema, SP). Para a avaliação de marcação intracelular de FoxP3, após marcação para os anticorpos de superfície, foi realizada permeabilização da membrana celular com um tampão de permeabilização contendo saponina.

A presença das formas fosforiladas das proteínas MAPKs ERK1/2 e p38 e RelA/p65 (NF-kB) também foi analisada por citometria de fluxo utilizando-se o kit da BD Phosflow T cell activation kit (BD Biosciences, San Jose, CA) com pequenas modificações. Os níveis intracelulares de proteínas fosforiladas foram avaliados utilizando-se anticorpo contra a subunidade de NF-kB-p65/RelA, marcado com PE e anticorpos anti ERK1/2 (pT202/pY204) e p38 MAPK (pT180/pY182) ambos Alexa Fluor® 647. As CMSP foram fixadas por 7 minutos com tampão *Lyse/Fix* 1X pré-aquecido à 37ºC, lavadas com tampão PBS 1X e permeabilizadas pelo *Permeabilization buffer III* por 30 minutos. Posteriormente, as células foram lavadas extensivamente (três vezes) com *Stain Buffer*, resuspenssas em 100uL de *Stain Buffer* sendo finalmente imunocoradas com anti-CD14-FITC, anti-CD3-PerCP e com cada um dos anticorpos monoclonais anti-fosfoproteínas, descritos acima, durante 60 minutos à temperatura ambiente.

As marcações foram examinadas usando o citômetro de fluxo FACSCanto II da BD (BD Biosciences, San Jose, CA). Um total de 30.000 eventos foi adquirido e os parâmetros foram analisados na população de monócitos e linfócitos através de *gate* na região ocupada classicamente por essas células no plot de tamanho *versus* granulosidade. As análises foram realizadas utilizando o programa BD FACS DIVA.

4.5.7 Análise das vias sinalizadoras de NF-κB e MAPK por Western Blot de células mononucleares do sangue periférico (CMSP)

Após a lavagem das células com PBS 1X, estas foram lisadas pela adição de 500 µl de solução de lise (0,5% p/v de NP-40, 100 mM de Tris/HCl pH 8,0, 10% de glicerol, 0,2 mM de EDTA, 1mM de $NaVO_3$, 1mM de DTT, 1mM de PMSF, 200mM de NaCl, 25 mM de NaF, leupeptina e aprotinina), e deixadas em banho de gelo por 15 minutos. Posteriormente, o lisado foi centrifugado a 12.000 rpm em microcentrífuga por 15 minutos a 4°C, sendo o sobrenadante aliquotado e guardado à -70°C até o momento de uso. A concentração das proteínas totais foi determinada pelo método de Bradford utilizando o "Kit Bio-Rad Assay" (Bio-Rad Laboratories USA). Os extratos proteicos totais (40µg) foram fracionados em gel de 10% de poliacrilamida/SDS e transferidos para membrana de nitrocelulose (Hybond™ ECL™, GE Healthcare). Posteriormente, as membranas foram bloqueadas com PBS-Tween 0,1% contendo 5% de leite em pó

desnatado, lavadas com PBS-Tween, e incubadas com o anticorpo de interesse na diluição de 1:1000 a 4°C por uma noite. Os anticorpos utilizados no presente projeto foram anticorpos anti as formas fosforiladas das proteínas ERK1/2 e p38 Cell Signaling Technology (Beverly, MA, USA). Após nova lavagem com PBS/Tween e incubação durante 1 hora à temperatura ambiente, com o anticorpo secundário respectivo, ligado à peroxidase (anti-coelho- Cell Signaling Technology, diluição 1:2000), as membranas foram lavadas novamente e incubadas em solução reveladora "ECL-Plus" (GE Healthcare), expostas contra filme de raio X (Hyperfilm ECL, Amersham) e reveladas utilizando-se revelador e fixador (Kodak), de acordo com indicações do fabricante. Para normalização da quantidade de proteína aplicada às diferentes canaletas, as membranas foram posteriormente incubadas com anticorpo anti β-actina (Sigma-Aldrich) seguidas do anticorpo secundário e reveladas da mesma forma descrita acima. Os níveis de fosforilação das MAPKs ERK1/2 e p38 foram quantificados através da análise densitométrica utilizando o programa Image J (Image Processing and Analysis in Java). Os resultados da quantificação destas proteínas foram normalizados pelos valores da proteína estrutural β-actina, em cada amostra. Alterações nos níveis de fosforilação foram expressos como índice de proteína fosforilada/β-actina, em unidades arbitrárias.

4.6. ANÁLISE ESTATÍSTICA

Na análise descritiva de variáveis categóricas as proporções foram calculadas e apresentadas. Foram verificadas se as variáveis contínuas possuíam distribuição normal através do teste Shapiro-Wilk. Essas variáveis contínuas são apresentadas como médias, medianas, desvios-padrão e faixa de variação. Para a comparação de variáveis categóricas entre os dois grupos de sujeitos (pacientes e controles), realizou-se o teste de $\chi 2$ de Pearson. Na comparação de variáveis contínuas entre dois grupos, empregaram-se o teste t de Student e o teste U de Mann-Whitney em variáveis de distribuição paramétrica e não-paramétrica, respectivamente. Quando a variável contínua independente tinha 3 ou mais categorias, utilizaram-se Kruskal- Wallis com correção de Dunn em variáveis de distribuição não-paramétrica e One-way ANOVA para variáveis de distribuição paramétrica.Para a avaliação dopapel de quimiocinas nos pacientes com o diagnóstico de TB utilizou-se análisede regressão logística binária entre as variáveis

preditoras e o diagnóstico de TB e de fase (mania ou eutimia). Foram consideradas variáveis preditoras as que tiveram p-valor inferior a 0,10. Foi estimada a odds ratio com intervalo de 95%. Para avaliar o ajuste domodelo foi utilizado o teste de Hosmer e Lemeshow.

As análises foram realizadas utilizando-se o programa estatístico SPSS versão 15.0 e versão 17.0, assim como Graphic Prism 4.0 para Windows. Um valor de p bilateral menor que 0,05 foi adotado como nível de significância estatística para todos os testes. Os gráficos foram construídos utilizando o programa Graphic Prism 4.0 para Windows.

5. RESULTADOS

Artigo 3: Comorbidades clínicas e psiquiátricas em pacientes com transtorno bipolar do tipo I.

Artigo 4: Increased BNDF levels in BD patients in late stage.

Artigo 5: Impaired nerve growth factor homeostasis in patients with bipolar disorder.

Artigo 6: Circulating levels of GDNF in bipolar disorder.

Artigo 7: Increased levels of adipokines in bipolar disorder.

Artigo 8: Chemokines in bipolar disorder: trait or state?

Artigo 9: Executive dysfunction in euthymic bipolar disorder patients and association with plasma biomarkers.

Resultados Adicionais:Avaliação de frequência de leucócitos, assim como de vias sinalizadoras intracelulares desencadeadas por citocinas pró-inflamatórias e fatores neurotróficos em pacientes com o diagnóstico de TB em comparação a controles.

Artigo 3: Comorbidades clínicas e psiquiátricas em pacientes com transtorno bipolar do tipo I

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ARTIGO ORIGINAL

Comorbidades clínicas e psiquiátricas em pacientes com transtorno bipolar do tipo I

Psychiatric and medical comorbidities in type 1 bipolar disorder patients

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RESUMO

Contexto: O transtorno bipolar tipo I está comumente associado a comorbidades clínicas e psiquiátricas, mas ainda há poucos dados disponíveis sobre pacientes brasileiros. **Objetivos:** O objetivo do presente estudo foi avaliar a prevalência de comorbidades clínicas e psiquiátricas em uma amostra brasileira de pacientes bipolares tipo I. O objetivo secundário foi investigar as associações de características clínico-demográficas e comorbidades com tentativas de suicídio. **Métodos:** Foram incluídos neste estudo 94 pacientes bipolares tipo I. O diagnóstico psiquiátrico foi determinado utilizando-se a avaliação *Mini International Neuropsychiatric Interview* (MINI-Plus). O diagnóstico de comorbidades clínicas foi baseado na história clínica e no acompanhamento de clínicos gerais. **Resultados:** As comorbidades mais prevalentes nos pacientes bipolares foram: transtorno de ansiedade generalizada (19,20%), dependência de substâncias (43,60%), hipertensão arterial (29,80%), *diabetes mellitus* (17,00%), dislipidemia (22,30%) e hipotireoidismo (19,10%). Não foram encontradas diferenças estatísticas em relação às características demográficas ou à prevalência de comorbidades nos grupos com e sem tentativa de suicídio. **Conclusão:** Pacientes bipolares atendidos em serviço psiquiátrico apresentam elevada prevalência de comorbidades psiquiátricas e clínicas. Nessa população, tentativas de suicídio não se associam com a presença de comorbidades ou características demográficas.

Palavras-chave
Transtorno bipolar, comorbidades, mania, suicídio.

ABSTRACT

Background: *Bipolar disorder type I is frequently associated with psychiatric and medical comorbidities, but data regarding Brazilian patients are lacking.* **Objectives:** *The aim of the present study was to evaluate the prevalence of psychiatric and medical comorbidities in a Brazilian sample of bipolar disorder patients type I. A secondary aim was to investigate the association of demographic characteristics and comorbidities with suicide attempts.* **Methods:** *Ninety four bipolar disorder type I patients were included in this study. Psychiatric diagnoses were performed following the Mini International Neuropsychiatric Interview (MINI-Plus) evaluation. The diagnosis of medical comorbidities was based on clinical history and general practice consultation.* **Results:** *The com-*

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monest comorbidities in bipolar disorder patients were generalized anxiety disorder (19.20%), substance dependence (43.60%), arterial hypertension (29.80%), diabetes mellitus (17.00%), dyslipidemia (22.30%) and hypothyroidism (19.10%). There were no differences in demographic characteristics or the prevalence of comorbidities when comparing patients with and without previous suicide attempt. **Conclusion:** *Bipolar disorder patients from a psychiatric unit present higher prevalence of psychiatric and clinical comorbidities. Previous suicide attempts were not associated with comorbidities or demographic characteristics.*

Keywords
Bipolar disorder, comorbidities, mania, suicide.

INTRODUÇÃO

O transtorno bipolar do tipo I se caracteriza por episódios recorrentes de mania e depressão e ocorre em 0,6% da população mundial[1]. Apesar dos avanços relacionados à terapêutica, os pacientes com transtorno bipolar, mesmo em períodos de eutimia, apresentam sintomas de humor residuais, assim como déficits cognitivos[2]. Estima-se que 15% a 20% das mortes em pacientes com transtorno bipolar possam ser atribuídas a suicídio[3]. Fatores sociodemográficos parecem ter pequena influência nas taxas de tentativa de suicídio e suicídio completo em pacientes com transtorno bipolar, entretanto a presença de comorbidades psiquiátricas é uma das principais variáveis associadas[4].

Comorbidade pode ser conceituada como a ocorrência de duas ou mais entidades nosológicas no mesmo paciente. A coocorrência de diagnósticos em um mesmo paciente pode influenciar o curso, a resposta ao tratamento e/ou o prognóstico da enfermidade. Pacientes bipolares tipo I apresentam, em média, 3,1 comorbidades psiquiátricas ao longo da vida[1]. Há um debate na literatura sobre se esse dado seria um artefato dos sistemas diagnósticos atuais, que empregam uma abordagem categórica, ou se refletiria a existência de entidades nosológicas distintas com um mesmo substrato neurobiológico[5]. Em relação à presença de comorbidades clínicas, descreve-se elevada prevalência de distúrbios metabólicos, cardiovasculares e endócrinos nesses pacientes[6]. Não se sabe se essas comorbidades clínicas se referem a condições ligadas ao transtorno bipolar *per se*, se seriam consequência do tratamento farmacológico ou uma combinação de ambos os fatores. Apesar de a presença de comorbidades psiquiátricas e clínicas ser importante na avaliação, no prognóstico e no curso do transtorno bipolar, a maioria dos estudos farmacoterápicos ignora esse fato.

Na população brasileira, ainda há poucos dados sobre a prevalência de comorbidades e sua influência no transtorno bipolar. O objetivo deste estudo é, portanto, identificar a prevalência de comorbidade sem uma amostra de pacientes com transtorno bipolar do tipo I provenientes de um serviço psiquiátrico. O objetivo secundário foi avaliar quais as características do transtorno bipolar e comorbidades (psiquiátricas e clínicas) estariam relacionadas com a presença de tentativas de suicídio.

MÉTODOS

Sujeitos

Foram incluídos no presente estudo 94 pacientes com o diagnóstico de transtorno bipolar tipo I. Os pacientes foram recrutados em serviço psiquiátrico de atendimento especializado em transtorno bipolar no Instituto de Previdência dos Servidores do Estado de Minas Gerais (IPSEMG), Belo Horizonte. Esse serviço é responsável pelo atendimento de pacientes provenientes da rede ambulatorial e de internação do Hospital Governador Israel Pinheiro, que pertence ao IPSEMG. O diagnóstico de transtornos psiquiátricos baseou-se na entrevista clínica estruturada *Mini International Neuropsychiatric Interview* (MINI-Plus)[7]. Devido ao fato de os pacientes bipolares serem acompanhados regularmente por clínicos gerais no mesmo serviço, foi extraído do prontuário dos pacientes o diagnóstico das seguintes comorbidades clínicas: 1) hipertensão arterial sistêmica; 2) *diabetes mellitus*; 3) hipotireoidismo; 4) dislipidemia.

As medidas antropométricas foram realizadas no mesmo dia da entrevista clínica. Tentativa de suicídio foi definida como qualquer ato de injúria, deliberadamente autoinfligido, independentemente do risco da letalidade envolvido, em que o paciente tenha a intenção consciente de terminar a própria vida[8].

Todos os procedimentos descritos no estudo receberam autorização do comitê de ética local. Todos os participantes tinham idade superior a 18 anos, e o consentimento livre e esclarecido foi obtido previamente à entrada no estudo. Não houve critério de exclusão para a entrada neste estudo.

Análise estatística

A análise descritiva foi usada para apresentar os dados clínicos e sociodemográficos da população. As variáveis contínuas foram apresentadas como médias e desvios-padrão. Os pacientes foram divididos em dois grupos segundo a presença de tentativas prévias de suicídio. As variáveis categóricas dos dados sociodemográficos e prevalências de comorbidades foram comparadas entre o grupo de pacientes com tentativas prévias de suicídio e o grupo sem tentativas prévias, empregando-se o teste do qui-quadrado de Pearson ou o teste exato de Fisher, quando apropriado.

O teste de Kolmogorov-Smirnov foi aplicado para verificar a distribuição de variáveis contínuas entre pacientes bipolares que apresentaram ou não tentativas de suicídio. Devido ao fato de os dados dos pacientes referentes a idade, escolaridade, índice de massa corporal, primeiro episódio de humor, primeiro episódio depressivo, primeiro episódio maníaco e número de internações hospitalares possuírem distribuição normal, o teste *t* de Student foi empregado para a comparação dos dois grupos. As análises foram realizadas utilizando-se o programa estatístico SPSS versão 17.0. Um valor de p bilateral menor que 0,05 foi adotado como nível de significância estatística para todos os testes.

RESULTADOS

Os dados sociodemográficos e clínicos dos participantes são apresentados na tabela 1. A prevalência do gênero feminino foi de 69,10%, e 32,30% dos pacientes eram solteiros. Apesar de os pacientes apresentarem idade média de 50,25 anos (DP = 12,02), apenas 68,10% se encontravam inativos laboralmente. A maioria dos pacientes apresentou longo tempo de evolução da doença (idade média ± DP em anos de 24,01 ± 12,77). Na presente amostra, 33,00% dos pacientes relataram tentativas de suicídio. O número médio de internações psiquiátricas foi de 4,18 (DP = 4,04) e o de tentativas de suicídio foi de 0,90 (DP = 1,58). Curiosamente, 24 pacientes não tinham apresentado, até o momento da entrevista, nenhum episódio depressivo.

Tabela 1. Dados sociodemográficos e clínicos da população de pacientes com transtorno bipolar tipo I

	Pacientes bipolares tipo I (n = 94)
Idade em anos (média ± DP)	50,25 ± 12,02
Escolaridade em anos (média ± DP)	10,13 ± 3,63
Gênero feminino	69,10%
Pacientes com histórico de tentativas de suicídio	33,00%
Número de tentativas (média ± DP)	0,90 ± 1,58
Status funcional	
Ativo	31,50%
Inativo	68,50%
Idade de início da doença em anos (média ± DP)	26,39 ±10,12
Idade do primeiro episódio depressivo (média ± DP)	26,02 ± 10,35
Idade do primeiro episódio maníaco (média ± DP)	29,74 ± 12,12
Duração da doença em anos (média ± DP)	24,01 ± 12,77
Número de internações (média ± DP)	4,18 ± 4,04
IMC (média ± DP)	29,22 ± 7,54
Medicamentos em uso atual (prevalência)	
Lítio	54,30%
Anticonvulsivantes	62,40%
Antipsicóticos	68,50%
Antidepressivos	9,50%

IMC: índice de massa corporal; DP: desvio-padrão.

A prevalência de comorbidades psiquiátricas e clínicas na amostra é apresentada na tabela 2. Cinquenta e nove (63,83%) pacientes apresentaram ao menos uma comorbidade psiquiátrica. Os transtornos mais prevalentes foram: transtorno de ansiedade generalizada (27,20%), dependência de álcool (35,50%) e tabaco (43,60%). Em relação a comorbidades clínicas, 52,13% dos pacientes apresentaram ao menos uma comorbidade.

Os pacientes bipolares foram subdivididos em dois grupos: pacientes que apresentaram tentativas de suicídio e pacientes que não apresentaram tentativas de suicídio. Não foi encontrada nenhuma associação significativa entre as variáveis investigadas e a presença ou ausência de tentativas de suicídio (ver tabela 3).

Tabela 2. Prevalência de comorbidades psiquiátricas e clínicas na população de pacientes com transtorno bipolar tipo I

	Pacientes bipolares tipo I (n = 94)
Qualquer comorbidade psiquiátrica	63,83%
TAG	27,20%
TOC	4,30%
Transtorno de pânico atual	5,30%
Dependência de substâncias ao longo da vida	
Álcool	35,50%
Tabaco	43,60%
Outras substâncias	5,30%
Hipertensão arterial sistêmica	29,80%
Diabetes mellitus	17,00%
Hipotireoidismo	19,10%
Dislipidemia	22,30%

TAG: transtorno de ansiedade generalizada; TOC: transtorno obsessivo-compulsivo.

DISCUSSÃO

O presente estudo avaliou as comorbidades clínicas e psiquiátricas em uma amostra clínica de pacientes bipolares do tipo I. Transtorno de ansiedade generalizada e dependência de substâncias foram as comorbidades psiquiátricas mais comuns. Hipertensão arterial sistêmica e dislipidemia foram as comorbidades clínicas mais prevalentes. História de tentativas de suicídio não se associou com comorbidades clínicas ou psiquiátricas.

Os dados referentes à prevalência de comorbidades psiquiátricas em pacientes com transtorno bipolar estão em consonância com a literatura[1], inclusive com outros estudos brasileiros[9-13]. Aparentemente, a presença de comorbidades em pacientes bipolares é mais regra do que exceção. Uma das hipóteses que poderia justificar tal dado pode ser um artefato dos sistemas diagnósticos contemporâneos que preconizam uma abordagem categórica. Essa abordagem, apesar de permitir maior confiabilidade dos diagnósticos psiquiátricos e melhorar a comunicação entre clínicos e

Tabela 3. Comparação entre dados sociodemográficos e comorbidades em pacientes bipolares tipo I com tentativa de suicídio e sem tentativa de suicídio

	Pacientes bipolares tipo I (n = 94)		Valor de p
	Sem tentativa de suicídio (n = 63)	Presença de tentativa de suicídio (n = 31)	
Idade em anos (média ± DP)	49,79 ± 12,59	51,19 ± 10,89	0,60 †
Escolaridade em anos (média ± DP)	10,16 ± 3,66	10,07 ± 3,64	0,91 †
Gênero feminino	69,80	67,70	0,84 **
Idade de início da doença em anos (média ± DP)	27,19 ± 10,40	24,79 ± 9,51	0,30 †
Primeiro episódio depressivo (média ± DP)	26,77 ± 10,67	24,74 ± 9,90	0,46 †
Primeiro episódio maníaco (média ± DP)	31,11 ± 12,94	26,68 ± 9,56	0,09 †
Duração da doença em anos (média ± DP)	22,54 ± 12,95	26,80 ± 12,12	0,14 †
Número de internações (média ± DP)	3,94 ± 4,45	4,64 ± 3,17	0,50 †
Episódio depressivo prévio	69,8	83,9	0,14 **
Presença de qualquer comorbidade psiquiátrica	60,32	70,97	0,31 **
TAG	24,60	32,3	0,23 **
TOC	1,6	9,7	0,10 *
Transtorno do pânico atual	1,6	3,2	0,54 *
Dependência de substâncias (atual)			
Álcool	4,8	6,5	1,00*
Tabaco	34,9	38,7	0,72 **
Outras substâncias	4,8	6,5	0,22*
Hipertensão arterial sistêmica	27,0	35,5	0,40 **
Diabetes mellitus	12,7	25,8	0,11 **
Hipotireoidismo	15,9	25,8	0,25 **
Dislipidemia	19,0	25,0	0,28 **

† Teste t; * teste exato de Fisher; ** teste do qui-quadrado de Pearson; DP = desvio-padrão; TAG: transtorno de ansiedade generalizada; TOC: transtorno obsessivo-compulsivo.

pesquisadores, apresenta algumas limitações[14]. Por exemplo, a definição individualizada de categorias diagnósticas como entidades nosológicas diferentes, ainda que baseada em consenso de especialistas, pode não ter validade discriminante que permita separar doenças distintas. Ademais, a ocorrência de dois transtornos em um mesmo paciente, em momentos diferentes ou concomitantes, pode sugerir mecanismo fisiopatológico subjacente em comum[6]. De nota, transtorno bipolar e transtornos de ansiedade apresentam aumento de atividade monoaminérgica[15], alterações de neuroplasticidade na amígdala e estruturas do sistema límbico[15], assim como genes em comum[16], o que sugere que possa haver um mesmo mecanismo fisiopatológico comum a dois transtornos.

Em relação à prevalência das comorbidades psiquiátricas descritas no presente trabalho, estudos epidemiológicos anteriores apontam a mesma prevalência de transtornos de ansiedade em pacientes bipolares[6]. Entretanto, há estudos discordantes, não havendo consenso na literatura[1,17]. A prevalência de qualquer transtorno de ansiedade nos pacientes bipolares é mais elevada que na população em geral (18,1%)[18]. Possíveis hipóteses associadas à discrepância dos dados são diferenças no desenho do estudo, por exemplo, estudos populacionais e estudos clínicos, assim como o uso de diferentes instrumentos para entrevista e diagnóstico.

Transtornos relacionados a substâncias apresentaram elevada prevalência na nossa população em comparação com estudos que usaram similares ferramentas de entrevista em pacientes bipolares[12] e mesmo quando comparados à população geral (3,8%)[18]. Os dados apresentados corroboram a prevalência de uso e/ou dependência de substâncias em pacientes bipolares na população brasileira[19]. Há inúmeras hipóteses que procuram explicar a elevada prevalência de dependência de substâncias associada ao transtorno bipolar. Em uma perspectiva baseada em crenças psicológicas, os pacientes com transtorno bipolar buscariam o uso de substâncias como uma forma de aliviar sintomas de humor considerados desagradáveis, como o taquipsiquismo e a irritabilidade[20]. Por outro lado, o uso nocivo de substâncias poderia deflagrar o primeiro episódio maníaco[21]. Ainda, o fato de o transtorno bipolar estar relacionado com maior impulsividade, envolvimento excessivo em atividades prazerosas e prejuízo de crítica durante o episódio maníaco poderia estimular a busca de substâncias psicoativas[22].

Em relação às comorbidades clínicas, a alta prevalência demonstrada no presente estudo está em conformidade com dados prévios na literatura em pacientes bipolares[23]. A sobreposição de comorbidades clínicas no paciente com transtorno bipolar tem provável etiologia multifatorial. Pesquisas têm demonstrado que a desregulação do humor e do apetite compartilha alguns substratos neurobiológicos[24]. Dados de neuroimagem, por exemplo, mostram que há sobreposição dos circuitos neurais ligados à regulação do humor e do comportamento alimentar, como as conexões entre a amígdala e o córtex pré-frontal[25,26]. Além disso, a hiperatividade persistente do eixo hipotálamo-hipófise-adrenal em pacientes bipolares pode estar relacionada a elevação da pressão arterial, aumento de resistência à insulina e dislipidemia[27,28]. É importante mencionar que o uso de medicamentos psicotrópicos, particularmente os antipsicóticos atípicos, também está ligado a síndrome metabólica, aumento do apetite e preferência por alimentos doces, assim como redução da atividade física[29]. Reconhece-se também que o uso do lítio está associado ao hipotireoidismo.

Em nosso estudo, não foram encontradas associações entre tentativas de suicídio e comorbidades clínicas ou psiquiátricas. Esse dado difere do relatado em outros trabalhos, que demonstraram que comorbidades psiquiátricas aumentam o risco de suicídio no transtorno bipolar[9,10,30]. É reconhecido que a inclusão de pacientes bipolares do tipo II nas amostras de estudo pode determinar o aumento da prevalência de tentativas de suicídio[11,31]. Ressalta-se ainda que, por se tratar de um estudo retrospectivo, pode existir viés de recordação.

Como limitações de nosso trabalho, destacam-se alguns pontos. O fato de se tratar de uma amostra obtida de serviço de atenção psiquiátrica especializada pode comprometer a generalização dos achados para pacientes com transtorno bipolar na comunidade ou acompanhados em outros contextos. O diagnóstico de comorbidades clínicas por meio do registro em prontuário pode ter subestimado a prevalência delas. A inclusão de pacientes bipolares em diferentes estados de humor (mania, eutimia e depressão) pode ter influenciado a avaliação da prevalência das comorbidades psiquiátricas. Deve-se considerar também a possibilidade de que o presente estudo apresente o viés de Berkson[32]. Segundo esse viés, pessoas portadoras de mais de uma doença ou transtorno tendem a procurar mais frequentemente tratamento médico, o que faz com que amostras clínicas possuam taxas de comorbidades maiores que a população geral. Por outro lado, pelo nosso conhecimento, este é o estudo brasileiro que investiga o maior número de comorbidades na maior amostra formada exclusivamente por pacientes portadores de transtorno bipolar tipo I. Novos estudos, com amostras ampliadas ou de base populacional, incluindo exames complementares ou de rastreio para o diagnóstico de comorbidades, são necessários.

CONCLUSÃO

Pacientes bipolares atendidos em centros psiquiátricos especializados apresentam elevada prevalência de comorbidades psiquiátricas e clínicas, particularmente transtornos de ansiedade, transtornos relacionados a substâncias, hipertensão arterial sistêmica e dislipidemia. Essas comorbidades não se associaram a tentativas de suicídio. Portanto, é importante que sejam incluídos na prática clínica exames complementares ou de rastreio para o diagnóstico de comorbidades clínicas em pacientes com transtorno bipolar.

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REFERÊNCIAS

1. Merikangas KR, Jin R, He JP, Kessler RC, Lee S, Sampson NA, et al. Prevalence and correlates of bipolar spectrum disorder in the world mental health survey initiative. Arch Gen Psychiatry. 2011;68(3):241-51.
2. Robinson LJ, Thompson JM, Gallagher P, Goswami U, Young AH, Ferrier IN, et al. A meta-analysis of cognitive deficits in euthymic patients with bipolar disorder. J Affect Disord. 2006;93:105-15.
3. Baldessarini RJ, Pompili M, Tondo L. Suicide in bipolar disorder: risks and management. CNS Spectr. 2006;11(6):465-71.
4. Kilbane EJ, Gokbayrak NS, Galynker I, Cohen L, Tross S. A review of panic and suicide in bipolar disorder: does comorbidity increase risk? J Affect Disord. 2009;115:1-10.
5. Altamura AC, Serati M, Albano A, Paoli RA, Glick ID, Dell'Osso B. An epidemiologic and clinical overview of medical and psychopathological comorbidities in major psychoses. Eur Arch Psychiatry Clin Neurosci. 2011;261(7):489-508. Epub 2011 Feb 18.
6. Krishnan KR. Psychiatric and medical comorbidities of bipolar disorder. Psychosom Med. 2005;67:1-8.
7. Amorim P. Mini International Neuropsychiatric Interview (MINI): validação de entrevista breve para diagnóstico de transtornos mentais. Rev Bras Psiquiatr. 2000;22:106-15.
8. Asberg M, Träskman L, Thorén P. 5-HIAA in the cerebrospinal fluid. A biochemical suicide predictor? Arch Gen Psychiatry. 1967;33(10):1193-7.
9. Cardoso BM, Kauer Sant'Anna M, Dias VV, Andreazza AC, Ceresér CM, Kapczinski F. The impact of co-morbid alcohol use disorder in bipolar patients. Alcohol. 2008;42:451-7.
10. Nery-Fernandes F, Quarantini LC, Galvão-De-Almeida A, Rocha MV, Kapczinski F, Miranda-Scippa A. Lower rates of comorbidities in euthymic bipolar patients. World J Biol Psychiatry. 2009;10(4 Pt 2):474-9.
11. Neves FS, Malloy-Diniz LF, Corrêa H. Suicidal behavior in bipolar disorder: what is the influence of psychiatric comorbidities. J Clin Psychiatry. 2009;70(1):13-8.
12. Da Silva Magalhães PV, Gomes FA, Kunz M, Kapczinski F. Birth cohort and dual diagnosis effects on age-at-onset in Brazilian patients with bipolar I disorder. Acta Psychiatr Scand. 2009;120:492-5.
13. Da Silva Magalhães PV, Kapczinski NS, Kapczinski F. Correlates and impact of obsessive-compulsive comorbidity in bipolar disorder. Compr Psychiatry. 2010;51:353-6.
14. First MB. Paradigm shifts and the development of the diagnostic and statistical manual of mental disorders: past experiences and future aspirations. Can J Psychiatry. 2010;55(11):692-700.

15. MacKinnon DF, Zamoiski R. Panic comorbidity with bipolar disorder: what is the manic--panic connection? Bipolar Disord. 2006;8:648-64.

16. Contreras J, Hare E, Pacheco A, Escamilla M, Raventos H. Is subclinical anxiety an endophenotype for bipolar I patients? A study from a Costa Rican sample. J Affect Disord. 2010;122(3):267-72.

17. McElroy SL, Altshuler LL, Suppes T, Keck PE Jr, Frye MA, Denicoff KD, et al. Axis I psychiatric comorbidity and its relationship to historical illness variables in 288 patients with bipolar disorder. Am J Psychiatry. 2001;158:420-6.

18. Kessler RC, Chiu WT, Demler O, Merikangas KR, Walters EE. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry. 2005;62(6):617-27.

19. Cardoso BM, Kauer Sant'Anna M, Dias VV, Andreazza AC, Ceresér KM, Kapczinski F. The impact of co-morbid alcohol use disorder in bipolar patients. Alcohol. 2008;42(6):451-7.

20. Weiss RD, Kolodziej M, Griffin ML, Najavits LM, Jacobson LM, Greenfield SF. Substance use and perceived symptom improvement among patients with bipolar disorder and substance dependence. J Affect Disord. 2004;79(1-3):279-83.

21. Strakowski SM, DelBello MP, Fleck DE, Adler CM, Anthenelli RM, Keck PE, et al. Effects of co--occurring alcohol abuse on the course of bipolar disorder following a first hospitalization for mania. Arch Gen Psychiatry. 2005;62(8):851-8.

22. Strakowski SM, DelBello MP, Fleck DE, Arndt S. The impact of substance abuse on the course of bipolar disorder. Biol Psychiatry. 2000;48:477-85.

23. Beyer J, Kuchibhatla M, Gersing K, Krishnan KR. Medical comorbidity in a bipolar outpatient clinical population. Neuropsychopharmacology. 2005;30(2):401-4.

24. Soreca I, Frank E, Kupfer DJ. The phenomenology of bipolar disorder: what drives the high rate of medical burden and determines long-term prognosis? Depress Anxiety. 2009;26(1):73-82.

25. Killgore WD, Young AD, Femia LA, Bogorodzki P, Rogowska J, Yurgelun-Todd DA. Cortical and limbic activation during viewing of high- versus low-calorie foods. Neuroimage. 2003;19(4):1381-94.

26. Langan C, McDonald C. Neurobiological trait abnormalities in bipolar disorder. Mol Psychiatry. 2009;14:833-46.

27. Watson S, Gallagher P, Ritchie JC, Ferrier IN, Young AH. Hypothalamic-pituitary-adrenal axis function in patients with bipolar disorder. Br J Psychiatry. 2004;184:496-502.

28. Whitworth JA, Williamson PM, Mangos G, Kelly JJ. Cardiovascular consequences of cortisol excess. Vasc Health Risk Manag. 2005;1(4):291-9.

29. Teixeira PJR, Rocha FL. Efeitos adversos metabólicos de antipsicóticos e estabilizadores de humor. Rev Psiquiatr RS. 2006;28(2):186-96.

30. Hawton K, Sutton L, Haw C, Sinclair J, Harriss L. Suicide and attempted suicide in bipolar disorder: a systematic review of risk factors. J Clin Psychiatry. 2005;66(6):693-704.

31. Baek JH, Park DY, Choi J, Kim JS, Choi JS, Ha K, et al. Differences between bipolar I and bipolar II disorders in clinical features, comorbidity, and family history. J Affect Disord. 2011;131(1-3):59-67.

32. Walter SD. Berkson's bias and its control in epidemiologic studies. J Chronic Dis. 1980;33(11-12):721-5.

Artigo 4: Increased BNDF levels in BD patients in late stage

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Increased BNDF levels in long-term bipolar disorder patients

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Abstract

Introduction: Bipolar disorder (BD) is a prevalent, chronic and progressive illness. There is a growing body of evidence indicating that brain-derived neurotrophic factor (BDNF) plays an important role in the pathophysiology of BD.

Objective: The aim of this study was to evaluate BDNF plasma levels in BD patients with long term illness in comparison with controls.

Methods: 87 BD type I patients and 58 controls matched by age, gender and education level were enrolled in this study. All subjects were assessed by the Mini-International Neuropsychiatric Interview and the patients by the Young Mania Rating Scale and the Hamilton Depression Rating Scale. The plasma levels of BDNF was measured by ELISA.

Results: BD patients had 23.4 mean years of disease. BD patients in mania presented 1.90-fold increase in BDNF plasma levels ($p = 0.001$), while BD patients in remission presented 1.64-fold increase in BDNF plasma levels ($p = 0.03$) in comparison with control. BDNF plasma levels was not influenced by age, length of illness or medications in use.

Conclusions: The present study suggets that long-term BD exhibit increased BDNF circulating levels.

Key-words: Bipolar disorder; neurotrophic factor; BDNF; mania; pathophisiology.

Resumo

Introdução: Transtorno bipolar (TB) é uma doença prevalente, crônica e progressiva. Um grande número de evidências indicam que o fator neurotrófico derivado do cérebro (BDNF) tem um importante papel na fisiopatologia do TB.

Objetivo: O objetivo deste estudo foi avaliar níveis plasmáticos de BDNF em pacientes com TB com longo tempo de doença em comparação com controles.

Métodos: Oitenta e sete pacientes com TB tipo I e 58 controles equiparados por idade, sexo e nível educacional foram envolvidos neste estudo. Todos os sujeitos foram valaidos pelo *Mini-International Neuropsychiatric Interview* e pacientes foram avaliados pela escala de mania de YOUNG e escala de depressão de Hamilton. Os níveis plasmáticos de BDNF foram mensurados de ELISA.

Resultados: Pacientes com TB tinham idade média de 23,4 anos de doença. Pacientes com TB em mania apresentaram um aumento de 1,90 vezes nos níveis plasmáticos de BDNF ($p = 0,001$), enquanto pacientes com TB em remissão apresentaram aumento de 1,64 vezes nos níveis plasmáticos de BDNF ($p = 0,03$) em comparação com controles. Níveis plasmáticos de BDNF não foram influenciados pela idade, tempo de doença ou medicações em uso.

Conclusões: O presente estudo sugere que pacientes com TB e longo tempo de doença apresentam aumento dos níveis circulantes de BDNF.

Palavras chave: Transtorno bipolar; fator neurotrófico; BDNF; mania; fisiopatologia.

Introduction

Bipolar disorder (BD) is a prevalent, severe and chronic disorder that affects mood and cognitive functions. Despite the old Kraepelin concept that BD has a cyclic and permanent course [1], recent evidence has shown an accelerated and progressive course in BD illness [2]. The physiopathology of BD is unknown but a body of evidence suggests a complex interaction among susceptibility genes, environmental stressors and biochemical mechanisms [3]. The modulation of brain-derived neurotrophic factor (BDNF) levels has been pointed as one of the major mechanisms enrolled in BD physiopathology. However there is scarce information regarding the circulating profile of BDNF in long-term BD. Accordingly the main aim of the present study was to evaluate plasma levels of BDNF in a sample of long-term BD patients in comparison with matched healthy subjects. Secondary analyses were performed in order to identify clinical and demographic factors associated with the variation of BDNF levels.

Methods

This is a cross-sectional study which evaluated 87 BD type I patients and 58 healthy controls matched by age and gender. Patients were consecutively recruited from outpatient and inpatient psychiatric settings at the Instituto de Previdência dos Servidores do Estado de Minas Gerais (IPSEMG), Belo Horizonte, Brazil during one year period. This clinic provides long term assistance for patients with chronic mental illnesses. All patients were medicated. The local institutional review board approved the study, which is in accordance with the Helsinki Declaration of 1975. All participants were more than 18 years old. All volunteers provided their written consent after a

complete explanation about the procedures involved in the research protocol was provided.

Patients and healthy controls were assessed with the Mini-International Neuropsychiatric Interview (M.I.N.I.-Plus) to confirm BD illness and other comorbid psychiatric disorders (in patients) or to exclude a history of psychiatric disorders (in controls) [4]. Patients with BD were also examined with the Hamilton Depression Rating Scale, 17-item version (HDRS) [5] and the Young Mania Rating Scale (YMRS) [6] to characterize the severity of depressive and manic symptoms, respectively. Remission was defined by HDRS and YMRS scores lower than 7 points for at least eight consecutive weeks. Clinical assessment of patients included the collection of the following demographic and clinical variables: gender, age, years of study, length of disease, medication in use and medical comorbidities. Length of illness was calculated by subtracting age at first major depressive or manic mood episode, as recorded in the clinical record or reported by the subject or family members, from the subject's current age in years. The healthy control group was recruited from the local population and did not have any personal psychiatric disorder (evaluated through M.I.N.I.-Plus) or family history of psychiatry disorder, suicide attempts or completed suicide. Subjects with dementia, infectious or autoimmune diseases, or who had used steroids, anti-inflammatory drugs or antibiotics four weeks before venipuncture were excluded from this research protocol. Control group was recruited from the local population and the subjects did not have any personal or family history of psychiatric disorders, suicide behavior, cognitive deficit or clinical diseases.

Ten milliliters of blood were drawn between 8 and 10 a.m. from each subject by venipuncture into a sodium heparin tube, on the same day of the clinical assessment.

The blood was immediately centrifuged at 3000 g for 10 min, 4 °C, twice. The plasma was collected and stored at -70 °C until assayed.

Plasma levels of BDNF were measured by Enzyme-linked immunosorbent assay (ELISA) according to the procedures supplied by the manufacturer (DuoSet, R&D Systems, Minneapolis, MN, USA). All samples were assayed in duplicates. Lower detection limits were reported by the manufacturer as 5 pg/mL for BDNF. Concentrations are expressed as pg/mL.

Statistical analyses were performed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). Association between dichotomous variables was assessed with a chi-square test. All variables were tested for normality of distribution by means of the Shapiro–Wilk test and all data were normally distributed. Two groups (patients vs. controls or mania vs. remission) were compared by a Student t-test. Difference among groups was evaluated using analysis of variance (ANOVA) followed by Dunn post-hoc test. Pearson's correlation analyses were performed to examine the relationship of plasma levels of BDNF with age, length of illness, severity of manic and depressive symptoms. All statistical tests were two-tailed and were performed using a significance level of $\alpha = 0.05$.

Results

The mean age of BD patients and their mean years of formal education were 49.56 years (± 12.34) and 10.08 years (± 3.53), respectively. The mean length of illness was 23.35 years (±12.64). Fifty nine out of 87 BD patients (67.8%) were female. The mean age of controls and their years of formal education were 46.79 years (± 9.43) and 10.16 years (± 3.71), respectively. Forty two out of 58 controls (72.4%) were female. There

were no statistical differences between BD group and controls regarding age, gender and years of study.

At the time of the interview, 48 patients were in mania and 39 patients in remission. BD patients with mania and in remission did not differ in many clinical variables, such as length of illness, medication in use, clinical and psychiatric co-morbidities. BD patients in mania presented higher scores in YMRS in comparison with remitted BD patients (25.23 ± 7.87 vs 1.12 ± 2.00, p = 0.001). BD patients in mania also presented higher scores in HDRS in comparison with BD patients in remission (5.25 ± 6.32 vs. 2.23 ± 2.77, p = 0.007).

BD patients compared with controls presented 1.78-fold increase in BDNF plasma levels (p <0.001). BD patients in mania presented 1.90-fold increase in BDNF plasma levels in comparison with healthy controls (p = 0.001, Dunn's post-hoc test). BD patients in remission presented 1.64-fold increase in BDNF plasma levels in comparison with healthy control (p = 0.03, Dunn's post-hoc test). BD patients in mania tended to have higher plasma levels than BD patients in remission, but this difference did not reach statistical difference (p=0.34). Regarding BD patients, BDNF plasma levels did not correlate with age (p = 0.63), length of illness (p = 0.67), severity of manic (p = 0.23) or depressive (p = 0.82) symptoms.

Plasma levels of BDNF did not differ in BD patients according to the presence of psychiatric and clinical co-morbidities, substance dependence, or the use of any mood stabilizing drug, i.e. atypical antipsychotics (p = 0.87), lithium (p = 0.13) or anticonvulsants (p = 0.82).

Discussion

The present study report the plasma BDNF levels of a large sample with long-term BD patients. Few studies assessed BD patients with long-term illness up to date. BD patients demonstrated increased BDNF plasma levels in comparison with healthy controls, regardless the mood state or medications in use.

A recent meta-analysis showed that BDNF levels decrease during mood states, and BDNF levels were negatively correlated with the severity of mood symptoms[7]. Moreover, BDNF levels were normal in patients in euthymia and negatively correlated with the length of BD [7]. The result of the present work is in opposite direction of these meta-analytic findings. It is worth mentioning, however, that this meta-analysis did not include BD patients with more than 20 years of illness.

Taken into account that circulating levels of BDNF may reflect its cerebral concentration [8], it is possible that higher BDNF concentration in the long course of BD may represent a reaction to the cerebral damage that occurred in the early years of the disease when neurodegenerative or neuroprogressive mechanisms seem to be more intense and BDNF levels would be decreased [9]. This assumption is in line with evidence from other neuropsychiatric disorders, such as long-term schizophrenia [10, 11], and experimental model of Alzheimer's disease [12]. A secondary hypothesis would be related with the effect of mood stabilizing drugs in BDNF levels. For instance, lithium therapy seems to increase the levels of BDNF in BD patients in mania [13].

This study has strengths and limitations that must be considered for the interpretation of the results. The diagnostic interviews of both patients and controls were performed using the same protocol, overcoming a limitation of previous similar studies. In addition, sample size and the exclusion of patients with other medical conditions, such as inflammatory diseases, can be considered strengths of the study. The use of medications by all BD patients is a limitation.

In conclusion our data reinforce the view that there is BDNF imbalance in long-term BD.

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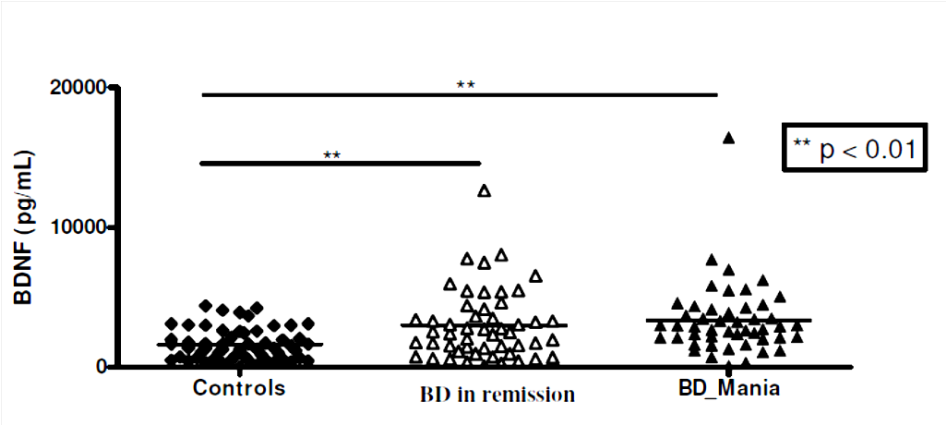
References

1. Mondimore FM. Kraepelin and manic-depressive insanity: an historical perspective. Int Rev Psychiatry 2005; 17 (1): 49-52
2. Berk M. Neuroprogression: pathways to progressive brain changes in bipolar disorder. Int J Neuropsychopharmacol. 2009; 12(4): 441-5.
3. Berk M, Kapczinski F, Andreazza AC, Dean OM, Giorlando F, Maes M, Yücel M, Gama CS, Dodd S, Dean B, Magalhães PV, Amminger P, McGorry P, Malhi GS. Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. Neurosci Biobehav Rev. 2011; 35(3): 804-17.
4. Amorim P. Mini International Neuropsychiatric Interview (MINI): validação de entrevista breve para diagnóstico de transtornos mentais. Rev Bras Psiquiatr. 2000; 22: 106-15.
5. Hamilton M. Development of a rating scale for primary depressive illness. Br J Soc Clin Psychol.1967; 6: 278-96.
6. Young RC, Biggs JT, Ziegler VE, Meyer DA. A rating scale for mania: reliability, validity and sensitivity. Br J Psychiatry.1978; 133: 429–35.
7. Fernandes BS, Gama CS, Ceresér KM, Yatham LN, Fries GR, Colpo G, de Lucena D, Kunz M, Gomes FA, Kapczinski F. Brain-derived neurotrophic factor as a state-marker of mood episodes in bipolar disorders: a systematic review and meta-regression analysis. J Psychiatr Res. 2011; 45(8): 995-1004.
8. Teixeira AL, Barbosa IG, Diniz BS, Kummer A. Circulating levels of brain-derived neurotrophic factor: correlation with mood, cognition and motor function. Biomark Med. 2010; 4(6): 871-87.
9. Barbosa IG, Huguet RB, Mendonça VA, Neves FS, Reis HJ, Bauer ME, Janka Z, Palotás A, Teixeira AL. Increased plasma levels of brain-derived

neurotrophic factor in patients with long-term bipolar disorder. Neurosci Lett. 2010; 475(2): 95-8.

10. Gama CS, Andreazza AC, Kunz M, Berk M, Belmonte-de-Abreu PS, Kapczinski F. Serum levels of brain-derived neurotrophic factor in patients with schizophrenia and bipolar disorder. Neurosci Lett. 2007; 420(1): 45-8.
11. Reis HJ, Nicolato R, Barbosa IG, Teixeira do Prado PH, Romano-Silva MA, Teixeira AL. Increased serum levels of brain-derived neurotrophic factor in chronic institutionalized patients with schizophrenia. Neurosci Lett. 2008; 439(2): 157-9.
12. Schulte-Herbrüggen O, Eckart S, Deicke U, Kühl A, Otten U, Danker-Hopfe H, Abramowski D, Staufenbiel M, Hellweg R. Age-dependent time course of cerebral brain-derived neurotrophic factor, nerve growth factor, and neurotrophin-3 in APP23 transgenic mice. J Neurosci Res. 2008; 86(12): 2774-83.
13. de Sousa RT, van de Bilt MT, Diniz BS, Ladeira RB, Portela LV, Souza DO, Forlenza OV, Gattaz WF, Machado-Vieira R. Lithium increases plasma brain-derived neurotrophic factor in acute bipolar mania: a preliminary 4-week study. Neurosci Lett. 2011; 494(1): 54-6.

Fig. 1.Plasma levels of BDNF in controls in comparison with BD patients in remission and BD patients with mania.



Artigo 5: Impaired nerve growth factor homeostasis in patients with bipolar disorder

Izabela Guimarães Barbosa, Rodrigo Barreto Huguet, Fernando Silva Neves, Helton José Reis, Moisés Evandro Bauer, Zoltá András, Antônio Lúcio Teixeira.

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ORIGINAL INVESTIGATION

Impaired nerve growth factor homeostasis in patients with bipolar disorder

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Abstract

Objective. Neuro-trophins are critically involved in neuro-plasticity, the impairment of which is a major role-player in bipolar disorder (BD), and their altered levels have been recently advocated in the patho-physiology of this affective malady. The aim of this study, therefore, was to evaluate the plasma levels of nerve growth factor (NGF) in BD patients in comparison with control subjects. *Methods.* Forty-nine BD type-I individuals (30 in mania and 19 in euthymia) and 36 healthy controls were assessed by Mini-plus, Young mania and Hamilton depression rating scales. NGF levels were detected by ELISA. *Results.* Plasma NGF concentrations were decreased in BD patients when compared to that seen with controls. BD individuals in mania had lower NGF levels than euthymic patients or controls. NGF levels were negatively correlated with the severity of mania. *Conclusions.* This is the first study to evaluate NGF levels in BD patients, providing further support to the hypothesis of impaired neuroplasticity in BD. These data also suggest that NGF measurement could be used for the biological marker for manic state.

Key words: *Biomarker, bipolar disorder, nerve growth factor (NGF), neuroplasticity, neurotrophin*

Introduction

Bipolar disorder (BD) is a severe psychiatric illness, affecting approximately 1% of the population worldwide. The cardinal feature that distinguishes BD from recurrent major depressive disorder is abnormal mood elevation. BD type-I patients present at least one episode of mania during the course of the disease (Belmaker 2004). The physiopathology of BD remains unclear; however, there is a growing body of evidence indicating impaired neuroplasticity (Zarate et al. 2006) and immunologic processes (Goldstein et al. 2009).

Neurotrophins are critically involved in neuro- and/or synaptic-plasticity and regulation of brain development. They also play key roles in differentiation and survival, as well as cell death associated with inflammation, ischemia and seizure (Chao 2003; Reichardt 2006). In addition to these well established

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functions in the central nervous system (CNS), neurotrophins have been implicated in cardiac development, angiogenesis, autoimmune diseases, and neuropsychiatric disorders (Reichardt 2006; Schulte-Herbrüggen et al. 2007; Nico et al. 2008; Alleva and Francia 2009). Nerve growth factor (NGF) was the first neurotrophin discovered in 1951 by Rita Levi-Montalcini, and is one of the best characterized members of this group. In the CNS, NGF can promote neuronal survival protecting sympathetic and cholinergic neurons against neurodegeneration, and also mediates activities such as learning and memory. It has a relevant signalling role in different cells of the human immune system (Nockher and Renz 2005).

Alterations in circulating levels of NGF have been described in a series of neuropsychiatric disorders, including Alzheimer's disease (AD), schizophrenia, schizophreniform disorder and psychological stress conditions (Schulte-Herbrüggen et al. 2007; Alleva and Francia 2009; Kale et al. 2009); however, NGF levels have not yet been investigated in BD. Therefore, the aim of the present study was to evaluate the plasma levels of NGF in BD patients and healthy subjects. Secondary analyses were performed in order to establish whether clinical and demographic factors were associated with NGF concentrations.

Methods

Participants

Forty-nine medicated BD type-I patients and 36 healthy controls matched for age, gender, ethnical and geographical areas, as well as level of education, were recruited for this study. BD subjects came from a public hospital as in- or out-patients. The control group was recruited from the community who did not have any personal or family history of psychiatry disorders, including suicide behaviour.

All procedures described in this study received approval from the local clinical research ethics committee, and are in accordance with the Helsinki Declaration of 1975. Written informed consent was obtained from all patients and healthy subjects.

All participants were over 18 years old. The following individuals were excluded from the study: subjects with dementia or previous diagnosis of other neurodegenerative disorders, infectious or autoimmune diseases, or who had used corticosteroids, anti-inflammatory drugs or antibiotics 4 weeks before sampling. Psychiatric diagnosis was based on DSM-IV criteria and was performed following a structured clinical interview (Mini-International Neuropsychiatric Interview, Mini-plus) (Sheehan et al. 1998; Amorim 2000). Healthy controls were also evaluated by Mini-plus to exclude any psychiatric condition. The severity of manic and depressive symptoms was assessed by the Young mania rating scale (YMRS) (Young et al. 1978) and the Hamilton depression rating scale, 17-item version (Ham-D) (Hamilton 1967), respectively. Euthymia was defined by YMRS score lower than 12, and Ham-D score lower than 7 points (Byer 2008).

Procedure

Five milliliters of blood were drawn from each subject by venipuncture into heparinized tube during clinical assessment. In order to rule out possible circadian rhythm-related changes in NGF levels, blood was taken between 08:00 and 10:00 h for each volunteer. Samples were immediately centrifuged twice at $3000 \times g$ for 10 min, and plasma was kept frozen at –70°C until assayed.

Plasma NGF levels were measured according to the procedure provided by the manufacturer using sandwich-ELISA kits for NGF (R&D Systems, Minneapolis, MN, USA). Undiluted samples were assayed in duplicate. The detection limits for these assays were 10 pg/ml, values bellow which were assumed to be zero. Concentrations are expressed as pg/ml (mean ± SD).

Statistical analysis

Descriptive statistics were used to report sociodemographic and clinical characteristics of the sample. Relationships between dichotomous variables were assessed using χ^2-test or Fisher's exact test, when appropriate. All variables were evaluated for normality of distribution by means of the Kolmogorov–Smirnov test. Because all variables were non-normally distributed, two groups were compared with the Mann–Whitney test, and three groups with the Kruskal–Wallis and Dunn's post-hoc test. Spearman's correlation analysis was performed to examine the relationship of NGF levels with age, length of illness, education, YMRS and Ham-D scores. All statistical tests were two-tailed and were performed using a significance level of $\alpha = 0.05$. Statistical analyses were performed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA).

Results

Thirty patients with mania and 19 with euthymia were included in this study. All patients were medicated. There were no significant differences in the proportion of gender or age between control and BD groups. Among BD patients, there were no marked variations among the age of first mood episode, length of disease, number of hospitalizations, or medications

Table I. Demographic and social features of control subjects and BD patients enrolled in the study.

Variables	Control subjects (n = 36)	BD patients		P value
		Mania (n = 30)	Euthymia (n = 19)	
Female gender (%)	52.80	60.00	57.90	0.43[c]
Age (years, mean ± SD)	42.94 ± 9.39	48.03 ± 13.66	45.00 ± 10.84	0.32[b]
Ham-D score (mean ± SD)	0.49 ± 1.36	4.00 ± 4.68	1.89 ± 1.79	0.02[b]
YMRS score (mean ± SD)	0	27.87 ± 6.13	1.37 ± 3.17	< 0.001[b]
Age of first mood episode (mean ± SD)	–	28.56 ± 11.24	24.42 ± 9.84	0.27[a]
Length of disease in years (mean ± SD)	–	19.71 ± 13.42	20.56 ± 10.08	0.49[a]
Medication in use (frequency, %)				
Lithium	–	43.30	68.40	0.14[d]
Anti-convulsants	–	56.70	68.40	0.55[d]
Anti-psychotics	–	73.30	63.20	0.53[d]
Anti-depressants	–	3.3	15.8	0.29[d]

n: number of patients; SD: standard deviation;
[a]Mann-Whitney test; [b]Kruskal-Wallis test; [c]χ^2-test; [d]Fisher-test.

in use. The demographic and clinical features of all groups are shown in Table 1.

BD patients presented lower NGF plasma levels than controls (81.93 ± 114.23, 108.78 ± 102.16, respectively, P = 0.007) (Figure 1). When comparing BD sub-groups (categorized according to mood state) with controls, NGF levels were lower in manic patients in comparison with euthymics and controls (mania: 78.37 ± 105.11, euthymia: 87.55 ± 130.16, controls: 108.78 ± 102.16) (Figure 2), reaching statistically significant difference between manic group and controls. There was no marked difference between BD patients in euthymia and controls, but there was a tendency of reduced levels in these patients.

Considering all BD patients, NGF plasma concentrations were positively correlated with the length of disease (ρ = 0.34, P = 0.03), however when the very same analysis was carried out for each sub-group (i.e. euthymia and mania separately), this correlation was not observed. There was no association between NGF and age, severity of depressive symptoms, or number of hospitalizations. Interestingly, NGF plasma levels were negatively correlated with the severity of mania (ρ = –0.40, P = 0.03).

NGF concentrations did not differ in BD categorized according to the presence of psychiatric comorbidities (i.e. generalized anxiety disorder, panic disorder, obsessive-compulsive disorder), dependence of substances or nicotine. Medications had no effect on NGF concentrations, e.g., lithium (used: 76.21 ± 107.47, not used: 88.04 ± 123.54, P = 0.85), valproic acid (used: 55.61 ± 54.34, not used: 105.21 ± 145.79, P = 0.22), carbamazepine (used: 166.38 ± 181.06, not used: 67.86 ± 95.17, P = 0.1), antipsychotics (used: 108.55 ± 145.21, not used: 54.21 ± 60.45, P = 0.74), antidepressants (used: 109.34 ± 107.10, not used: 79.50 ± 115.61, P = 0.45).

Statistical analyses performed after the exclusion of outliers (i.e. patients with extremely high NGF

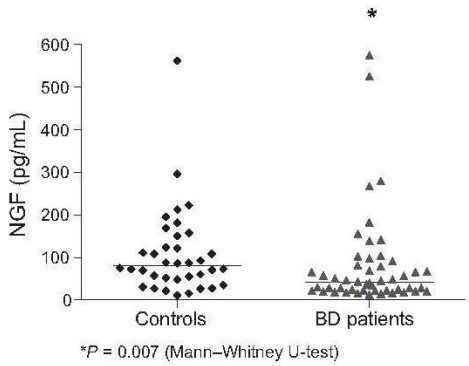

Figure 1. Plasma NGF levels in controls and bipolar disorder (BD) patients. *P = 0.007 (Mann–Whitney U-test).

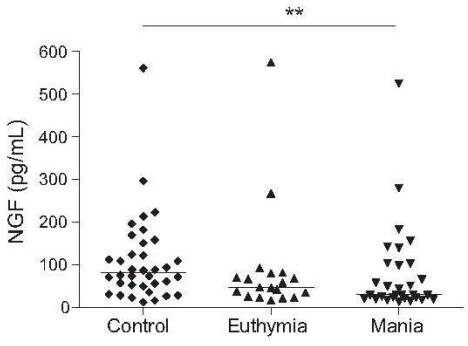

Figure 2. Plasma levels of NGF in control subjects and bipolar patients in euthymia and in mania. **P < 0.05 (Kruskal–Wallis with Dunn's post-hoc test).

levels) in each group did not show any significant difference in the parameters studied.

Discussion

To the best of our knowledge, this is the first study to assess NGF levels in BD patients. Plasma concentrations of NGF were found to be decreased in BD subjects in comparison with asymptomatic controls. Levels of NGF were notably lower in mania, and were negatively correlated with the severity of manic symptoms.

This result is in line with previous studies indicating decreased levels of neurotrophins, especially brain-derived neurotrophic factor (BDNF) in BD (Kapczinski et al. 2008). It also corroborates the view that plastic changes are more pronounced during mood episodes (Zarate et al. 2006). Indeed, there is a considerable amount of evidence indicating that structural changes in the CNS of BD patients are associated with the number of mood episodes (reviewed in Kapczinski et al. 2008).

NGF plays an important role in the development and maintenance of the sensory and sympathetic nervous system, as well as in the cholinergic functions of the CNS (Counts and Mufson 2005; Cuello et al. 2010; Niewiadomska et al. 2010). Cholinergic neurons within the nucleus basalis and the septal diagonal band complex provide the major source of cholinergic innervation to the cerebral cortex and hippocampus, respectively, and play a key role in memory and attention (Niewiadomska et al. 2010). In spite of the old Kraepelinean concept that BD does not evolve with cognitive decline, recent studies have demonstrated deficits in several cognitive domains in BD patients, especially attention, verbal memory and executive functioning (Goldberg and Chengappa 2009; Zarate and Manji 2008). These deficits seem to be a trait independent of the mood state or the psychotropic effect (Goldberg and Chengappa 2009). The cholinergic system is involved in the physiology of these cognitive domains commonly affected in BD patients (Zarate and Manji 2008; Goldberg and Chengappa 2009; Harvey 2009). Therefore, one possible mechanism involved in this neurodegenerative process might be the decreased availability of NGF (Schindowski et al. 2008). Low plasma levels of NGF in BD patients in mania also reinforces the theory that mood episodes are associated with increased pathogenic processes in BD patients, particularly mania episodes (Kapczinski et al. 2008). Unfortunately, there were no depressive BD patients in this group to corroborate this assumption.

There was a tendency of elevated plasma NGF levels during the course of disease ($\rho=0.34$, $P=0.03$) in our study. Interestingly, a biphasic fluctuation of NGF concentration has been reported with lower cortical levels at onset, and increase during the course of eurodegenerative diseases (Hellweg et al. 1998). Increased NGF levels may also be attributable to the long-term use of mood stabilizing agents and/or antipsychotics. In the present report, however, NGF plasma concentrations were not associated with the use of any of these medications, not corroborating the hypothesis of drug-related NGF changes. Moreover, several reports could not show any effect of lithium in the cerebral expression of NGF in rats (Omata et al. 2008). Conversely, some papers have found increased plasma NGF levels in chronic schizophrenia in patients treated with atypical antipsychotics but not in those using typical neuroleptics (Parikh et al. 2003). In the present study, none of the BD patients used atypical antipsychotics.

Limitations of the present study include the fact that (i) the sample was mainly composed of BD patients with higher length of disease, and the present results may not reflect the initial stages of the disorder; (ii) BD subjects in depression were not included; (iii) cognitive impairment in BD individuals were not assessed, and the results could reflect cognitive dysfunctions observed in BD; (iv) the results could have been influenced by medications; (v) patients with diabetes mellitus, cardiovascular disorders and/or metabolic syndrome were not excluded from this study because of the relatively high frequency of these co-morbidities in BD (McElroy et al. 2009; Teixeira and Rocha, 2007), however NGF levels may be altered in these diseases (Bulló et al. 2007; Kim et al. 2009; Nico et al. 2008). By contrast, sample size and the exclusion of patients with other medical conditions, such as inflammatory diseases, can be considered as strengths of the study. For the first time and despite these limitations, these results clearly indicate a role for NGF in BD pathology, as well as being a potential biomarker in manic state, however further studies are warranted to confirm these findings and hypotheses.

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Statement of interest

The authors declare that they have no conflict of interest.

References

Alleva E, Francia N. 2009. Psychiatric vulnerability: suggestions from animal models and the role of neurotrophins. Neurosci Biobehav Rev 33:525–536.

Amorim P. 2000. Mini international neuropsychiatric interview (MINI): validação de entrevista breve para diagnóstico de transtornos mentais. Rev Bras Psiquiatr 22:106–115.

Belmaker RH. 2004. Bipolar disorder. New Engl J Med 351: 476–486.

Byer LJ. 2008. An evidence-based medicine strategy for achieving remission in bipolar disorder. J Clin Psychiatry 69:31–37.

Bulló M, Peeraully MR, Trayhurn P, Folch J, Salas-Salvadó J. 2007. Circulating nerve growth factor levels in relation to obesity and the metabolic syndrome in women. Eur J Endocrinol 157(3):303–310.

Chao MV. 2003. Neurotrophins and their receptors: a converge point for many signaling pathways. Nat Rev Neurosci 4(4): 199–309.

Counts SE, Mufson EJ. 2005. The role of nerve growth factor receptors in cholinergic basal forebrain degeneration in prodromal Alzheimer disease. J Neuropathol Exp Neurol 64(4): 263–272.

Cuello AC, Bruno MA, Allard S, Leon W, Iulita MF. 2010. Cholinergic involvement in Alzheimer's disease. A link with NGF maturation and degradation. J Mol Neurosci 40(1–2):230–235.

Goldberg JF, Chengappa KN. 2009. Identifying and treating cognitive impairment in bipolar disorder. Bipolar Disord 11(Suppl 2):123–137.

Goldstein BI, Kemp DE, Soczynska JK, McIntyre RS. 2009. Inflammation and the phenomenology, pathophysiology, comorbidity, and the treatment of bipolar disorder: a systematic review of the literature. J Clin Psychiatry 70:1078–1090.

Hamilton M. 1967. Development of a rating scale for primary depressive illness. Br J Soc Clin Psychol 6:278–296.

Harvey PD. 2009. Pharmacological cognitive enhancement in schizophrenia. Neuropsychol Rev 19(3):324–335.

Hellweg R, von Richthofen S, Anders D, Baethge C, Röpke S, Hartung HD, Gericke CA. 1998. The time course of nerve growth factor content in different neuropsychiatric diseases – a unifying hypothesis. J Neural Transm 105:871–903.

Kale A, Joshi S, Pillai A, Naphade N, Nasrallah H, Mahadik SP. 2009. Reduced cerebrospinal fluid and plasma nerve growth factor in drug-naïve psychotic patients. Schizophr Res 115:209–214.

Kapczinski F, Frey BN, Kauer-Sant'Anna M, Grassi-Oliveira R. 2008. Brain-derived neurotrophic factor and neuroplasticity in bipolar disorder. Expert Rev Neurother 8(7):1101–1113.

Kim HC, Cho YJ, Ahn CW, Park KS, Kim JC, Nam JS, et al. 2009. Nerve growth factor and expression of its receptors in patients with diabetic neuropathy. Diabet Med 26(12):1228–1234.

McElroy SL, Frye MA, Suppes T, Dhavale D, Keck PE Jr, Leverich GS, et al. 2002. Correlates of overweight and obesity in 644 patients with bipolar disorder. J Clin Psychiatry 63(3): 207–213.

Nico B, Mangieri D, Benagiano V, Crivellato E, Ribatti D. 2008. Nerve growth factor as an angiogenic factor. Microvasc Res 75(2): 135–141.

Niewiadomska G, Mietelska-Porowska A, Mazurkiewicz M. 2010. The cholinergic system, nerve growth factor and the cytoskeleton. Behav Brain Res (in press, PMID: 20170684).

Nockher WA, Renz H. 2005. Neurotrophins in clinical diagnostic: pathophysioplogy and laboratory investigation. Clin Chim Acta 352:49–74.

Omata N, Murata T, Takamatsu S, Maruoka N, Mitsuya H, Yonekura Y, et al. 2008. Neuroprotective effect of chronic lithium treatment against hypoxia in specific brain regions with upregulation of cAMP response element binding protein and brain-derived neurotrophic factor but not nerve growth factor: comparasion with acute lithium treatment. Bipolar Disord 10:360–368.

Parikh V, Evans DR, Khan MM, Mahadik SP. 2003. Nerve growth factor in never-medicated first-episode psychotic and medicated chronic schizophrenic patients: possible implications for treatment outcome. Schizophr Res 60:117–123.

Reichardt LF. 2006. Neurotrophin-regulated signaling pathways. Philos Trans R Soc Lond B Biol Sci 361(1473):1545–1564.

Schindowski K, Belarbi K, Buée L. 2008. Neurotrophic factors in Alzheimer's disease: role of axonal transport. Genes Brain Behav 7:43–56.

Schulte-Herbrüggen O, Braun A, Rochlitzer S, Jockers-Scerübl MC, Hellweg R. 2007. Neurotophic factors – a tool for therapeutic strategies in neurological, neuropsychiatric and neuroimmunological diseases? Curr Med Chem 14(22):2318–2329.

Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. 1998. The Mini-international neuropsychiatric interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 59:4–57.

Teixeira PJ, Rocha FL. 2007. The prevalence of metabolic syndrome among psychiatric inpatients in Brazil. Rev Bras Psiquiatr 29(4):330–336.

Young RC, Biggs JT, Ziegler VE, Meyer DE. 1978. A rating scale for mania: reliability, validity and sensitivity. Br J Psychiatry 133:429–435.

Zarate CA, Manji HK. 2008. Bipolar disorder: candidate drugs targets. Mt Sinai J Med 75:226–247.

Zarate CA Jr, Singh J, Manji HK. 2006. Cellular plasticity cascades: targets for the development of novels therapeutics for bipolar disorder. Biol Psychiatry 59:1006–1020.

Artigo 6: Circulating levels of GDNF in bipolar disorder

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ABSTRACT

Neurotrophic factors regulate the survival and growth of neurons, and influence synaptic efficiency and plasticity. Several studies suggest the existence of a relationship between changes in neurotrophic levels and bipolar disorder (BD). The glial cell-line derived neurotrophic factor (GDNF) influences monoaminergic neurons and glial cells, but its role in BD patients is controversial. In order to elucidate it we evaluated plasma levels of GDNF in a sample of 70 BD patients (35 in mania and 35 in euthymia) and compared with 50 healthy controls matched for age, gender and educational levels. GDNF plasma levels were measured by enzyme-linked immunosorbent assay (ELISA). Patients were assessed by a Mini-International Neuropsychiatric Interview (MINI-plus), Young Mania and Hamilton Depression Rating Scales. Plasma GDNF levels were significantly increased in BD patients in euthymia compared with BD patients in mania and healthy controls ($p < 0.05$). GDNF plasma levels were correlated with age ($\rho = 0.30$, $p < 0.05$) and negatively correlated with manic symptoms in BD patients ($\rho = -0.54$, $p < 0.05$). Our results provide evidence that peripheral levels of GDNF are related with different mood states in BD, reinforcing the involvement of neurotrophic factors in its physiopathology.

Bipolar disorder (BD) is a chronic, recurrent and one of the most severe psychiatric illnesses. The cardinal feature that distinguishes BD from recurrent major depressive disorder is the presence of at least one episode of mania or hypomania during lifetime. BD type I presents at least one mania episode and affects approximately one percent of the population worldwide [7].

Growing evidence shows that BD is associated with abnormalities in neural circuits and synapses, as well in plastic processes [23]. *Post-mortem* study of the brain of BD patients demonstrates abnormalities in prefrontal and cingulate grey matter areas mostly characterized by reduced number, density and size of glial population [20]. Dysregulation of neurotrophins imbalance has been suggested as one of the mediators and markers of neuroanatomical brain changes in BD [14].

Neurotrophins play an important role in synaptic plasticity and have shown to be altered in patients with BD. Brain-derived neurotrophic factor (BDNF) is the most studied neurotrophin in BD. In a recent meta-analysis, Lin [15] showed that levels of BDNF are reduced during mania and depression when compared to healthy controls, but not in euthymic patients. A related neurotrophin that has also been associated with synaptic plasticity is the glial cell-line derived neurotrophic factor (GDNF) [2,9]. Until now only a few studies have evaluated GDNF levels in BD patients [19,21,26,31] and it is still unclear whether GDNF levels are associated with BD and/or with different mood states.

In order to clarify the role of GDNF in BD, the aims of this study were to evaluate plasma levels of GDNF in a sample of BD patients and to investigate whether mood state (i.e. mania) influences GDNF circulating levels in BD.

Abbreviations: BD, bipolar disorder; BDNF, brain-derived neurotrophic factor; DSM-IV-TR, diagnostic and statistical manual of mental disorders; text, revision; ELISA, enzyme-linked immunosorbent assay; GDNF, glial cell-line derived neurotrophic factor; HDRS, Hamilton Depression Rating Scale; MINI-Plus, Mini-International Neuropsychiatric Interview; YMRS, Young Mania Rating Scale.

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Table 1
Clinical and demographic features of controls and bipolar disorders (BD) subjects.

	Healthy controls ($N = 50$)	BD patients ($N = 70$)		p-Values
		Mania ($N = 35$)	Euthymia ($N = 35$)	
Female gender (frequency, %)	70.00	62.86	77.14	>0.05[a]
Age in years (mean ± SD)	47.48 ± 7.64	52.54 ± 12.41	47.29 ± 9.07	>0.05[c]
Education level in years (mean ± SD)	9.76 ± 3.31	10.49 ± 2.91	9.63 ± 4.02	>0.05[c]
YMRS (mean ± SD)	–	25.80 ± 7.05	0.83 ± 1.67	<0.0001[b]
HDRS (mean ± SD)	–	3.26 ± 3.72	2.43 ± 2.81	>0.05[b]
Age of first mood episode (mean ± SD)	–	28.58 ± 10.36	24.66 ± 9.60	>0.05[b]
Age of first depressive episode (mean ± SD)	–	29.06 ± 10.95	25.37 ± 9.46	>0.05[b]
Age of first manic episode (mean ± SD)	–	27.31 ± 10.43	32.06 ± 12.60	>0.05[b]
Duration of illness (mean ± SD)	–	26.69 ± 15.17	22.51 ± 11.15	>0.05[b]
Number of hospitalizations (mean ± SD)	–	10.70 ± 13.47	6.37 ± 5.01	>0.05[b]
Suicide attempt (frequency, %)	–	20.00	40.00	>0.05[a]
Medication in use (frequency, %)				
Lithium	–	70.00	48.28	>0.05[a]
Anticonvulsants	–	66.67	56.62	>0.05[a]
Antipsychotics	–	50.00	41.40	>0.05[a]

N: number; YMRS: Young Mania Rating Scale; HDRS: Hamilton Depression Rating Scale; BD: bipolar disorder.
[a] Chi-square test.
[b] Mann–Whitney test.
[c] Kruskal–Wallis test.

Seventy BD type I patients (35 in mania and 35 in euthymia) and fifty healthy controls matched for gender, age and years of study were recruited for this study in a public hospital as in-patient or out-patient admission. The control group was recruited from the local population and they did not have any personal or family history of psychiatric disorders, suicide behavior, cognitive deficit or clinical diseases.

All procedures described in this study received approval from the local clinical research ethics committee and are in accordance with the Helsinki Declaration of 1975. Written informed consent was obtained from all patients and healthy subjects prior to conducting any study procedures.

All participants were over the legal age of 18 years old. Subjects with dementia and previous diagnosis of any neurodegenerative disorders, infectious or autoimmune diseases, or who had used corticosteroids, non-steroidal anti-inflammatory drugs or antibiotics 4 weeks before the entry into the study were excluded. As GDNF levels could be influenced by chronic renal failure [18], no subjects with this condition was included in the study. Psychiatric diagnoses were based on DSM-IV-TR criteria [1] and were performed following a structured clinical interview (Mini-International Neuropsychiatric Interview, MINI-plus) [3,25]. Healthy controls were assessed by MINI-plus to exclude any psychiatric condition. The severity of manic and depressive symptoms was assessed by the Young Mania Rating Scale (YMRS) [28] and the Hamilton Depression Rating Scale, 17-item version (HDRS) [10], respectively. Euthymia was defined by YMRS score and HDRS score lower than 7 points for at least four weeks.

All blood samples were drawn at the moment of the clinical assessment. Five milliliters of blood was drawn from each subject by venipuncture into a vacuum tube containing heparin, and was immediately centrifuged twice at 3000 × g for 10 min, and plasma was kept frozen at −70 °C until assayed. Plasma concentration of GDNF was measured according to the procedure provided by the manufacturer using sandwich ELISA kits for GDNF (DuoSet, R&D Systems, Minneapolis, MN, USA). All samples were assayed in duplicate. The detection limits for these assays were 10 pg/mL. Concentration is expressed as pg/mL.

Statistical analyses were performed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to report socio-demographic and clinical characteristics of the sample. Association between dichotomous variables was assessed with chi-square test or Fisher's exact test when appropriate. All variables were tested for normality of distribution by means of the Kolmogorov–Smirnov test and all data were non-normally distributed. Two groups (patients vs. controls) were compared by Mann–Whitney test. Differences between three groups (patients in mania vs. patients in euthymia vs. controls) were compared with Kruskal–Wallis test. Multiple comparisons among levels were checked with Dunn's post hoc test. Spearman's correlation analysis was performed to examine the relationship of GDNF levels with age, length of illness, education. All statistical tests were two-tailed and were performed using a significance level of alpha = 0.05.

The demographic and clinical features of all groups are shown in Table 1. There were no statistical significant differences between BD patients groups and the healthy control group regarding gender, age and years of study. Among BD patients, there were no marked variations among the age of first mood episode, length of disease, number of hospitalizations or medications in use. All patients were medicated.

There was no significant differences in GDNF plasma levels between BD patients and healthy controls (mean ± SD, pg/ml; 55.78 ± 78.42, 39.78 ± 57.89, respectively, $p > 0.05$) (Fig. 1A). When comparing BD sub-groups (categorized according to mood state) with healthy controls, GDNF plasma levels were higher in euthymic BD patients in comparison with manic and healthy controls (mean ± SD, pg/ml; euthymia: 76.74 ± 95.26; mania: 34.09 ± 48.80; controls: 40.52 ± 58.99; $p < 0.05$), post hoc analysis (euthymia vs. mania, $p < 0.05$; euthymia vs. healthy control, $p < 0.05$; mania vs. healthy control, $p > 0.05$) (Fig. 1B). There was no significant difference between BD patients in mania and controls, but there was a tendency of reduced levels in patients. There was a predominance of female subjects in all groups, but there was no difference in GDNF plasma levels when comparing gender.

GDNF plasma levels were positively correlated with age in BD patients ($\rho = 0.30$, $p < 0.05$); BD patients in euthymia ($\rho = 0.39$, $p < 0.05$); and BD patients in mania ($\rho = 0.47$, $p < 0.05$). GDNF plasma levels were negatively correlated with YMRS in BD patients ($\rho = -0.54$, $p < 0.05$); BD patients in euthymia ($\rho = -0.47$, $p < 0.05$); and BD patients in mania ($\rho = -0.33$, $p > 0.05$). There was no association between GDNF levels and severity of depressive symptoms or duration of illness. Correlation between age and plasma levels of GDNF was not observed in healthy controls.

GDNF levels did not differ in BD categorized according to the presence of clinical comorbities (blood arterial hypertension, diabetes mellitus, dislipidemia, and thyroid diseases), psychiatric

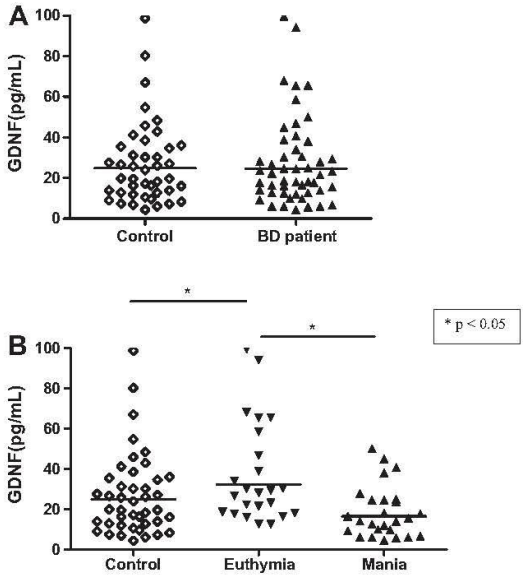

Fig. 1. (A) Plasma GDNF levels in controls and bipolar disorder (BD) patients. (B) Plasma GDNF levels in controls, euthymia and mania.

comorbities (i.e. generalized anxiety disorder, panic disorder, previously or actual episode of psychosis), dependence of substances (including alcohol and tobacco), previous attempt of suicide and previous depressive episode. Plasma GDNF levels did not differ in BD patients according to the medication in use, such as lithium, anti-convulsants or anti-psychotics (data not show).

In the present study, plasma levels of GDNF were significantly increased in euthymic BD patients in comparison with healthy controls and BD patients in mania. In BD patients, plasma levels of GDNF were negatively correlated with the severity of mania and positively correlated with age. Our results are in line with the hypothesis that altered levels of neurotrophic factors are related with mood changes in BD patients [5,6,14,15,27]. However it seems that GDNF may present a different balance in comparison with BDNF levels in BD [15].

This is the first study to show higher plasma levels of GDNF in euthymic BD patients in comparison with healthy controls. Otsuki et al. [19] did not find any difference in RNA levels of GDNF when the authors compared BD patients in depression or after their remission and controls. Rosa et al. [21] reported increased serum levels of GDNF in BD patients in depression and mania compared with healthy controls, without difference between euthymic patients and healthy controls. Two studies showed decreased levels of GDNF in BD patients in euthymia [26] and mania or depression [31]. Interestingly, Zhang et al. [31] showed that BD patients evolved with increased serum levels of GDNF after remission of a depression or mania episode.

In the past, the role of GDNF in the development and maintenance of the nigrostriatal system and its relation with dopaminergic system was only implicated in Parkinson's disease physiopathology. Otherwise recent evidence has suggested that the up-regulation of GDNF by astrocytes and microglia may be a protective mechanism to restrain neuronal loss observed in different types of brain diseases [17,22]. Repeated mood episodes and aging seem to present a cumulative load to BD patients and may be associated with neurotoxicity [13]. Therefore one possible explanation to our results is that mood episodes might be toxic to the brain, reducing GDNF levels. As the person gets older, in order to prevent detrimental effects of mood episodes, GDNF levels would be increased during euthymic states. Reinforcing our results, two previous studies with major depression patients have shown decreased levels of GDNF during acutely ill episode and increased levels of GDNF after mood recovery [29,30].

There are some limitations in our study. First, all patients were medicated (mood stabilizing agents, antipsychotics and/or antidepressants) and it is not possible to exclude the effect of drugs in circulating levels of GDNF. Zhang et al. [31] did not find any effect of medication in GDNF levels. Conversely, data from *in vitro* studies demonstrate increased levels of GDNF in the presence of antidepressants and mood stabilizing agents [4,8,11,24]. Due to the high probability of disease relapse upon drug withdrawal, it is not recommended or ethically acceptable to interrupt drugs in order to rule out the impact of psychotropic drugs in BD patients. Second, it is unknown whether peripheral GDNF levels correlate with GDNF concentration in the central nervous system [12,16]. The number of subjects included in the present study and the strict inclusion criteria must be considered strengths of our work.

In conclusion, our results are in line with previous studies and suggest the role of neurotrophic factors in BD physiopathology.

Contributors

IGB evaluated all subjects and wrote the first draft of the manuscript. RBH selected patients to entry the study. LPS and MEB helped to design the study and contributed to its discussion. LAC contributed to discussion as well. MNS undertook the statistical analyses. NPR performed the laboratory measurements. ALT conceived and managed the study. All authors approved the final version of the manuscript.

Role of the funding source

Brazilian funding agencies had no participation in the study design and in the interpretation of the results.

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References

[1] American Psychiatric Association, Diagnostic and Statistical Manual of Mental Disorders, Text Revision: DSM-IV-TR, fourth ed., American Psychiatric Press, Washington, DC, 2000.
[2] M.S. Airaksinen, The GDNF family: signaling biological functions and therapeutic value, Nat. Rev. Neurosci. 3 (2002) 383–394.
[3] P. Amorim, Mini International Neuropsychiatric Interview (MINI): validação de entrevista breve para diagnóstico de transtornos mentais, Rev. Bras. Psiquiatr. 22 (2000) 106–115.
[4] F. Angelucci, L. Aloe, P. Jiménez-Vasquez, A.A. Mathé, Lithium treatment alters brain concentrations of nerve growth factor, brain-derived neurotrophic factor and glial cell line-derive neurotrophic factors and glial cell line-derived neurotrophic factor in a rat model of depression, Int. J. Neuropsychopharmacol. 6 (2003) 225–231.
[5] I.G. Barbosa, R.B. Huguet, V.A. Mendonça, F.S. Neves, H.J. Reis, M.E. Bauer, Z. Janka, A. Palotás, A.L. Teixeira, Increased plasma levels of brain-derived neurotrophic factor in patients with long-term bipolar disorder, Neurosci. Lett. 475 (2010) 95–98.
[6] I.G. Barbosa, R.B. Huguet, F.S. Neves, H.J. Reis, M.E. Bauer, Z. Janka, A. Palotás, A.L. Teixeira, Impaired nerve growth factor homeostasis in patients with bipolar disorder, World J. Biol. Psychiatry 12 (2011) 228–232.

[7] R.H. Belmaker, Bipolar disorder, N. Engl. J. Med. 351 (2004) 476–486.
[8] P.S. Chen, G.S. Peng, G. Li, S. Yang, X. Wu, C.C. Wang, B. Wilson, R.B. Lu, P.W. Gean, D.M. Chuang, J.S. Hong, Valproate protects dopaminergic neurons in midbrain neuron/glia cultures by stimulating the release of neurotrophic factors from astrocytes, Mol. Psychiatry 11 (2006) 1116–1125.
[9] A. Ducray, S.H. Krebs, B. Schaller, R.W. Seiler, M. Meyer, H.R. Widmer, GDNF-family ligands display distinct action profiles on cultured GABAergic and serotonergic neurons of rat ventral mesencephalon, Brain Res. 1069 (2006) 104–112.
[10] M. Hamilton, Development of a rating scale for primary depressive illness, Br. J. Soc. Clin. Psychol. 6 (1967) 278–296.
[11] K. Hisaoka, M. Takebayashi, M. Tsuchioka, N. Maeda, Y. Nakata, S. Yamawaki, Antidepressants increase glial cell line-derived neurotrophic production through monoamine-independent activation of protein tyrosine kinase and extracellular signal-regulated kinase in glial cells, J. Pharmacol. Exp. Ther. 321 (2007) 148–157.
[12] A.J. Kastin, V. Akerstrom, W. Pan, Glial cell line-derived neurotrophic factor does not enter normal mouse brain, Neurosci. Lett. 340 (2003) 239–241.
[13] F. Kapczinski, E. Vieta, A.C. Andreazza, B.N. Frey, F.A. Gomes, J. Tramontina, M. Kauer-Sant'Anna, R. Grassi-Oliveira, R.M. Post, Allostatic load in bipolar disorder: implications for pathophysiology and treatment, Neurosci. Biobehav. Rev. 32 (2008) 675–692.
[14] F. Kapczinski, B.N. Frey, M. Kauer-Sant'Anna, R. Grassi-Oliveira, Brain-derived neurotrophic factor and neuroplasticity in bipolar disorder, Expert Rev. Neurother. 8 (2008) 1101–1113.
[15] P.Y. Lin, State-dependent decreased in levels of brain-derived neurotrophic factor in bipolar disorder: a meta-analytic study, Neurosci. Lett. 466 (2009) 139–143.
[16] C. Lundborg, M. Hahan-Zoric, B. Bilber, E. Hansson, Glial cell line-derived neurotrophic is increased in cerebrospinal fluid but decreased in blood during long-term pain, J. Neuroimmunol. 220 (2010) 108–113.
[17] C.A. Miller, Stress and depressed? Check your GDNF for epigenetic repression, Neuron 69 (2011) 188–190.
[18] H. Onodera, T. Nagata, M. Kanazawa, Y. Taguma, Y. Itoyama, Increased plasma GDNF levels in patients with chronic renal diseases, Nephrol. Dial. Transplant. 14 (1999) 1604–1605.
[19] K. Otsuki, S. Uchida, T. Watanuki, Y. Wakabayashi, M. Fujimoto, T. Matsubara, H. Funato, Y. Watanabe, Altered expression of neurotrophic factor in patients with major depression, J. Psychiatr. Res. 42 (2008) 1145–1153.
[20] G. Rajkowska, A. Halaris, L.D. Selemon, Reductions in neuronal and glial density characterize the dorsolateral prefrontal cortex in bipolar disorder, Biol. Psychiatry 49 (2001) 741–752.
[21] A.R. Rosa, B.N. Frey, A.C. Andreazza, K.M. Ceresér, A.B. Cunha, J. Quevedo, A. Santin, C. Gottfried, C.A. Gonçalves, E. Vieta, K. Kapczinski, Increased serum glial cell line-derived neurotrophic factor immunocontent during manic and depressive episodes in individuals with bipolar disorder, Neurosci. Lett. 407 (2006) 146–150.
[22] A. Saavedra, G. Baltazar, E.P. Duarte, Driving GDNF expression: the green and red traffic lights, Prog. Neurobiol. 86 (2008) 186–215.
[23] R.J. Schloesser, J. Huang, P.S. Klein, H.K. Manji, Cellular plasticity cascades in the pathophysiology and treatment of bipolar disorder, Neuropsychopharmacology 33 (2008) 110–133.
[24] Z. Shao, L.E. Dyck, H. Wang, X.M. Li, Antipsychotic drugs cause glial cell line-derived neurotrophic factor secretion from C6 glioma cells, J. Psychiatry Neurosci. 31 (2006) 32–37.
[25] D.V. Sheehan, Y. Lecrubier, K.H. Sheehan, P. Amorim, J. Janavs, E. Weiller, T. Hergueta, R. Baker, G.C. Dunbar, The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10, J. Clin. Psychiatry 59 (1998) 4–57.
[26] M. Takebayashi, K. Hisaoka, A. Nishida, M. Tsuchioka, I. Miyoshi, T. Kozuru, S. Hikasa, Y. Okamoto, H. Shinno, S. Morinobu, S. Yamawaki, Decreased levels of whole blood glial cell line-derived neurotrophic factor (GDNF) in remitted patients with mood disorders, Int. J. Neuropsychopharmacol. 9 (2006) 607–612.
[27] A.L. Teixeira, I.G. Barbosa, B.S. Diniz, A. Kummer, Circulating levels of brain-derived neurotrophic factors: correlation with mood, cognition and motor function, Biomarkers Med. 4 (2010) 871–887.
[28] R.C. Young, J.T. Biggs, V.E. Ziegler, D.A. Meyer, A rating scale for mania: reliability validity and sensitivity, Br. J. Psychiatry 133 (1978) 429–435.
[29] X. Zhang, Z. Zhang, C. Xie, G. Xi, H. Zhou, Y. Zhang, W. Sha, Effect of treatment on serum glial cell line-derived neurotrophic factor in depressed patients, Prog. Neuropsychopharmacol. Biol. Psychiatry 32 (2008) 86–90.
[30] X. Zhang, Z. Zhang, W. Sha, C. Xie, G. Xi, H. Zhou, Y. Zhang, Eletroconvulsive therapy increases glial cell-line derived neurotrophic factor (GDNF) serum levels in patients with drug-resistant depression, Psychiatry Res. 170 (2009) 273–275.
[31] X. Zhang, Z. Zang, W. Sha, C. Xie, G. Xi, H. Zhou, Y. Zhang, Effect of treatment on serum glial cell line-derived neurotrophic factor in bipolar patients, J. Affect. Disord. 126 (2010) 326–329.

Artigo 7: Increased levels of adipokines in bipolar disorder

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Increased levels of adipokines in bipolar disorder

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ABSTRACT

Bipolar disorder (BD) is associated with considerable higher chronic medical comorbidities, overweight and obesity. Adipokines are adipocyte-derived secretory factors which have functions in immune response and seem to be associated with both BD and overweight. The aim of this study was to evaluate the plasma levels of adipokines (adiponectin, resistin and leptin) and TNF-α and its receptors (sTNFR1 and sTNFR2) in BD overweight patients in comparison with overweight controls. Thirty euthymic BD type-I patients and thirty controls matched by age, gender and body-mass index (BMI) were assessed by Mini-International Neuropsychiatric Interview, Young Mania and Hamilton Depression rating scales (YMRS and HDRS, respectively). Plasma levels of adiponectin, resistin, leptin, TNF-α and its soluble receptors were measured by ELISA. BD patients presented increased plasma levels of adiponectin ($p < 0.001$), leptin ($p < 0.001$) and sTNFR1 (p 0.01). Plasma levels of adipokines were not correlated neither with clinical parameters nor TNF-α, sTNFR1 and sTNFR2 plasma levels. This study provides further support to the hypothesis of the immune/inflammatory imbalance in BD.

1. Introduction

Bipolar disorder (BD) is a medical illness characterized by episodic recurrent mania or hypomania and major depression (Belmaker, 2004). BD patients present more chronic medical comorbidities than controls, resulting in increased premature mortality from cardiovascular, respiratory, and endocrine causes (Roshanaei-Moghaddam & Katon, 2009; Carney & Jones, 2006; Krishnan, 2005). Clinical and epidemiological studies have also shown that more than half of BD patients are either overweight or obese (McElroy et al., 2002; Elmslie et al., 2000).

Despite mood stabilizers and antipsychotics are associated with increased weight, since the pre-psychopharmacology era, authors, such as Kretschmer, associated a somatic typology, i.e. *habitus picnicus* characterized by abdominal fat pattern, with an increased risk for developing manic-depressive psychosis (JAMA, 1968). Recent evidence shows that BD illness is associated with increased weight regardless of treatment with antipsychotics and mood stabilizers (Maina et al., 2008).

The neurobiological mechanisms underlying the relationship between obesity and mood symptoms in BD are still unknown. A series of mechanisms may sustain the relationship between BD and overweight and obesity, including endocrine dysregulation, behavioral patterns, like physical inactivity and overeating, and pro-inflammatory state (Fagiolini et al., 2008; Soczynska et al., 2011). There is accumulating evidence that monocyte–macrophage system, T cell system and inflammatory pathways are altered in BD (Berk et al., 2011; Dean et al., 2011; Drexhage et al., 2011; Goldstein et al., 2009). Obesity is also considered a chronic low-grade pro-inflammatory state (Ouchi et al., 2011; Karalis et al., 2009).

Adipokines, like adiponectin, resistin and leptin, are mediators produced by the adipose tissue that play several roles in energetic homeostasis, insulin sensitivity and also the immune response (Ouchi et al., 2011; Soczynska et al., 2011). Despite the vast literature showing the role of adipokines in obesity, their role in BD is still undetermined. Their relation with other inflammatory parameters is also under investigated in BD. Therefore the aim of the present study was to investigate adipokines levels and inflammatory parameters in a sample of euthymic BD type I patients in comparison with controls.

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2. Methods

2.1. Subjects

This study included thirty euthymic BD type I patients and thirty controls matched by age, gender and educational level. Given that circulating levels of adipokines in humans reflect the degree of their adiposity (Karalis et al., 2009), BD patients were also matched to controls by body mass index (BMI). Patients were recruited from an outpatient psychiatric clinic specialized in BD. All patients were medicated. All subjects were clinically evaluated with the Mini-International Neuropsychiatric Interview (M.I.N.I.-Plus) (Amorim, 2000; Sheehan et al., 1998). The severity of depressive and manic symptoms was assessed, respectively, with the Hamilton Depression Rating Scale, 17-item version (HDRS) (Hamilton, 1967) and the Young Mania Rating Scale (YMRS) (Young et al., 1978). Euthymia was defined by YMRS and HDRS scores lower than 7 points, for at least four weeks. Participants were excluded if they presented mood disorders due to a general medical condition, infectious or autoimmune diseases. In addition, participants who had used corticosteroids, anti-inflammatory or antibiotics in the four weeks prior to the study were excluded.

Clinical assessment of subjects included the collection of demographic and clinical data, and anthropometric measurement. BMI was calculated by dividing the weight (in kilograms) by the squared height (in meters) (BMI = kg/m^2). All subjects included were overweight. Overweight was defined as a BMI greater than or equal to 25 (WHO, 2011).

Written informed consent was obtained from all participants. The local institutional review board approved the study, which is in accordance with the Helsinki Declaration of 1975. All participants were at least 18 years old.

2.2. Samples

Ten milliliters of blood were drawn from each subject by venipuncture into a sodium heparin tube (Vacuplast, Huangyn, China) on the same day of the clinical assessment. All procedures were performed between 8 and 10 am to minimize biological differences due to glucocorticoid variation and circadian rhythms (Sukumaran et al., 2011). The blood was immediately centrifuged at 3000 g for 10 min, 4 °C, twice. The plasma was collected and stored at −80 °C until assayed.

Plasma levels of adiponectin, resistin, leptin, tumor necrosis factor alpha (TNF-α) and its soluble receptors (sTNFR1 and sTNFR2) were measured by enzyme-linked immunosorbent assay (ELISA), according to the procedures supplied by the manufacturer (DuoSet, R&D Systems, Minneapolis, MN, USA). All samples were assayed in duplicate. Detection limits were defined at 5 pg/mL for adiponectin, resistin, leptin; 3 pg/mL for TNF-α; and 10 pg/mL for sTNFR1 and sTNFR2. Concentrations are expressed as pg/mL.

2.3. Data analysis and statistical evaluation

Association between dichotomous variables was assessed by chi-square test or Fisher's exact test when appropriate. All variables were tested for normality of distribution by means of the Shapiro–Wilk test. Two groups were compared by *t*-test when the variables were normally distributed. Pearson's correlation analyses were performed to examine the relationship of adiponectin, resistin and leptin levels with age, years of study, length of illness, BMI, YMRS, HDRS, plasma levels of TNF-α, sTNFR1 and sTNFR2. All statistical tests were two-tailed using a significance level of $\alpha = 0.05$. Outliers were defined as values higher than two standard deviations from the mean were excluded from the analysis. Statistical analyses were performed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA).

3. Results

Demographic and clinical features of all participants ($n = 60$) are shown in Table 1. There was no significant difference between BD patients and controls regarding age, gender and educational level. The mean BMI of the control group was 28.25 kg/m^2 (SD = 5.74), and mean BMI of patients was 29.14 kg/m^2 (SD = 6.94), not differing statistically.

BD patients presented mean YMRS and HDRS 1.13 (SD = 1.63) and 1.87 (SD = 2.87), respectively. The mean age of the first mood episode was 24.14 (SD = 8.56) years and the mean length of disease was 25.54 (SD = 12.56) years. Seventy percent of patients were in use of at least two mood stabilizers, 43% of patients were in use of lithium, 60% were in use of anticonvulsants and 70% using antipsychotics. BD patients did not differ from controls in the frequency of arterial hypertension and diabetes mellitus. BD patients presented higher frequency of dyslipidemia ($p = 0.04$) and hypothyroidism ($p = 0.005$).

BD patients presented higher adiponectin ($p < 0.001$) (Table 1 and Fig. 1A), leptin (pnbspltnbsp0.001) (Table 1 and Fig. 1B) and sTNFR1 plasma levels (pnbsp=nbsp0. 01) (Table 1) when compared to controls. There was no significant difference between BD patients and controls in plasma levels of resistin, TNF-alpha and sTNFR2.

Plasma levels of adipokines, TNF-α and its soluble receptors (sTNFR1 and sTNFR2) did not differ among BD patients according to the presence of psychiatric comorbidities (*i.e.* generalized anxiety disorder, panic disorder or social phobia), dependence of substances (including nicotine, alcohol, cannabis or cocaine).

Also, plasma levels of adipokines did not differ among BD patients according to the presence of medical comorbidities (*i.e.* arterial hypertension, diabetes mellitus, dyslipidemia or hypothyroidism) or medications in use (*i.e.* lithium, anticonvulsants, antipsychotics, antihypertensives, antidiabetogenic drugs, antilipidemic drugs or thyroid hormones). Plasma levels of adipokines were not correlated with age, years of study, length of illness, BMI, YMRS, HDRS, plasma levels of TNF-α, sTNFR1 and sTNFR2.

4. Discussion

In the present study, BD patients exhibited increased plasma levels of adiponectin, leptin and sTNFR1 in comparison with controls. The increased levels of adiponectin, leptin and sTNFR1 were not explained by group differences in gender, age or BMI. Moreover psychiatric medication, psychiatric or medical comorbidities did not seem to interfere with the levels of these molecules.

The present study is concordant with Elmslie et al. (2009) that found increased adiponectin plasma levels in overweight BD patients in comparison with overweight controls. Conversely, Hung et al. (2007) reported decreased levels of adiponectin in non-obese depressive BD patients in comparison with controls. Adiponectin is known as an anti-inflammatory adipokine exclusively synthesized by adipocytes and is present at high levels in the blood (Ouchi et al., 2011). Adiponectin levels are usually decreased in the circulation of obese individuals as its expression is downregulated in dysfunctional adipocytes within the context of obesity (Ouchi et al., 2011; Soczynska et al., 2011; Meier & Gressner, 2004). As BMI was controlled in BD patients and controls, the increased plasma levels of adiponectin in BD is somehow surprising. Further, there was no association between adiponectin levels and medications in use, comorbid psychiatric or medical conditions, suggesting that higher adiponectin plasma levels seem to be linked to BD illness.

Table 1
Clinical and demographic features of controls and BD euthymic patients.

Variables	Control subjects ($N = 30$)	BD patients ($N = 30$)	p value
Female gender (%)	60.0	76.7	0.17‡
Age in years (mean ± SD)	47.13 ± 7.36	49.03 ± 10.87	0.55†
Body mass index in kg/m^2 (mean ± SD)	28.26 ± 5.74	29.14 ± 6.94	0.60†
Educational level in years (mean ± SD)	9.13 ± 3.22	9.73 ± 3.48	0.49†
Medical comorbidities			
Arterial hypertension (%)	16.7	30.0	0.36‡
Diabetes Mellitus (%)	10.0	26.7	0.18‡
Dyslipidemia (%)	13.3	40.0	0.04‡
Hypothyroidism (%)	0.0	26.7	0.005‡
Adipocine levels pg/mL (mean ± SD)	8282.48 ± 5335.94	37,013.08 ± 4107.97	<0.001†
Resistin levels pg/mL (mean ± SD)	2293.17 ± 1007.29	2147.21 ± 1068.63	0.59†
Leptin levels pg/mL (mean ± SD)	1485.27 ± 626.92	2130.88 ± 359.20	<0.001†
TNF-alpha levels pg/mL [median (P25-P75)]	76.50 (10.25–233.75)	115.00 (5.25–295.00)	0.54††
sTNFR1 levels pg/mL (mean ± SD)	666.00 (493.00–1794.25)	1288.50 (701.50–2344.20)	0.01††
sTNFR2 levels pg/mL (mean ± SD)	2327.00 (1524.50–4334.50)	2722.50 (1216.00–4001.50)	0.73†

Legend: † Pearson chi-square, ‡ Student t-test; †† Mann–Whitney test.

Conflicting results also emerge in relation to plasma levels of leptin in BD. Three studies found decreased levels of leptin in BD patients (Kurt et al., 2007; Atmaca et al., 2002a,c), while two others showed similar leptin levels in comparison with controls (Tsai et al., 2007; Gergerlioglu et al., 2006). The result in the present study is in line with Tsai et al. (2007) which showed increased leptin levels in BD patients after remission from a manic episode. Leptin is involved in the regulation of food intake, body weight and energy expenditure, with increased levels being consistently reported in obesity (Meier and Gressner, 2004).

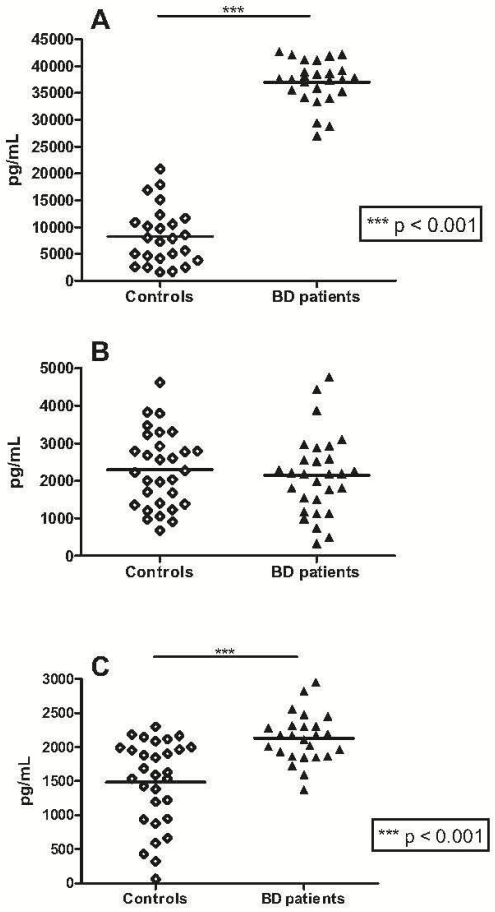

Fig. 1. Plasma levels of adipokines in euthymic BD patients in comparison with controls. A. Adiponectin; B. Resistin; C. Leptin.

In the present study the main factor associated with adiponectin and leptin levels, *i.e.* BMI, was controlled in BD patients and controls. Therefore a subjacent mechanism in BD patients may explain the increased levels of these adipokines. Adiponectin and leptin elevation could be associated with the imbalance of the inflammatory state observed in BD patients (Berk et al., 2011; Goldstein et al., 2009). Leptin is considered a pro-inflammatory cytokine, being associated with TNF-α (Ouchi et al., 2011). Otherwise, increased adiponectin levels have been appointed as one of the mechanisms responsible to decrease pro-inflammatory cytokines, notably TNF-α (Soczynska et al., 2011; Ouchi et al., 2011). The mechanisms underlying this effect are not completely understood, but may involve decrease of nuclear factor-kappa B (NFκB) activation, a transcription factor which plays a pivotal role in the regulation of inflammatory/immune responses (Ouchi et al., 2000). Therefore, increase in adiponectin levels could be one mechanism to compensate the elevation of inflammatory parameters in BD. Indeed, the inflammatory response is increased in BD patients (Berk et al., 2011; Goldstein et al., 2009). In the present study we confirmed that BD patients presented increased plasma levels of sTNFR1, despite no altered levels of TNF-α (Barbosa et al., 2011). TNF-α is less stable than its soluble receptors (sTNFRs), being degraded soon after release in peripheral tissues (Kronfol & Remick, 2000). As sTNFRs are induced by TNF-α, their increased concentrations in plasma may reflect the activity of TNF-α, even when TNF-α itself is not detected (Coelho et al., 2008). In line with a previous study, plasma levels of sTNFR1, but not sTNFR2, were increased in BD patients (Barbosa et al., 2011). TNFR1 has a ubiquitous distribution and mediates most actions of TNF-α. By contrast, TNFR2 seems to be confined mainly in hematopoietic cells. Furthermore, TNF-α binding to TNFR1 may result in activation of NFκB. TNFR2 does not directly engage NFκB activation, but the stimulation of this receptor induces endogenous membrane bound TNF-α that subsequently activates TNFR1 (Figiel, 2008).

There are some limitations in our study. (i) All patients were medicated, and adipokine levels could be influenced by mood stabilizers and antipsychotics (Peña et al., 2008; Himmerich et al., 2005; Atmaca et al., 2002a,b,c). (ii) Some of the patients were in use of antidiabetic drugs. It is not known whether hypoglycemic drugs can increase adiponectin levels (Sofer et al., 2011; Araki et al., 2006). The use of hypoglycemic drugs was not associated with changes in adiponectin levels in our study, which is

concordant with Baptista et al. (2007) that did not find any interference of hypoglycemic drugs in adipokine levels in psychotic patients. (iii) It is not possible to exclude whether increased leptin levels in BD is associated with hypothyroidism or the use of triiodothyronine (Yun et al., 2011; Siemińska et al., 2010; Cabanelas et al., 2010). The effect of hypothyroidism or the use of triiodothyronine on adiponectin levels has not been clarified yet (Cabanelas et al., 2010; Iglesias & Díez, 2007). (iv) BD patients presented higher frequency of arterial hypertension and diabetes. Although this difference did not reach statistical significance in the present study, this trend may potentially be significant in larger samples.

5. Conclusion

In conclusion, our results corroborate the view that inflammatory mechanisms, including adipokines, may contribute to BD physiopathology. The changes in these peripheral markers in BD reinforce the idea of a systemic illness and a different profile in inflammatory mechanisms linked with obesity.

Role of the funding source

Funding agencies had no role in study delineation, data collection and analysis, decision to submit the paper to the present journal, or preparation of the manuscript.

Contributors

IGB and RBH recruited and evaluated all subjects enrolled in the study. ASM and NPR performed the laboratory measurements. IGB and PVM analyzed the data. IGB and NPR wrote the first draft of the manuscript. ALT, FK and LPS conceived, designed and managed the study. All authors revised and approved the final version of the manuscript.

Conflict of interest

The authors report no conflict of interest.

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References

Amorim P. Mini International Neuropsychiatric Interview (MINI): validação de entrevista breve para diagnóstico de transtornos mentais. Revista brasileira de psiquiatria 2000;22:106–15.
Araki T, Emoto M, Teramura M, Yokoyama H, Mori K, Hatsuda S, et al. Effect of adiponectin on carotid arterial stiffness in type 2 diabetic patients treated with pioglitazone and metformin. Metabolism: Clinical and Experimental 2006;55:996–1001.
Atmaca M, Kuloglu M, Tezcan E, Ustundag B, Bayik Y. Serum leptin and cholesterol levels in patients with bipolar disorder. Neuropsychobiology 2002a;46:176–9.
Atmaca M, Kuloglu M, Tezcan E, Ustundag B. Weight gain and serum leptin levels in patients on lithium treatment. Neuropsychobiology 2002b;46:67–9.
Atmaca M, Kuloglu M, Tezcan E, Ustundag B, Gecici O, Firidin B. Serum leptin and cholesterol values in suicide attempters. Neuropsychobiology 2002c;45:124–7.
Baptista T, Rangel N, Fernández V, Carrizo E, El Fakih Y, Uzcátegui E, et al. Metformin as an adjunctive treatment to control body weight and metabolic dysfunction during olanzapine administration: a multicentric, double-blind, placebo-controlled trial. Schizophrenia Research 2007;93:99–108.
Barbosa IG, Huguet RB, Mendonça VA, Sousa LP, Neves FS, Bauer ME, et al. Increased plasma levels of soluble TNF receptor I in patients with bipolar disorder. European Archives of Psychiatry and Clinical Neuroscience 2011;261:139–43.
Belmaker RH. Bipolar disorder. New England Journal of Medicine 2004;351:476–86.
Berk M, Kapczinski F, Andreazza AC, Dean OM, Giorlando F, Maes M, et al. Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. Neuroscience and Biobehavioral Reviews 2011;35:804–17.
Cabanelas A, Cordeiro A, Santos Almeida NA, Monteiro de Paula GS, Coelho VM, Ortiga-Carvalho TM, et al. Effect of triiodothyronine on adiponectin expression and leptin release by white adipose tissue of normal rats. Hormone and Metabolic Research 2010;42:254–60.
Carney CP, Jones LE. Medical comorbidity in women and men with bipolar disorders: a population-based controlled study. Psychosomatic Medicine 2006;68:684–91.
Coelho FM, Reis HJ, Nicolato R, Romano-Silva MA, Teixeira MM, Bauer ME, et al. Increased serum levels of inflammatory markers in chronic institutionalized patients with schizophrenia. Neuroimmunomodulation 2008;15:140–4.
Dean O, Giorlando F, Berk M. N-acetylcysteine in psychiatry: current therapeutic evidence and potential mechanisms of action. Journal of Psychiatry and Neuroscience 2011;36:78–86.
Drexhage RC, Hoogenboezem TH, Versnel MA, Berghout A, Nolen WA, Drexhage HA. The activation of monocyte and T cell networks in patients with bipolar disorder. Brain, Behavior and Immunity 2011;25:1206–13.
Elmslie JL, Silverstone JT, Mann JI, Williams SM, Romans SE. Prevalence of overweight and obesity in bipolar patients. The Journal of Clinical Psychiatry 2000;61:179–84.
Elmslie JL, Porter RJ, Joyce PR, Hunt PJ, Shand BI, Scott RS. Comparison of insulin resistance, metabolic syndrome and adiponectin in overweight bipolar patients taking sodium valproate and controls. The Australian and New Zealand Journal of Psychiatry 2009;43:53–60.
Fagiolini A, Chengappa KN, Soreca I, Chang J. Bipolar disorder and the metabolic syndrome: causal factors, psychiatric outcomes and economic burden. CNS Drugs 2008;22:655–69.
Figiel I. Pro-inflammatory cytokine TNF-alpha as a neuroprotective agent in the brain. Acta Neurobiologiae Experimentalis 2008;68:526–34.
Gergerlioglu HS, Savas HA, Celik A, Savas E, Yumru M, Tarakcioglu M, et al. Atypical antipsychotic usage-related higher serum leptin levels and disabled lipid profiles in euthymic bipolar patients. Neuropsychobiology 2006;53:108–12.
Goldstein BI, Kemp DE, Soczynska JK, McIntyre RS. Inflammation and the phenomenology, pathophysiology, comorbidity, and treatment of bipolar disorder: a systematic review of the literature. The Journal of Clinical Psychiatry 2009;70:1078–90.
Hamilton M. Development of a rating scale for primary depressive illness. The British Journal of Social and Clinical Psychololgy 1967;6:278–96.
Himmerich H, Koethe D, Schuld A, Yassouridis A, Pollmächer T. Plasma levels of leptin and endogenous immune modulators during treatment with carbamazepine or lithium. Psychopharmacology 2005;179:447–51.
Hung YJ, Hsieh CH, Chen YJ, Pei D, Kuo SW, Shen DC, et al. Insulin sensitivity, proinflammatory markers and adiponectin in young males with different subtypes of depressive disorder. Clinical Endocrinology 2007;67:784–9.
Iglesias P, Díez JJ. Influence of thyroid dysfunction on serum concentrations of adipocytokines. Cytokine 2007;40:61–70.
Karalis KP, Giannogonas P, Kodela E, Koutmani Y, Zoumakis M, Teli T. Mechanisms of obesity and related pathology: linking immune responses to metabolic stress. The FESB Journal 2009;276:5747–54.
Krishnan KR. Psychiatric and medical comorbidities of bipolar disorder. Psychosomatic Medicine 2005;67:1–8.
Kronfol Z, Remick DG. Cytokines and the brain: implications for clinical psychiatry. The American Journal of Psychiatry 2000;157:683–94.
Kurt E, Guler O, Serteser M, Cansel N, Ozbulut O, Altinbaş K, et al. The effects of electroconvulsive therapy on ghrelin, leptin and cholesterol levels in patients with mood disorders. Neuroscience Letters 2007;426:49–53.
Maina G, Salvi V, Vitalucci A, D'Ambrosio V, Bogetto F. Prevalence and correlates of overweight in drug-naïve patients with bipolar disorder. Journal of Affective Disorders 2008;110:149–55.
McElroy SL, Frye MA, Suppes T, Dhavale D, Keck Jr PE, Leverich GS, et al. Correlates of overweight and obesity in 644 patients with bipolar disorder. The Journal of Clinical Psychiatry 2002;63:207–13.
Meier U, Gressner AM. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. Clinical Chemistry 2004;50:1511–25.
[No authors listed] Ernst Kretschmer (1888–1964) body and mind. JAMA: The Journal of the American Medical Association 1968;205:929–30.
Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, et al. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. Circulation 2000;102:1296–301.
Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nature Reviews Immunology 2011;11:85–97.
Peña R, Marquina D, Serrano A, Elfakih Y, de Baptista EA, Carrizo E, et al. Frequency of abnormal correlation between leptin and the body mass index during first and second generation antipsychotic drug treatment. Schizophrenia Research 2008;106:315–9.
Roshanaei-Moghaddam B, Katon W. Premature mortality from general medical illnesses among persons with bipolar disorder: a review. Psychiatric Services (Washington, D.C.) 2009;60:147–56.
Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. The Journal of Clinical Psychiatry 1998;59(Suppl. 20):22–33.

Siemińska L, Wojciechowska C, Kos-Kudła B, Marek B, Kajdaniuk D, Nowak M, et al. Serum concentrations of leptin, adiponectin, and interleukin-6 in postmenopausal women with Hashimoto's thyroiditis. Endokrynologia Polska 2010;61:112–6.

Soczynska JK, Kennedy SH, Woldeyohannes HO, Liauw SS, Alsuwaidan M, Yim CY, et al. Mood disorders and obesity: understanding inflammation as a pathophysiological nexus. Neuromolecular Medicine 2011;13:93–116.

Sofer E, Boaz M, Matas Z, Mashavi M, Shargorodsky M. Treatment with insulin sensitizer metformin improves arterial properties, metabolic parameters, and liver function in patients with nonalcoholic fatty liver disease: a randomized, placebo-controlled trial. Metabolism: Clinical and Experimental 2011;60:1278–84.

Sukumaran S, Jusko WJ, DuBois DC, Almon RR. Mechanistic modeling of the effects of glucocorticoids and circadian rhythms on adipokine expression. The Journal of Pharmacology and Experimental Therapeutics 2011;337:734–46.

Tsai SY, Lee HC, Chen CC. Hyperinsulinaemia associated with beta-adrenoceptor antagonist in medicated bipolar patients during manic episode. Progress in Neuro-Psychopharmacology & Biological Psychiatry 2007;31:1038–43.

World Health Organization. Obesity and overweight. Fact sheet # 311, 2011 March [online]. Available from URL: http://www.who.int/mediacentre/factsheets/fs311/en/; 2011. Accessed 26.08.11.

Young RC, Biggs JT, Ziegler VE, Meyer DA. A rating scale for mania: reliability, validity and sensitivity. The British Journal of Psychiatry: The Journal of Mental Science 1978;133:429–35.

Yun JE, Won S, Mok Y, Cui W, Kimm H, Jee SH. Association of the leptin to high-molecular-weight adiponectin ratio with metabolic syndrome. Endocrine Journal 2011;58:807–15.

Artigo 8: Chemokines in bipolar disorder: trait or state?

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ORIGINAL PAPER

Chemokines in bipolar disorder: Trait or state?

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Abstract Recent evidence has suggested that inflammatory and immune mechanisms may play a role in the pathophysiology of bipolar disorder (BD). Only a few studies have assessed the profile of chemokines, a family of chemotactic cytokines related to the recruitment of leukocytes, in BD. The objective of our study was to evaluate the plasma levels of chemokines in BD patients in different mood states in comparison with healthy controls. Seventy BD type I patients (35 in euthymia and 35 in mania), and 50 healthy controls matched by age, gender, and education level were enrolled in this study. All subjects were assessed by the Mini-International Neuropsychiatry Interview and the patients by the Young Mania Rating Scale and the Hamilton Depression Rating Scale. The plasma levels of CCL2, CCL3, CCL11, CCL24, CXCL8, and CXCL10 were measured by enzyme-linked immunosorbent assay. BD patients presented higher plasma levels of CCL11 (1.69-fold increase; $p < 0.001$), CCL24 (1.40-fold increase; $p = 0.02$), CXCL10 (1.45-fold increase; $p < 0.001$) and decreased plasma levels of CXCL8 (8.68-fold decrease $p < 0.001$). Logistic regression stressed the main effect of increased plasma levels of CXCL10 (OR = 1.009, 95 % CI = 1.000–1.018, $p = 0.042$) and CCL11 (OR = 1.002, 95 % CI = 1.001–1.003, $p = 0.003$) and decreased plasma levels of CXCL8 (OR = 0.995, 95 % CI = 0.990–0.999, $p = 0.013$) to BD. This study reinforces the view that BD is associated with an immune dysfunction.

Keywords Bipolar disorder · Chemokines · Immunology · Inflammation · Mania

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Introduction

Bipolar disorder (BD) is a prevalent, severe and chronic disorder that affects not only mood, but also neurovegetative and cognitive functions [1]. Recent studies have proposed a role for the immune system in BD [2–4]. There is accumulating evidence that BD is linked to Th1/Th2 imbalance [5, 6], and a recent study demonstrated an increased number of regulatory T cells in these patients [7]. Treatment strategies targeting inflammatory mechanisms have also been identified as promising in BD [2, 8].

Chemokines, or chemotactic cytokines, are small proteins, classically defined by their ability to direct the movement of circulating leukocytes to sites of inflammation or injury. Chemokines are classified on the basis of differences in their structure and corresponding functional

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specificity. The two main families are the following: CC and CXC chemokines: CC chemokines attract mononuclear cells to sites of chronic inflammation, whereas CXC chemokines attract mainly polymorphonuclear cells to sites of acute inflammation [9]. Chemokines have been implicated not only in leukocyte recruitment, but also in apoptosis, angiogenesis, and neurogenesis [10]. In the central nervous system (CNS), chemokines and their receptors are found in microglia and neurons from the hypothalamus, nucleus accumbens, hippocampus, thalamus, cortex and cerebellum [11].

There is still scarce evidence of the involvement of chemokines in BD. For instance, the functional polymorphism A-2518G of the CCL3 gene has been found to be associated with BD illness [12]. In a *postmortem* study, the expression of CCL3 was downregulated in the dorsolateral prefrontal cortex and the parietal cortex of BD patients [13]. Only a few studies have evaluated circulating levels of chemokines in BD. BD patients in mania demonstrated increased plasma levels of CXCL8 [14], while BD patients in euthymia demonstrated increased plasma levels of CXCL10 [15].

This study aims to investigate the plasma levels of the chemokines CCL2, CCL3, CCL11, CCL24, CXCL8, and CXCL10 in BD type I patients in different mood states (i.e., euthymia and mania) compared with healthy controls. A secondary aim was to evaluate whether plasma levels of chemokines were correlated with clinical parameters or associated with BD state or trait.

Methods

Participants

This study included 70 BD type I patients (35 in euthymia and 35 in mania), and 50 healthy controls matched by age, gender and educational level. Patients were recruited from an outpatient and inpatient psychiatric clinic specializing in BD at Instituto de Previdência dos Servidores do Estado de Minas Gerais (IPSEMG), Belo Horizonte, Brazil. All patients were medicated. The local institutional review board approved the study, which is in accordance with the Helsinki Declaration of 1975. All participants were more than 18 years old. All volunteers provided their written consent after a complete explanation about the procedures involved in the research protocol was provided.

Patients and healthy controls were assessed with the Mini-International Neuropsychiatric Interview (M.I.N.I.-Plus) to confirm BD illness and other comorbid psychiatric disorders (in patients) or to exclude a history of psychiatric disorders (in controls) [16, 17]. BD patients were also examined with the Hamilton Depression Rating Scale, 17-item version (HDRS) [18] and the Young Mania Rating Scale (YMRS) [19] to characterize the severity of depressive and manic symptoms, respectively. Euthymia was defined by HDRS and YMRS scores lower than 7 points for at least eight consecutive weeks. Clinical assessment of patients included the collection of the following demographic and clinical variables: gender, age, years of study, length of disease, suicide attempts, number of hospitalizations, lifetime psychosis, medication in use, and medical comorbidities. The healthy control group was recruited from the local population and did not have any personal psychiatric disorder (evaluated through M.I.N.I.-Plus) or family history of psychiatry disorder, suicide attempts or completed suicide. Subjects with dementia, infectious or autoimmune diseases, or who had used steroids, anti-inflammatory drugs or antibiotics 4 weeks before venipuncture were excluded from this research protocol.

Measurement of chemokines

Ten milliliters of blood was drawn between 8 and 10 a.m. from each subject by venipuncture into a sodium heparin tube, on the same morning of their clinical assessment. The blood was immediately centrifuged at 3,000*g* for 10 min, 4 °C, twice. The plasma was collected and stored at −80 °C until assayed.

For analysis, plasma samples were thawed, and excess protein was removed by acid/salt precipitation, as routinely performed in our laboratory [20, 21]. Briefly, equal volume of plasma and 1.2 % trifluoroacetic acid/1.35 M NaCl were mixed and left at room temperature for 10 min. Samples were then centrifuged for 5 min at 3,000*g*, and the supernatants were adjusted for salt content (0.14 M sodium chloride and 0.01 M sodium phosphate) and pH (7.4), for the determination of chemokine levels. The concentration of chemokines was measured according to the procedures supplied by the manufacturer and using sandwich enzyme-linked immunosorbent assay (ELISA) according to the procedures supplied by the manufacturer (DuoSet, R&D Systems, Minneapolis, MN, USA). The detection limit of these assays was 10 pg/mL.

Statistical analysis

Statistical analyses were performed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to report socio-demographic and clinical characteristics of the sample. Association between dichotomous variables was assessed with Pearson's Chi-square test or Fisher's exact test when appropriate. All variables were tested for normality of distribution by means of the Kolmogorov–Smirnov test and all data were non-normally distributed. Differences between two groups

were compared with Mann–Whitney tests. Differences between three groups were analyzed by Kruskal–Wallis followed by Dunn's post hoc test. Spearman's correlation analysis was performed to examine the relationships between the plasma levels of chemokines with age, length of illness, YMRS, and HDRS. The magnitude of correlation was classified according to Munro (low = 0.26–0.49; moderate = 0.50–0.69; high = 0.70–0.89; very high = 0.90–1.00) [22].

Differences between BD patients and healthy controls or BD patients in mania and BD patients in euthymia were further examined with logistic regression modeling (stepwise backwards logistic regression analysis). According to the backward elimination procedure, variables with the highest p value were progressively deleted from the model. The final model retained variables with a significance level ≤ 0.05. The goodness of fit of the final model was tested by the Hosmer–Lemeshow method, and odds ratios with 95 % confidence intervals are shown for each independent variable retained in the model. All p values were two-tailed and a significance level of $\alpha = 0.05$ was chosen.

Results

Demographic, clinical features, and chemokine plasma levels

The mean age of the BD group and its mean age of first mood episode were 49.79 years ± 11.84) and 26.32 years (±9.97), respectively. The mean length of illness was 23.37 years (±12.30). Forty-five out of 70 BD patients (64.3 %) were women. The demographic and clinical features of healthy controls, BD patients in euthymia and in mania are shown in Table 1. There were no marked differences in the age of the first mood episode, length of illness, number of hospitalizations, history of psychosis or suicide attempts, medications in use or clinical comorbidities between BD patients in euthymia and in mania (Table 1).

In comparison with controls, BD patients presented higher plasma levels of CCL11 (mean ± SD, pg/ml; 980.63 ± 533.03, 579.25 ± 420.53, respectively, $p < 0.001$), CCL24 (1203.04 ± 1191.10, 856.63 ± 744.78, respectively, $p = 0.02$) and CXCL10 (197.85 ± 64.22, 136.85 ± 77.44, respectively, $p < 0.001$), but lower plasma levels of CXCL8 (49.19 ± 92.12, 425.92 ± 617.53, respectively, $p < 0.001$). When comparisons were carried out after categorizing BD patients according to mood state, the results remained unchanged, except CCL24 levels were found to be higher in euthymic BD patients in comparison with healthy controls ($p < 0.01$, post hoc analysis), but similar in mania and control groups (Fig. 1d).

Plasma levels of chemokines did not differ in BD patients according to the presence of psychiatric comorbidities or substance dependence. Plasma levels of chemokines did not differ in BD patients according to the medications in use, such as lithium, valproic acid or antipsychotics.

Correlation analysis and logistic regression models

Considering BD patients, age was positively correlated with CCL2 ($\rho = 0.31$; $p = 0.02$); and length of illness was positively correlated with CCL2 ($\rho = 0.27$; $p = 0.04$) and CCL24 ($\rho = 0.31$; $p = 0.02$).

The first model of logistic regression was performed to assess the likelihood of subjects presenting BD. The model contained four independent variables (plasma levels of CCL11, CCL24, CXCL8, and CXCL10). The full model containing all predictors was statistically significant, χ^2 (4, $N = 88$) = 31.07, $p < 0.001$, indicating that the model was able to distinguish between BD patients and healthy controls. The model as a whole explained between 39.2 % (Cox & Snell R Square) and 52.3 % (Nagelkerke R Square) of the variance of subjects, and correctly classified 77.3 % of cases. As shown in Table 2, only three of the independent variables made a unique statistically significant contribution to the model (plasma levels of CCL2, CCL11 and CXCL8).

The second model of logistic regression was performed to assess the likelihood that BD patients are in mania. The model contained two independent variables (plasma levels of CCL24 and CXCL10). The full model containing all predictors was not statistically significant, χ^2 (2, $N = 70$) = 6.21, $p = 0.05$, indicating that the model was not able to distinguish between BD patients in euthymia and BD patients in mania.

Discussion

To the best of our knowledge, this is the first study to assess a series of circulating chemokines in BD patients, including those in mania. BD patients demonstrated increased plasma levels of CCL11, CCL24, CXCL10, and decreased plasma levels of CXCL8 in comparison with healthy controls. There was no significant difference between BD patients in euthymia and in mania. Increased plasma levels of CCL11 and CXCL10 and decreased plasma levels of CXCL8 were associated with BD trait. Length of illness was correlated with CCL2 and CCL24, while age was correlated only with CCL2.

The finding of an imbalance of plasma levels of chemokines in BD patients fits with the literature describing the immunologic and inflammatory abnormalities associated with this illness. The results of the present work

Table 1 Demographic and clinical features of healthy controls ($n = 50$) and bipolar disorder (BD) patients ($n = 70$)

	Healthy controls ($N = 50$)	BD patients		p value
		Euthymia ($N = 35$)	Mania ($N = 35$)	
Female gender (frequency, %)	72.00	71.43	57.15	0.30†
Age in years (mean ± SD)	46.72 ± 9.14	47.31 ± 10.65	52.26 ± 12.59	0.71†††
Education level in years (mean ± SD)	10.08 ± 3.69	10.82 ± 3.21	9.86 ± 4.31	0.33†††
YMRS (mean ± SD)	–	1.26 ± 2.13	26.83 ± 7.00	<0.0001††
HDRS (mean ± SD)	–	2.54 ± 2.80	3.34 ± 3.84	0.64†††
Length of illness in years (mean ± SD)	–	23.66 ± 11.62	23.00 ± 13.31	0.77††
Number of hospitalizations (mean ± SD)	–	3.81 ± 3.19	4.43 ± 4.50	0.97††
Suicide attempt (frequency, %)	–	40.00	20.00	0.12‡
Lifetime psychosis (frequency, %)	–	77.14	60.00	0.20‡
Medication in use (frequency, %)				
Lithium	–	65.71	42.86	0.09‡
Anticonvulsants	–	60.00	60.00	1.00‡
Antipsychotics	–	62.86	71.43	0.61‡
Medical comorbidities (frequency, %)				
Arterial hypertension	–	22.86	28.57	0.79‡
Diabetes mellitus	–	22.86	14.29	0.54‡
Dyslipidemia	–	25.71	8.57	0.11‡
Hypothyroidism	–	11.43	25.71	0.22‡

N number, *YMRS* Young Mania Rating Scale, *HDRS* Hamilton Depression Rating Scale, *BD* bipolar disorder

‡ Fisher's exact test

† Pearson's Chi-square test

†† Mann–Whitney test

††† Kruskal–Wallis test

reinforce the concept of both Th1 and Th2 hyperactivity in BD. Regarding the Th1 profile CXCL10 levels were increased in BD patients in mania and euthymia in this study. The observation of increased CXCL10 levels is concordant with findings by Brietzke et al. [13]. It is well known that the role of CXCL10 is to promote the chemotaxis of activated Th1 cells. Th1 hyperactivity has been previously demonstrated in BD patients [5, 23, 24]. The increased plasma levels of CCL11 and CCL24 suggest Th2 hyperactivity. CCL11 and CCL24 bind to the chemokine receptor CCR3 which is expressed on eosinophils and other cell types, including mast cells and lymphocytes that secrete IL-4 and IL-5 [25]. Increased IL-4 levels and Th2 hyperactivity was previously demonstrated in BD patients as well [6, 23]. In the present study, plasma levels of CCL24 were higher in BD patients in euthymia than healthy controls and BD patients in mania. This finding is unexpected and deserves further investigation.

CXCL8 is a chemokine produced by monocytes, macrophages and endothelial cells, and increased peripheral levels have been associated with auto-immune disorders and schizophrenia [26, 27]. Peripherally, CXCL8 induces transmigration and degranulation of neutrophils [28]. Previous studies evaluating CXCL8 in BD patients are contradictory. One study identified increased plasma levels in depressive and manic states [14], while another study identified no alteration in euthymic patients [15].

Our results are in line with the concept of the activation of the immune system in BD illness. The finding that chemokine imbalance is related to BD trait and not to mood state emphasizes the view that there are neurobiological traits in BD [29]. One important issue is whether peripheral measurement of chemokines reflects what is happening in the CNS. In addition, current knowledge is limited regarding the physiological role of chemokines in the CNS and the mechanisms of regulation and balance of these molecules in psychiatric illnesses. Although previous studies have suggested that chemokine plasma levels may reflect brain chemokine secretion [30], there are no definitive conclusions on this topic. BD has been associated with progressive changes in the structure and function of the brain, and inflammatory mechanisms have been hypothesized to be one of the pathways involved in this process [3]. Moreover, anti-inflammatory therapies have been associated with potentiation of the efficacy mood stabilizing drugs [10]. Additionally, it is not known whether the

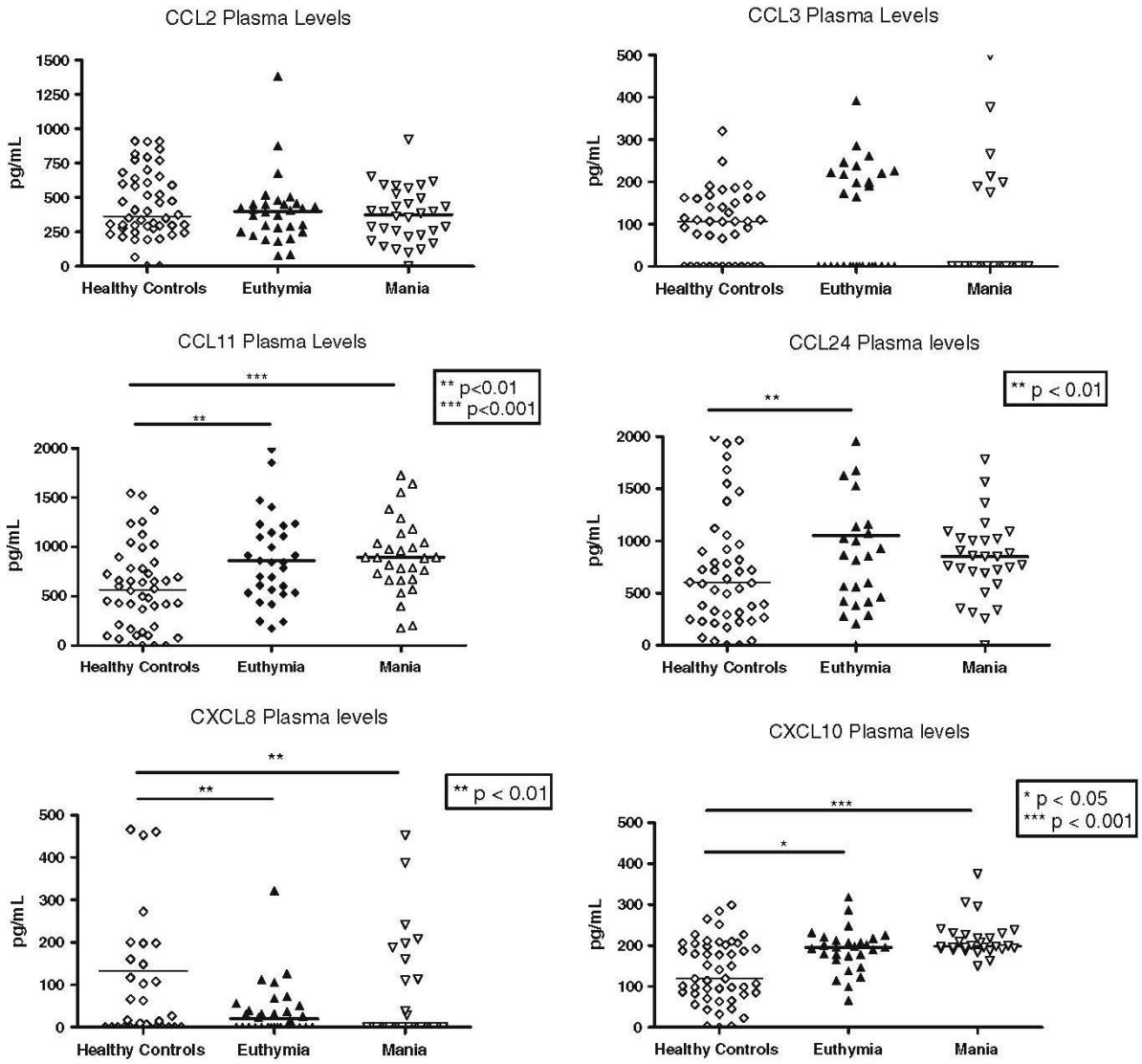

Fig. 1 Plasma levels of chemokines (pg/ml) in healthy controls ($n = 50$) and BD patients in euthymia ($n = 35$) and in mania ($n = 35$). **a** CCL2; **b** CCL3; **c** CCL11; **d** CCL24; **e** CXCL8; and **f** CXCL10. $^{*}p < 0.05$ (Kruskal–Walis, Dunn's post hoc test); $^{**}p < 0.01$ (Kruskal–Walis, Dunn's post hoc test); $^{***}p < 0.001$ (Kruskal–Walis, Dunn's post hoc test)

Table 2 Logistic regression predicting likelihood of bipolar disorder

	B	SE	Wald	*df*	*p* value	Odds ratio	95 % CI for odds ratio	
							Lower	Upper
CCL11 plasma levels	0.002	0.001	8.87	1	0.003	1.002	1.001	1.003
CXCL8 plasma levels	−0.005	0.002	6.13	1	0.013	0.995	0.990	0.999
CXCL10 plasma levels	0.009	0.005	4.12	1	0.042	1.009	1.000	1.018
Constant	−2.300	1.032	4.97	1	0.026	0.100		

immune profile is the same at the onset of the illness and after several episodes or after a long duration of BD [3]. The positive correlation of CCL2 and CCL24 levels and length of illness found in the present study reinforces the postulate of an increased pro-inflammatory state in the late stages of illness [31].

This study has strengths and limitations that must be considered for the interpretation of the results. The diagnostic interviews of both patients and controls were performed using the same protocol, overcoming a limitation of previous similar studies. In addition, sample size and the exclusion of patients with other medical conditions, such as inflammatory diseases, can be considered strengths of the study. All patients were medicated, being complex to exclude an eventual effect of the treatment on the levels of chemokines. However, the use of mood stabilizers and antipsychotics did not seem to influence chemokine levels in the present study. Other studies also failed to describe any influence of medication on inflammatory parameters in BD patients, corroborating the assumption that immunological changes in BD patients are associated with disease status [2, 32, 33]. Moreover, Liu and coworkers observed that immune parameters were significantly increased in BD patients during the pre-medication, medication, and the remission stages in comparison with controls [6]. The best approach to define trait or state in BD illness would be a longitudinal study, assessing the same patients in mania and in remission. Despite the cross-sectional design of the present study, as during euthymia and mania nearly no differences regarding the chemokine levels were found, the current results suggest that chemokines imbalance is a trait marker of BD.

In conclusion, our data reinforce the view that there is an immunological imbalance associated with BD trait.

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Conflict of interest None.

References

1. Altamura AC, Serati M, Albano A, Paoli RA, Glick ID, Dell'Osso B (2011) An epidemiologic and clinical overview of medical and psychopathological comorbidities in major psychoses. Eur Arch Psychiatry Clin Neurosci 261:489–508
2. Barbosa IG, Huguet RB, Mendonça VA, Sousa LP, Neves FS, Bauer ME, Teixeira AL (2011) Increased plasma levels of soluble TNF receptor I in patients with bipolar disorder. Eur Arch Psychiatry Clin Neurosci 261:139–143
3. Berk M, Kapczinski F, Andreazza AC, Dean OM, Giorlando F, Maes M, Yücel M, Gama CS, Dodd S, Dean B, Magalhães PV, Amminger P, McGorry P, Malhi GS (2011) Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. Neurosci Biobehav Rev 35:804–817
4. Goldstein BI, Kemp DE, Soczynska JK, McIntyre RS (2009) Inflammation and the phenomenology, pathophysiology, comorbidity, and treatment of bipolar disorder: a systematic review of the literature. J Clin Psychiatry 70:1078–1090
5. Kim YK, Myint AM, Lee BH, Han CS, Lee SW, Leonard BE, Steinbusch HW (2004) T-helper types 1, 2, and 3 cytokine interactions in symptomatic manic patients. Psychiatry Res 129(3):267–272
6. Liu HC, Yang YY, Chou YM, Chen KP, Shen WW, Leu SJ (2004) Immunologic variables in acute mania of bipolar disorder. J Neuroimmunol 150:116–122
7. Drexhage RC, Hoogenboezem TH, Versnel MA, Berghout A, Nolen WA, Drexhage HA (2011) The activation of monocyte and T cell networks in patients with bipolar disorder. Brain Behav Immun 25(6):1206–1213
8. Nery FG, Monkul ES, Hatch JP, Fonseca M, Zunta-Soares GB, Frey BN, Bowden CL, Soares JC (2008) Celecoxib as an adjunct in the treatment of depressive or mixed episodes of bipolar disorder: a double-blind, randomized, placebo-controlled study. Hum Psychopharmacol 23(2):87–94
9. Charo IF, Ransohoff RM (2006) The many roles of chemokines and chemokine receptors in inflammation. N Engl J Med 354:610–621
10. Gonzalez-Perez O, Jauregui-Huerta F, Galvez-Contreras AY (2010) Immune system modulates the function of adult neural stem cells. Curr Immunol Rev 6:167–173
11. Adler MW, Rogers TJ (2005) Are chemokines the third major system in the brain? J Leukoc Biol 78:1204–1209
12. Altamura AC, Mundo E, Cattaneo E, Pozzoli S, Del'Osso B, Vergani C, Trabattoni D, Arosio B, Clerici M (2010) The MCP-1 gene (SCYA2) and mood disorders preliminary results of a case-control association study. NeuroImmunoModulation 17:126–131
13. Nakatani N, Hattori E, Ohnishi T, Dean B, Iwayama Y, Matsumoto I, Kato T, Osumi N, Higuchi T, Niwa S, Yoshikawa T (2006) Genome-wide expression analysis detects eight genes with robust alterations specific to bipolar I disorder: relevance to neuronal network perturbation. Hum Mol Genet 15:1949–1962
14. O'Brien SM, Scully P, Scott LV, Dinan TG (2006) Cytokine profiles in bipolar affective disorder: focus on acutely ill patients. J Affect Disord 90:263–267
15. Brietzke E, Kauer-Sant'anna M, Teixeira AL, Kapczinski F (2009) Abnormalities in serum chemokine levels in euthymic patients with bipolar disorder. Brain Behav Immun 23:1079–1082
16. Amorim P (2000) Mini International Neuropsychiatric Interview (MINI): validação de entrevista breve para diagnóstico de transtornos mentais. Rev Bras Psiquiatr 22:106–115
17. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, Dunbar GC (1998) The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 59:4–57
18. Hamilton M (1967) Development of a rating scale for primary depressive illness. Br J Soc Clin Psychol 6:278–296
19. Young RC, Biggs JT, Ziegler VE, Meyer DA (1978) A rating scale for mania: reliability, validity and sensitivity. Br J Psychiatry 133:429–435
20. Teixeira AL, Reis HJ, Nicolato R, Brito-Melo G, Correa H, Teixeira MM, Romano-Silva MA (2008) Increased serum levels of CCL11/eotaxin in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 32(3):710–714
21. Scalzo P, de Miranda AS, Guerra Amaral DC, de Carvalho Vilela M, Cardoso F, Teixeira AL (2011) Serum levels of chemokines in Parkinson's disease. NeuroImmunoModulation 18:240–244
22. Munro BH (2005) Statistical methods for health care research, 5th edn. Lippincott/Raven Publishers, Philadelphia
23. Brietzke E, Stertz L, Fernandes BS, Kauer-Sant'anna M, Mascarenhas M, Escosteguy Vargas A, Chies JA, Kapczinski F (2009) Comparison of cytokine levels in depressed, manic and euthymic patients with bipolar disorder. J Affect Disord 116(3):214–217

24. Ortiz-Domínguez A, Hernández ME, Berlanga C, Gutiérrez-Mora D, Moreno J, Heinze G, Pavón L (2007) Immune variations in bipolar disorder: phasic differences. Bipolar Disord 9(6):596–602
25. Rothenberg ME, Hogan SP (2006) The eosinophil. Annu Rev Immunol 24:147–174
26. Ellman LM, Deicken RF, Vinogradov S, Kremen WS, Poole JH, Kern DM, Tsai WY, Schaefer CA, Brown AS (2010) Structural brain alterations in schizophrenia following fetal exposure to the inflammatory cytokine interleukin-8. Schizophr Res 121(1–3): 46–54
27. Zhang XY, Zhou DF, Cao LY, Zhang PY, Wu GY, Shen YC (2004) Changes in serum interleukin-2, -6, and -8 levels before and during treatment with risperidone and haloperidol: relationship to outcome in schizophrenia. J Clin Psychiatry 65(7): 940–947
28. Semple BD, Kossmann T, Morganti-Kossmann MC (2010) Role of chemokines in CNS health and pathology: a focus on the CCL2/CCR2 and CXCL8/CXCR2 networks. J Cereb Blood Flow Metab 30(3):459–473
29. Langan C, McDonald C (2009) Neurobiological trait abnormalities in bipolar disorder. Mol Psychiatry 14(9):833–846
30. Cavčić A, Tešović G, Gorenec L, Grgić I, Benić B, Lepej SZ (2011) Concentration gradient of CXCL10 and CXCL11 between the cerebrospinal fluid and plasma in children with enteroviral aseptic meningitis. Eur J Paediatr Neurol 15(6):502–507
31. Drexhage RC, Knijff EM, Padmos RC, Heul-Nieuwenhuijzen L, Beumer W, Versnel MA, Drexhage HA (2010) The mononuclear phagocyte system and its cytokine inflammatory networks in schizophrenia and bipolar disorder. Expert Rev Neurother 10(1):59–76
32. Kim YK, Jung HG, Myint AM, Kim H, Park SH (2007) Imbalance between pro-inflammatory and anti-inflammatory cytokines in bipolar disorder. J Affect Disord 104:91–95
33. Su KP, Leu SJ, Yang YY, Shen WW, Chou YM, Tsai SY (2002) Reduced production of interferon-gamma but not interleukin-10 in bipolar mania and subsequent remission. J Affect Disord 71:205–209

Artigo 9: Executive dysfunction in euthymic bipolar disorder patients and association with plasma biomarkers

Izabela Guimarães Barbosa, Natália Pessoa Rocha, Rodrigo Barreto Huguet,Rodrigo A. Ferreira, João Vinícius Salgado, Livia A. Carvalho, Carmine M. Pariante, Antônio Lúcio Teixeira.

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Brief report

Executive dysfunction in euthymic bipolar disorder patients and its association with plasma biomarkers

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ABSTRACT

Background: Despite the old Kraepelinean concept that bipolar disorder (BD) does not evolve with cognitive decline, the presence of cognitive impairment, especially executive dysfunction has been recognized in BD patients. Brain-derived neurotrophic factor (BDNF) and pro-inflammatory molecules are important contributors to the pathophysiology of BD, and imbalance in peripheral levels of these molecules may be implicated in the cognitive decline observed in BD patients. We aimed to investigate the executive performance of BD type I euthymic patients and its relation with the plasma levels of BDNF, TNF-α and its related soluble receptors (sTNFR1 and sTNFR2).

Methods: We evaluated executive functioning through the Frontal Assessment Battery (FAB). Plasma levels of BDNF, TNF-α, sTNFR1 and sTNFR2 were measured using enzyme-linked immunosorbent assay (ELISA) in 25 euthymic type I BD patients and 25 age and gender matched healthy controls.

Results: BD patients had an impairment in executive functioning ($p<0.006$), particularly sensitivity of interference ($p=0.02$), inhibitory control ($p=0.02$), and increased BDNF plasma levels ($p=0.001$) in comparison with controls. Plasma levels of TNF-α were correlated with inhibitory control in BD patients ($\rho=0.50$, $p=0.02$) while motor programming was negatively correlated with sTNFR2 plasma levels ($\rho=-0.47$, $p=0.02$) in controls. Executive function correlated with age and MMSE, but not with BDNF, neither was influenced by psychiatric and clinical comorbidities nor medications in use.

Conclusion: BDNF is altered in BD but do not correlate with executive functioning.

1. Introduction

Bipolar disorder (BD) is a severe and chronic mood disorder characterized by the presence of mania or hypomania. There is a growing body of evidence suggesting that BD patients demonstrate cognitive impairment and a compromise of memory, attention and executive function (Harvey et al., 2010; Yatham et al., 2010). Executive dysfunction seems to be present in the early stages of BD and tends to be exacerbated during depression and after manic episodes, indicating that it may regarded as a state marker of the disease

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(Elshahawi et al., 2011; López-Jaramillo et al., 2010; Torres et al., 2010).

The pathophysiology of BD and its associated cognitive impairment are not fully understood. The imbalance in neurotrophic factors and pro-inflammatory molecules has been proposed as one of the possible mechanisms involved (Goldstein et al., 2009; Kapczinski et al., 2008). Brain-derived neurotrophic factor (BDNF) and inflammatory markers have been recognized to play a role in cognitive processes, including executive function (Schofield et al., 2009; Tramontina et al., 2009). No previous studies have evaluated cognitive impairment and levels of BDNF and inflammatory markers in BD patients.

The present study aimed at investigating the cognitive performance, that is, executive functioning, of a sample of BD patients. A secondary aim was to evaluate whether plasma levels of BDNF and inflammatory markers were associated with executive functioning. We hypothesized that plasma levels of BDNF and TNF-α would be associated with poorer executive performance in BD.

2. Methods

2.1. Subjects

This study included twenty-five BD type I euthymic patients, and twenty five healthy controls matched by age, gender and level of education. Patients were recruited from an outpatient psychiatric clinic. All patients were medicated. Patients and healthy controls were assessed with the Mini-International Neuropsychiatric Interview (M.I.N.I.-Plus) to confirm BD and other comorbid psychiatry disorders (in patients) and to exclude a history of psychiatric disorders (in controls) (Amorim, 2000). The severity of depressive and manic symptoms was assessed by the 17-item version of the Hamilton Depression Rating Scale (HDRS) (Moreno and Moreno, 1998) and the Young Mania Rating Scale (YMRS) (Vilela et al., 2005), respectively. Euthymia was defined by YMRS and HDRS scores lower than 7 points, for at least 4 weeks.

Participants were excluded if they presented with a mood disorder due to a general medical condition, if they had undergone previous neurosurgery, if they had any other neurologic disorder and/or neurodegenerative condition, *delirium*, dementia, significant sensory impairment, infectious or autoimmune diseases. In addition, participants who had used corticosteroids, anti-inflammatories or antibiotics in the 4 weeks prior to the study were excluded.

Cognitive examination included the Mini-Mental State Examination (MMSE) (Brucki et al., 2003) and the Frontal Assessment Battery (FAB) (Beato et al., 2007). The MMSE is a brief battery for cognitive screening, comprising 30 items from different domains such as orientation, attention, memory and language. FAB is a brief exam to evaluate executive function and consists of six sub-tests that explore neurocognitive processes related to the frontal lobes: conceptualization; mental flexibility: motor programming; sensitivity of interference; inhibitory control; and environmental autonomy. In each sub-test, scores range from 0 (worst) to 3 (best). The total FAB score is calculated by the sum of the scores of each of the six subtests.

Written informed consent was obtained from all participants. The local institutional review board approved the study, which is in accordance with the Helsinki Declaration of 1975. All participants were at least 18 years old.

2.2. BDNF, TNF-α, sTNFR1 and sTNFR2 assessment

Ten milliliters of blood were drawn between 8 and 10 a.m. from each subject by venepuncture into a sodium heparin tube, on the same day of the clinical assessment. The blood was immediately centrifuged at 3000 *g* for 10 min, 4 °C, twice. The plasma was collected and stored at −70 °C until assayed.

Plasma levels of BDNF, TNF-α, sTNFR1 and sTNFR2 were measured by Enzyme-linked immunosorbent assay (ELISA) according to the procedures supplied by the manufacturer (DuoSet, R&D Systems, Minneapolis, MN, USA). All samples were assayed in duplicate. Lower detection limits were reported by the manufacturer as 5 pg/mL for BDNF, 3 pg/mL for TNF-α, and 10 pg/mL for sTNFR1 and sTNFR2. Concentrations are expressed as pg/mL.

2.3. Statistical analysis

Association between dichotomous variables was assessed with a chi-square test or Fisher's exact test when appropriate. All variables were tested for normality of distribution by means of the Shapiro–Wilk test and all data were non-normally distributes. Two groups (patients vs. controls) were compared by a Mann–Whitney test. Spearman's correlation analyses were performed to examine the relationship of MMSE, FAB and FAB sub-tests with age, years of study, length of illness, number of suicide attempts, number of hospitalizations, and plasma levels of BDNF, TNF-α, sTNFR1 and sTNFR2. All statistical tests were two-tailed and were performed using a significance level of $\alpha = 0.05$. Statistical analyses were performed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Socio-demographic, clinic and cognitive results

The demographic, clinical and cognitive features of all groups are shown in Table 1. No subject was diagnosed with cognitive impairment according to cut-off scores of MMSE adjusted to the educational level relevant for the Brazilian population (Brucki et al., 2003). BD patients presented a poorer performance on the FAB scores in comparison to healthy controls ($p = 0.006$). The analysis of the sub-tests demonstrated that BD patients presented lower scores in sensitivity to interference ($p = 0.02$) and inhibitory control ($p = 0.02$).

Regarding BD patients, FAB performance was negatively correlated with age ($\rho = -0.42$, $p = 0.02$), and positively correlated with years of study ($\rho = 0.45$, $p = 0.03$) and with MMSE score ($\rho = 0.46$, $p = 0.03$). There were positive correlations between sensitivity of interference and years of study ($\rho = 0.50$, $p = 0.02$) and sensitivity of interference and MMSE score ($\rho = 0.44$, $p = 0.04$). Considering healthy controls, FAB total performance, conceptualization and mental

Table 1
Clinical, demographic features and cognitive performance of BD euthymic patients and healthy.

	BD euthymic patients (n = 25)	Healthy controls (n = 25)	p Values
Gender (male/female)	8/17	11/14	0.56[a]
Age in years (mean ± SD)	50.88 ± 9.11	48.04 ± 7.08	0.21
Body Mass Index (BMI) (mean ± SD)	29.12 ± 6.64	28.77 ± 6.20	0.80
Education level in years (mean ± SD)	9.96 ± 3.60	9.88 ± 3.42	0.75
YMRS (mean ± SD)	1.08 ± 1.53	–	–
HDRS (mean ± SD)	1.52 ± 1.64	–	–
MMSE (mean ± SD)	28.48 ± 1.39	29.00 ± 1.04	0.18
FAB (mean ± SD)	12.80 ± 2.87	14.92 ± 1.91	0.006
Conceptualization	2.24 ± 0.93	2.24 ± 0.78	0.78
Mental flexibility	2.04 ± 0.84	2.36 ± 0.76	0.17
Programming	2.36 ± 1.04	2.76 ± 0.44	0.29
Sensitivity to interference	**2.40 ± 1.00**	**2.84 ± 0.62**	**0.02**
Inhibitory control	**1.00 ± 1.04**	**1.76 ± 1.16**	**0.02**
Environmental autonomy	3.00 ± 0.00	3.00 ± 0.00	1.00
Length of illness in years (mean ± SD)	27.88 ± 11.80	–	–
Age of first mood disorder (mean ± SD)	22.60 ± 7.06	–	–
Age of first manic episode (mean ± SD)	25.42 ± 8.84	–	–
Age of first depressive episode (mean ± SD)	22.15 ± 8.82	–	–
Number of hospitalizations (mean ± SD)	5.25 ± 3.28	–	–
Number of suicide attempt (mean ± SD)	3.33 ± 3.31	–	–
History of psychosis (frequency %)	80.00	–	–
Medication in use (frequency %)			
Lithium	60.00	–	–
Anticonvulsants	52.00	–	–
Antipsychotics	56.00	–	–
Typical	12.00	–	–
Atypical	44.00	–	–
Antidepressants	12.00	–	–

Abbreviations: n = number; FAB = battery assessment frontal; HDRS = Hamilton Depression Rating Scale; MMSE = mini-mental state examination; SD = standard deviation; YMRS = Young Mania Rating Scale.
[a] Qui-square test.

flexibility were positively correlated with years of study ($\rho = 0.52$, $p = 0.01$; $\rho = 0.40$, $p = 0.05$; $\rho = 0.56$, $p = 0.004$, respectively), and motor programming was positively correlated with MMSE score ($\rho = 0.44$, $p = 0.03$).

FAB and MMSE scores did not differ in BD patients according to the presence of psychiatric co-morbidities, substance dependence, previous episodes of depression or according to medication in use (data not shown).

3.2. Plasma BDNF, TNF-α, sTNFR1 and sTNFR2 levels

BD patients presented higher BDNF plasma levels than healthy controls ($p = 0.001$) (Fig. 1A). There were no significant differences between plasma levels of TNF-α, sTNFR1 and sTNFR2 between BD patients and healthy controls (Fig. 1B, C and D, respectively).

In BD patients, TNF-α plasma levels were positively correlated with inhibitory control ($\rho = 0.50$, $p = 0.02$). Considering healthy controls, motor programming was negatively correlated with sTNFR2 plasma levels ($\rho = -0.47$, $p = 0.02$). There were no other significant correlations between the molecules assessed and the other cognitive parameters evaluated.

Plasma levels of BDNF, TNF-α, sTNFR1 and sTNFR2 did not differ in BD patients according to the presence of psychiatric co-morbidities, substance dependence, and previous episode of depression or psychosis. Moreover, BDNF, TNF-α, sTNFR1 and sTNFR2 plasma levels did not differ in BD patients according to medication in use.

4. Discussion

To the best of our knowledge, this is the first study to assess simultaneously cognitive, neurotrophic and inflammatory parameters in a sample of euthymic BD patients. BD patients demonstrated an impairment in executive functions and increased BDNF plasma levels in comparison with healthy controls. Executive function was correlated with age and MMSE, but not with BDNF plasma levels, and neither was influenced by psychiatric and clinical comorbidities, nor medications in use. Interestingly, TNF-α plasma levels correlated with inhibitory control in BD patients.

BD patients in euthymia present executive functioning compromise (Glahn et al., 2007; Thompson et al., 2009). The impairment in inhibitory response is the only parameter identified as a cognitive endophenotype of BD, even present in first-degree relatives of BD patients (Bora et al., 2009). In the present study, BD patients exhibited marked impairment in tasks of sensitivity to interference and inhibitory control.

FAB scores were negatively correlated with age. This correlation may be explained either by the number of mood episodes in BD patients or by age-related cognitive changes (Zhou et al., 2011). It is well known that high numbers of mood episodes seem to be "toxic" to the brain and, hence, to be associated with cognitive impairment in BD patients (Kapczinski et al., 2010). In the present study, however, the number of psychiatric hospitalizations (an indirect index of severe mood episodes) did not influence cognitive performance.

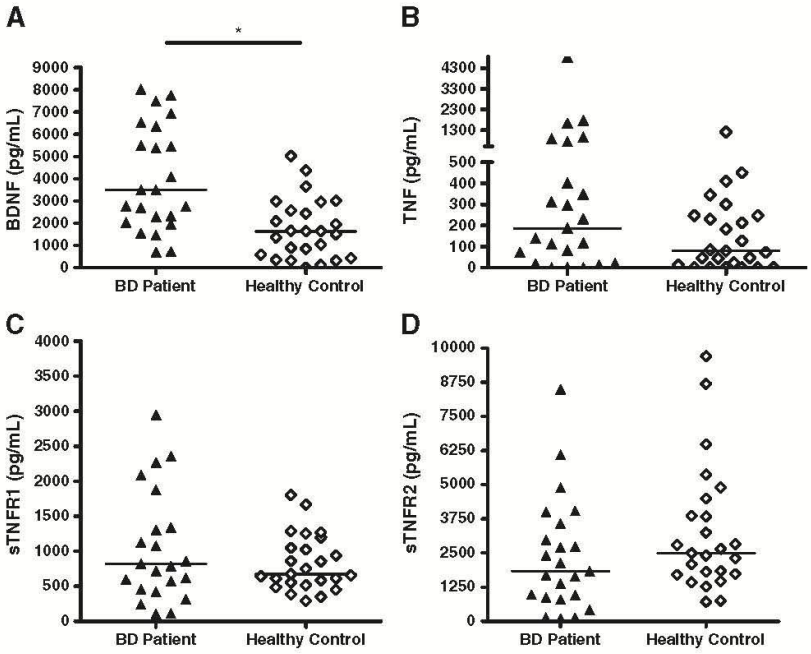

Fig. 1. A. Plasma BDNF levels in BD patients in euthymia and healthy controls. B. Plasma TNFα levels in BD patients in euthymia and healthy controls. C. Plasma sTNFR1 levels in BD patients in euthymia and healthy controls. D. Plasma sTNFR2 levels in BD patients in euthymia and healthy controls.

Plasma levels of BDNF were increased in BD patients. This result has been shown before with patients with a similar duration of disease to the current study (Barbosa et al., 2010), and seems to be associated with the long-term use of mood stabilizing agents and/or represents a reaction to the cerebral damage that occurred in the early years of the disease (Manji et al., 2000; Schulte-Herbrüggen et al., 2008). It is well known that mood and cognition are affected in BD and BDNF is a neurotrophic factor of paramount importance in them (Post, 2007). The lack of association between BDNF levels and executive parameters in the present work reinforces the view that cognitive functioning depends on a complex network involving neuronal circuits, neurotransmitters and neurotrophic factors, rather than a single molecule or marker (Reichardt, 2006; Sandi, 2004).

Several studies have consistently demonstrated elevated levels of inflammatory molecules during manic and depressive states, however the results are more controversial regarding euthymia (Kauer-Sant'anna et al., 2009; Kim et al., 2007; Ortiz-Domínguez et al., 2007). In the present work, levels of TNF-α and its soluble receptors in euthymic BD patients were similar to control subjects. TNF-α plasma levels were correlated with inhibitory control in BD. This finding is interesting, but merits further investigation as it is not possible to rule out a type I error due to multiple comparisons.

Some limitations of the present study must be considered. The small sample sizes prevented more sophisticated data analysis and limited the statistical power of the study. As all patients were medicated, it was impossible to exclude the effect of treatment on cognitive performance and plasma levels of the molecules assessed. It is worth mentioning the prospective study by Mur et al. (2008) that did not identify any influence of lithium on the executive dysfunction in BD patients in comparison with healthy controls. Another characteristic which was not controlled for in this study and which may have influenced cognitive performance was low premorbid intellectual functioning. We controlled for educational level, which correlates highly with premorbid IQ. Another limitation of this study is the lack of a comprehensive neuropsychological evaluation including other cognitive measures like visual and verbal memory. By contrast, the strict exclusion criteria and the analysis of cognitive, neurotrophic and inflammatory parameters together can be regarded as strengths of the study.

Impaired executive functioning in stable BD patients was not associated with BDNF plasma levels but it could be associated with a pro-inflammatory state, at least in specific cognitive measures related to inhibitory control. The inclusion of larger samples and more comprehensive neurocognitive batteries would allow for further testing of this hypothesis.

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Conflict of interest

All authors declare that they have no conflicts of interest.

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References

Amorim, P., 2000. Mini International Neuropsychiatric Interview (MINI): validação de entrevista breve para diagnóstico de transtornos mentais. Revista Brasileira de Psiquiatria 22 (3), 106–115.

Barbosa, I.G., Huguet, R.B., Mendonça, V.A., Neves, F.S., Reis, H.J., Bauer, M.E., Janka, Z., Palotás, A., Teixeira, A.L., 2010. Increased plasma levels of brain-derived neurotrophic factor in patients with long-term bipolar disorder. Neuroscience Letters 475 (2), 95–98.

Beato, R.G., Nitrini, R., Formigoni, A.P., Caramelli, P., 2007. Brazilian version of the Frontal Assessment Batery (FAB): preliminary data on administration to healthy elderly. Dementia & Neuropsychologia 1, 59–65.

Bora, E., Yucel, M., Pantelis, C., 2009. Cognitive endophenotypes of bipolar disorder: a meta-analysis of neuropsychological deficits in euthymic patients and their first-degree relatives. Journal of Affective Disorders 113 (1–2), 1–20.

Brucki, S.M., Nitrini, R., Caramelli, P., Bertolucci, P.H., Okamoto, I.H., 2003. Suggestions for utilization of the mini-mental state examination in Brazil. Arquivos de Neuro-Psiquiatria 61 (3B), 777–781.

Elshahawi, H.H., Essawi, H., Rabie, M.A., Mansour, M., Beshry, Z.A., Mansour, A.N., 2011. Cognitive functions among euthymic bipolar I patients after a single manic episode versus recurrent episode. Journal of Affective Disorders 130 (1–2), 80–91.

Glahn, D.C., Bearden, C.E., Barquil, M., Barret, J., Reichenberg, A., Bowden, C.L., Soares, J.C., Velligan, D.I., 2007. The neurocognitive signature of psychotic bipolar disorder. Biological Psychiatry 62 (8), 910–916.

Goldstein, B.I., Kemp, D.E., Soczynska, J.K., McIntyre, R.S., 2009. Inflammation and the phenomenology, pathophysiology, comorbidity, and treatment of bipolar disorder: a systematic review of the literature. The Journal of Clinical Psychiatry 70 (8), 1078–1090.

Harvey, P.D., Wingo, A.P., Burdick, K.E., Baldessarini, R.J., 2010. Cognition and disability in bipolar disorder: lessons from schizophrenia research. Bipolar Disorders 12 (4), 364–375.

Kapczinski, F., Frey, B.N., Kauer-Sant'Anna, M., Grassi-Oliveira, R., 2008. Brain-derived neurotrophic factor and neuroplasticity in bipolar disorder. Expert Review of Neurotherapeutics 8 (7), 1101–1113.

Kapczinski, F., Dal-Pizzol, F., Teixeira, A.L., Magalhaes, P.V., Kauer-Sant'Anna, M., Klamt, F., Pasquali, M.A., Quevedo, J., Gama, C.S., Post, R., 2010. A systemic toxicity index developed to assess peripheral changes in mood episodes. Molecular Psychiatry 15 (8), 784–786.

Kauer-Sant'anna, M., Kapczinski, F., Andreazza, A.C., Bond, D.J., Lam, R.W., Young, L.T., Yatham, L.N., 2009. Brain-derived neurotrophic factor and inflammatory markers in patients with early vs. late-stage bipolar disorder. The International Journal of Neuropsychopharmacology 12 (4), 447–458.

Kim, Y.K., Jung, H.G., Myint, A.M., Kim, H., Park, S.H., 2007. Imbalance between pro-inflammatory and anti-inflammatory cytokines in bipolar disorder. Journal of Affective Disorders 104 (1–3), 91–95.

López-Jaramillo, C., Lopera-Vásquez, J., Gallo, A., Ospina-Duque, J., Bell, V., Torrent, C., Martínez-Arán, A., Vieta, E., 2010. Effects of recurrence on the cognitive performance of patients with bipolar I disorder: implications for relapse prevention and treatment adherence. Bipolar Disorders 12 (5), 557–567.

Manji, H.K., Moore, G.J., Rajkowska, G., Chen, G., 2000. Neuroplasticity and cellular resilience in mood disorders. Molecular Psychiatry 5 (6), 578–593.

Moreno, R.A., Moreno, D.H., 1998. Escalas de depressão de Montgomery & Asberg (MADRS) e de Hamilton (HAM-D). Revista de Psiquiatria Clínica (São Paulo) 25 (5), 262–272.

Mur, M., Portella, M.J., Martínez-Arán, A., Pifarré, J., Vieta, E., 2008. Neuropsychological profile in bipolar disorder: a preliminary study of monotherapy lithium-treated euthymic bipolar patients evaluated at 2-year interval. Acta Psychiatrica Scandinavica 118 (5), 373–381.

Ortiz-Domínguez, A., Hernández, M.E., Berlanga, C., Gutiérrez-Mora, D., Moreno, J., Heinze, G., Pavón, L., 2007. Immune variations in bipolar disorder: phasic differences. Bipolar Disorders 9 (6), 596–602.

Post, R.M., 2007. Role of BDNF in bipolar and unipolar disorder: clinical and theoretical implications. Journal of Psychiatric Research 41 (12), 979–990.

Reichardt, L.F., 2006. Neurotrophin-regulated signalling pathways. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 361 (1473), 1545–1564.

Sandi, C., 2004. Stress, cognitive impairment and cell adhesion molecules. Nature Reviews. Neuroscience 5 (12), 917–930.

Schofield, P.R., Williams, L.M., Paul, R.H., Gatt, J.M., Brown, K., Luty, A., Cooper, N., Grieve, S., Dobson-Stone, C., Morris, C., Kuan, S.A., Gordon, E., 2009. Disturbances in selective information processing associated with the BDNF Val66Met polymorphism: evidence from cognition, the P300 and fronto-hippocampal systems. Biological Psychology 80 (2), 176–188.

Schulte-Herbrüggen, O., Eckart, S., Deicke, U., Kühl, A., Otten, U., Danker-Hopfe, H., Abramowski, D., Staufenbiel, M., Hellweg, R., 2008. Age-dependent time course of cerebral brain-derived neurotrophic factor, nerve growth factor, and neurotrophin-3 in APP23 transgenic mice. Journal of Neuroscience Research 86 (12), 2774–2783.

Thompson, J.M., Gray, J.M., Crawford, J.H., Young, A.H., Ferrier, I.N., 2009. Differential deficit in executive control in euthymic bipolar disorder. Journal of Abnormal Psychology 118 (1), 146–160.

Torres, I.J., DeFreitas, V.G., DeFreitas, C.M., Kauer-Sant'Anna, M., Bond, D.J., Honer, W.G., Lam, R.W., Yatham, L.N., 2010. Neurocognitive functioning in patients with bipolar I disorder recently recovered from a first manic episode. The Journal of Clinical Psychiatry 71 (9), 1234–1242.

Tramontina, J.F., Yates, D., Magalhaes, P.V., Trentini, C., Satn'anna, M.K., Fries, G.R., Bock, H., Saraiva-Pereira, M.L., Kapczinski, F., 2009. Brain-derived neurotrophic factor gene val66met polymorphism and executive functioning in patients with bipolar disorder. Revista Brasileira de Psiquiatria 31 (2), 136–140.

Vilela, J.A., Crippa, J.A., Del-Ben, C.M., Loureiro, S.R., 2005. Reliability and validity of a Portuguese version of the Young Mania Rating Scale. Brazilian Journal of Medical and Biological Research 38 (9), 1429–1439.

Yatham, L.N., Torres, I.J., Malhi, G.S., Frangou, S., Glahn, D.C., Bearden, C.E., Burdick, V.E., Mínez-Arán, A., Dittmann, S., Goldberg, J.F., Ozerdem, A., Aydemir, O., Chengappa, K.N., 2010. The International Society for Bipolar Disorders — Battery for Assessment of Neurocognition (ISBD-BANC). Bipolar Disorders 12 (4), 351–363.

Zhou, S.S., Fan, J., Lee, T.M., Wang, C.Q., Wang, K., 2011. Age-related differences in attentional networks of alerting and executive control in young, middle-aged, and older Chinese adults. Brain and Cognition 75 (2), 205–210.

RESULTADOS ADICIONAIS

5.1. Avaliação de diferentes populações de leucócitos em pacientes com o diagnóstico de transtorno bipolar

Foram avaliados diferentes populações de leucócitos em células mononucleares de sangue periférico (CMSP) de 21 pacientes e 21 controles. As características sóciodemográficas da população estudada estão descritos na tabela 4.

Tabela 4. Características sóciodemográficas da população estudada.

	Pacientes (N = 21)	Controles (N = 21)	Valor de p
Sexo feminino (proporção %)	71,4	67,7	0,74 †
Idade em anos (média ± DPM)	55,05 ± 10,64	51,95 ± 5,12	0,43 ††
Escolaridade (média ± DPM)	10,05 ± 3,75	9,43 ± 3,61	0,29 ††
Tempo de evolução de doença (média ± DPM)	30,70 ± 14,38	---	---
Medicações em uso (proporção %)			
Lítio	67,7	---	---
Anticonvulsivantes	57,2	---	---
Antipsicóticos	33,3	---	---

Abreviações: N= número de pacientes; DPM= desvio-padrão da média

† teste do chi-quadrado de Pearson

†† teste de Mann-Whitney

Como mostrado na tabela 4, a população estudada apresenta idade média de 55,05 anos (pacientes) e 51,95 anos (controles). Pacientes não diferiram de controles em relação a gênero, idade ou escolaridade. Os pacientes apresentaram tempo longo de evolução da doença (média de 30,70 anos), e 11 entre os 21 pacientes (52,4%) estavam em uso de medicações, sendo pelo menos dois estabilizadores de humor. Entre os pacientes 15 estavam em episódio de eutimia e 6 estavam em episódio de mania.

Foram avaliados a frequência de monócitos $CD14^+$, linfócitos B $CD19^+$, linfócitos T citotóxicos $CD3^+CD8^+$, linfócitos T auxiliares $CD3^+CD4^+$ e células T reguladoras $CD4^+CD25^+FOXp3^+$ um CMSP. Na Figura 2 estão representados gráficos de ponto do tipo dot-plot demonstrativos das subpopulações analisadas (**Figura 2**).

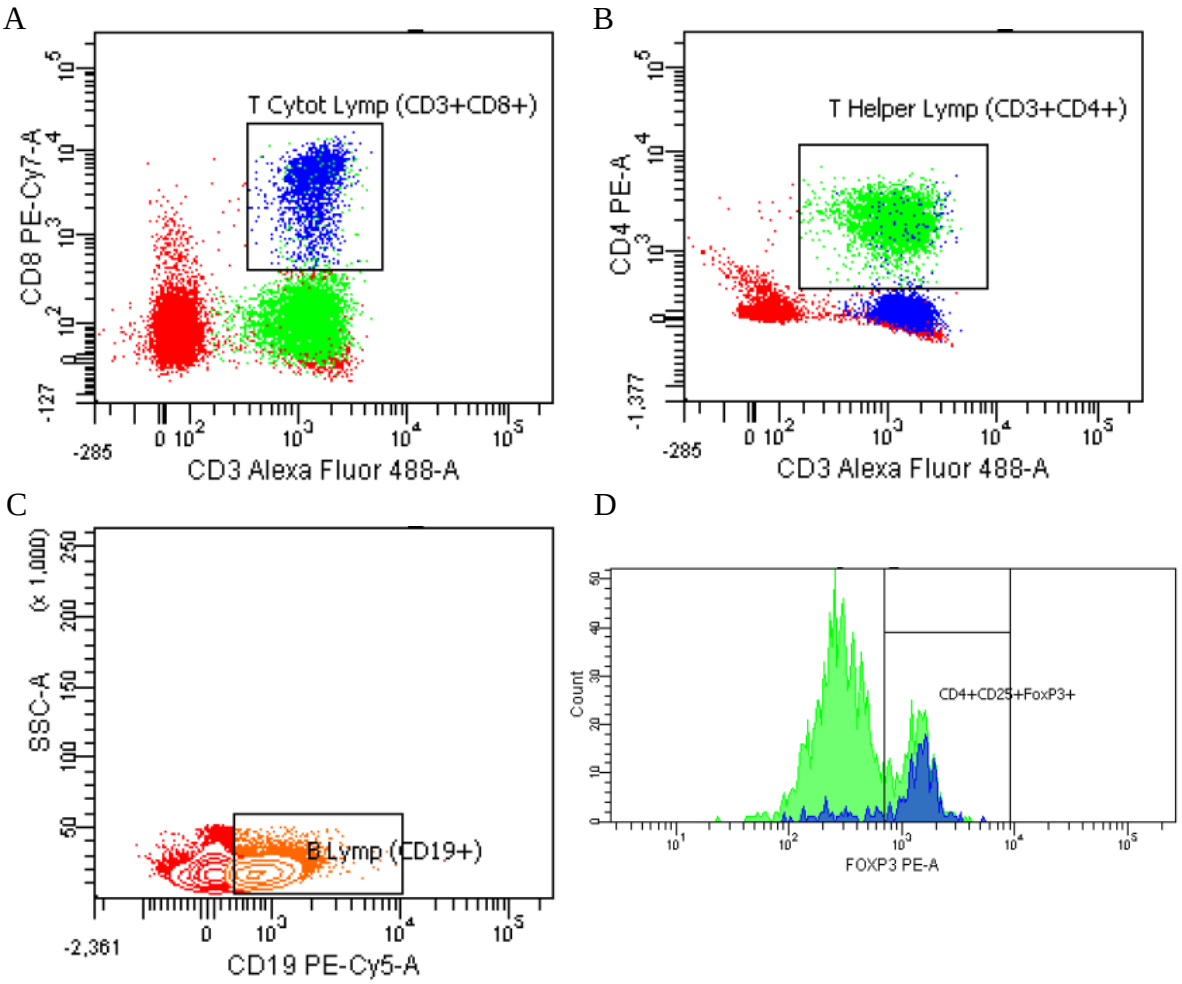

Figura 2. Estratégia de análise para as diferentes populações de CMSP. Após a seleção da população de interesse em gráficos pontuais de tamanho *versus* granulosidade (FSCxSSC), gráficos de fluorescência foram construídos para análise da frequência dos diferentes subtipos de leucócitos. Linfócitos T citotóxicos (A) e linfócitos T auxiliares (B) foram analisados em gráficos de fluorescência (FL1xFL5 e FL1xFL2, respectivamente). Os linfócitos B (C) foram analisados em gráficos de granulosidade versus fluorescência 3. Para a análise das células T reguladoras, após seleção de células duplo positivas $CD4^+CD25^+$ em gráficos de ponto tipo dot-plot, histogramas foram construídos para seleção das células $FoxP3^+$ nessa população (D).

Em pacientes com o diagnóstico de TB encontrou-se aumento na frequência de monócitos $CD14^+$ em comparação ao grupo controle (respectivamente 18,25 ± 10,42; 12,33 ± 7,65, p = 0,03, teste de Mann-Whitney) (**Figura 3a**). Quando avaliou-se os linfócitos T citotóxicos $CD3^+CD8^+$, essas células encontram-se em menor frequência em pacientes com o diagnóstico de TB quando comparado ao controle (respectivamente 12,08 ± 7,05; 18,47 ± 7,01, p = 0,04, teste de Mann-Whitney) (**Figura 3c).** Com relação aos linfócitos B $CD19^+$, linfócitos T auxiliares $CD3^+CD4^+$ e células T reguladoras $CD4^+CD25^+FOXp3^+$ os pacientes com o diagnóstico de Tb não apresentaram diferença em comparação ao controle (**Figura 3b, 3d e 3e**, respectivamente).

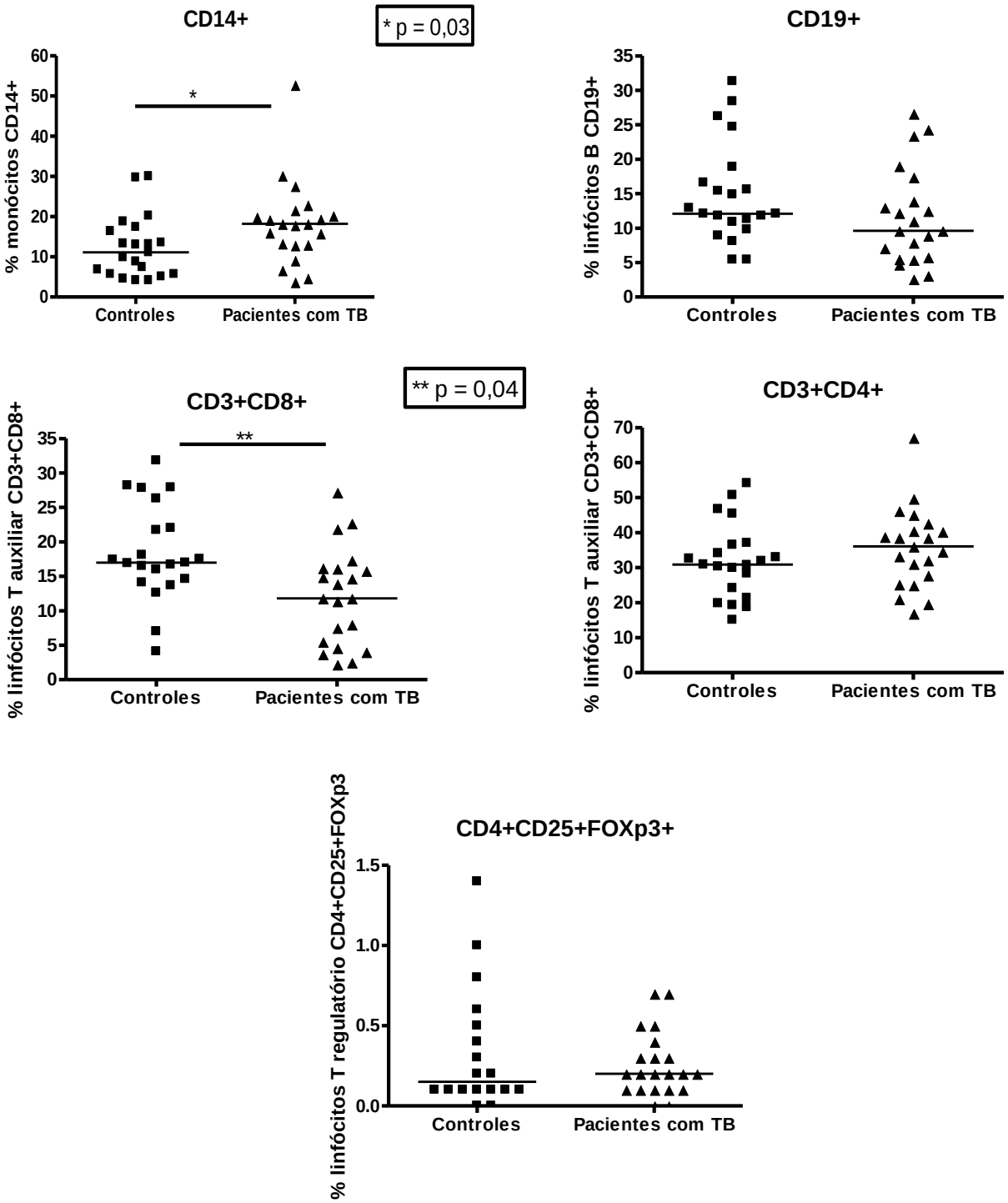

Figura 3. Frequência de leucócitos em CMSP de pacientes com diagnóstico de TB e indivíduos controles. A) monócitos $CD14^{+}$; B) linfócitos B $CD19^{+}$; C) linfócitos T citotóxicos $CD3^{+}CD8^{+}$; D) linfócitos T auxiliares $CD3^{+}CD4^{+}$ e E) células T reguladoras $CD4^{+}CD25^{+}FOXp3^{+}$ de 21 indivíduos controles (■) e 21 pacientes com diagnóstico de TB (▲).

A tabela 5 mostra a associação entre as diferentes populações leucocitárias em relação a idade e tempo de evolução de doença (**Tabela 5**). Conforme observado nesta tabela, não foram encontradas correlações entre a frequência de populações leucocitárias e a idade dos indivíduos controles, bem dos pacientes com o diagnóstico de TB. A frequência de populações leucocitárias de pacientes com diagnóstico de TB também não apresentou correlação com o tempo de evolução de (**Tabela 5**).

Tabela 5. Correlações entre a frequência de populações leucocitárias e idade e tempo de evolução de doença entre controles e pacientes com o diagnóstico de TB

		Controles	Pacientes com TB	
		Idade	Idade	Evolução
$CD14^+$	rho	0,079	0,070	- 0,142
	p	0,734	0,764	0,551
$CD19^+$	rho	-0,008	- 0,113	0,010
	p	0,972	0,626	0,966
$CD3^+CD8^+$	rho	-0,116	- 0,314	- 0,075
	p	0,617	0,165	0,753
$CD3^+CD4^+$	rho	-0,339	- 0,197	0,098
	p	0,133	0,393	0,682
$CD4^+CD25^+$	rho	0,008	- 0,256	- 0,148
	p	0,973	0,262	0,535
$CD4^+CD25^+FoxP3^+$	rho	0,144	- 0,016	- 0,149
	p	0,535	0,946	0,530

5.2. Avaliação de vias sinalizadoras intracelulares desencadeadas por citocinas pró-inflamatórias e fatores neurotróficos em pacientes com o diagnóstico de TB

Além da investigação dos níveis circulantes de moléculas ligadas a plasticidade sináptica e sobrevivência neuronal, foi realizada uma avaliação de vias intracelulares associadas a tais fenômenos e estimuladas por alteração dos fatores neurotróficos e moléculas do sistema imunológico. Para esta análise, foram incluídos 12 controles e 15 pacientes com o diagnóstico de TB em eutimia. As características sóciodemográficas da população estudada estão descritas na tabela 6.

Tabela 6. Características sóciodemográficas da população estudada

	Pacientes (N = 15)	Controles (N = 12)	Valor de p
Sexo feminino (proporção %)	73,3	75,0	0,92 †
Idade em anos (média ± DPM)	52,00 ± 8,73	53,00 ± 5,19	0,08 ††
Escolaridade (média ± DPM)	10,80 ± 3,12	8,92 ± 3,37	0,68 ††
Tempo de evolução de doença (média ± DPM)	27,13 ± 13,38	---	---
HAMD (média ± DPM)	2,20 ± 1,66	---	---
YOUNG (média ± DPM)	2,33 ± 4,40	---	---
Medicações em uso (proporção %)			
Lítio	66,7	---	---
Anticonvulsivantes	53,3	---	---
Antipsicóticos	40,0	---	---

Abreviações: N= número de pacientes; DPM= desvio-padrão da média

† teste do chi-quadrado de Pearson

†† teste de Mann-Whitney

Conforme exposto na Tabela 6, a população estudada apresenta idade média de 52,00 anos (pacientes) e 53,00 anos (controles). Pacientes com o diagnóstico de TB não diferiram de controles em relação a gênero, idade ou escolaridade. Os pacientes apresentaram tempo longo de evolução do TB (média de 27,13) anos. Os 15 pacientes estudados estavam em uso de medicações e 60% (9/15) estavam em uso de pelo menos dois estabilizadores de humor.

A análise por *Western blot* utilizando extratos proteicos obtidos de CMSP foi realizada para a avaliação dos níveis de fosforilação de p38 (**Figura 4a**) e ERK1/2 (**Figura 4b**). Conforme demonstrado na figura 4, pacientes com o diagnóstico de TB apresentaram aumento dos níveis de fosforilação de p38 e ERK1/2 em comparação aos controles. Para tal avaliação, foi utilizada a análise densitométrica dos dado, e os dados são apresentados em média ± desvio padrão médio em unidades arbitrárias. Dados de p38 (pacientes com TB: 2,47 ± 0,48; controles: 0,50 ± 0,17, $p < 0,001$) e de ERK1/2 (pacientes com TB: 3,8 ± 1,42; controles: 0,63 ± 0,18, $p = 0,003$).

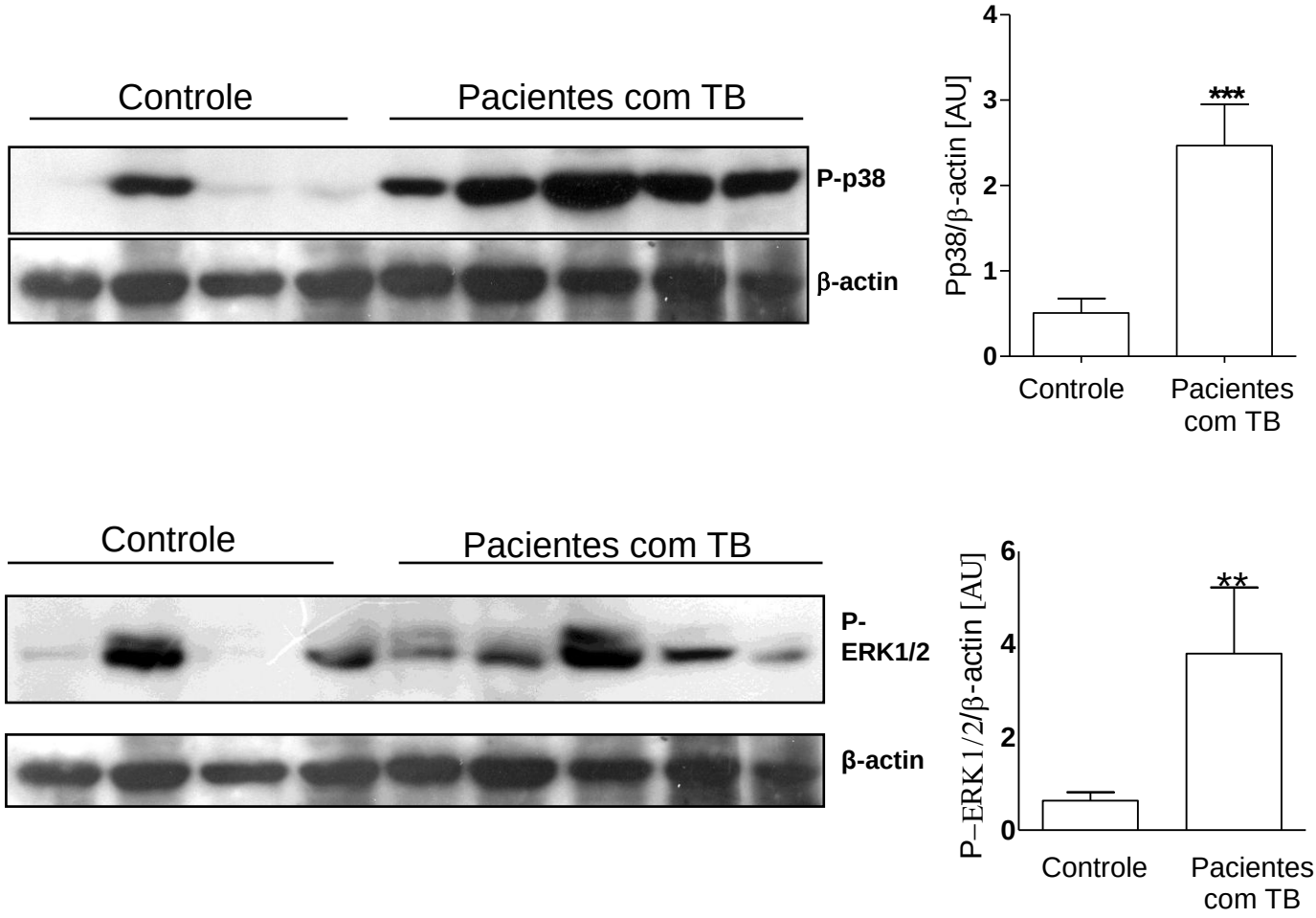

Figura 4. Análise dos níveis de fosforilação de p38 e ERK1/2 em extratos proteicos de CMSP em pacientes com o diagnóstico de TB em comparação com controles por *Western blot*. A) p38 e B) ERK1/2.

A avaliação das proteínas fosforiladas p38, ERK1/2 e NF-κB/p65 também foi avaliada em CPMS por FACS. Conforme demonstrado na figura 5, pacientes com o diagnóstico de TB apresentaram aumento na frequência de fosforilação de p38 (p = 0,01), e ERK1/2 (p = 0,04) e NF-κB/p65 (p = 0,05) em comparação com controles (**Figura 5**).

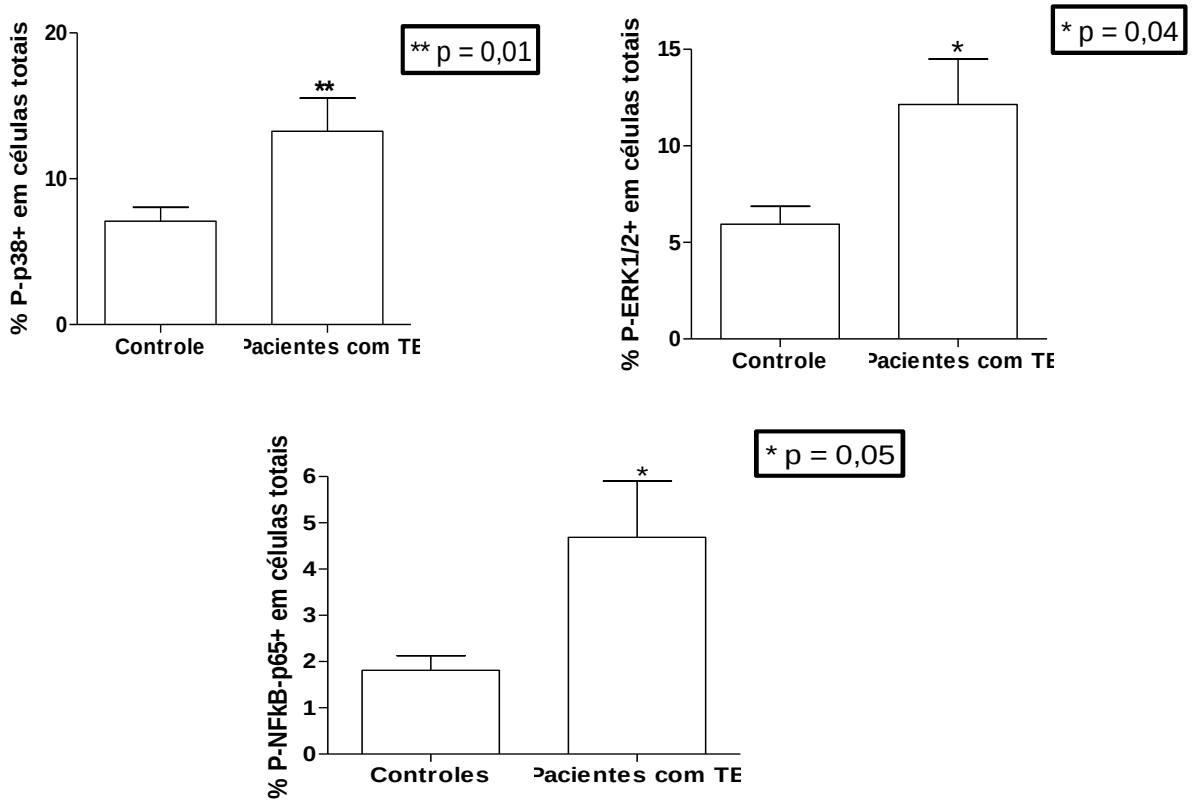

Figura 5. Frequência de proteínas fsforiladas das vias MAPKs e NF-kB por citometria de fluxo em CPMS de pacientes com o diagnóstico de TB em comparação com controles. A) p38; B) ERK1/2 e C) NF-κB/p65.

6.DISCUSSÃO

Este trabalho envolveu pacientes com o diagnóstico de TB tipo I em acompanhamento em ambulatório especializado para o tratamento de transtorno bipolar, em regime de internação hospitalar, hospital dia e controles. É um estudo transversal com o objetivo de investigaros fatores neurotróficos e marcadores imunológicos em pacientes com o diagnóstico de transtorno bipolar tipo I.

6.1. Caracterização demográfica e clínicados pacientes com o diagnóstico de transtorno bipolar

Foram incluídos no presente estudo 94 pacientes com o diagnóstico de TB tipo I. A maioria dos pacientes com o diagnóstico de TB era composta por mulheres, com idade média de 50,25 (± 12,02) anos. Apesar de os pacientes com o diagnóstico de TB apresentarem elevado grau de escolaridade10,13 (± 3,63) anos, somente 31,5% estava exercendo algum tipo de função laboral, sugerindo comprometimento ocupacional pela doença. Em revisão da literatura realizada por Huxley e Baldessarini (2007) conclui-se que entre 57 e 65 % de pacientes com o diagnóstico de TB estão desempregados. A presença de déficit cognitivo tem sido apontada como o principal fator preditor de incapacidade laborativa (DEPP et al, 2012).

A maioria dos pacientes com o diagnóstico de TB apresentou idade de início da doença por volta de 26,39 (± 10,12) anos, iniciando principalmente com episódios depressivos, assim como descrito na literatura (SOUERY et al, 2011). Os pacientes avaliados apresentavam longo tempo de evolução da doença 24,01 (± 12,77) anos e em média 4,18 internações hospitalares.

Em relação à presença de comorbidades clínicas, conforme discutido no artigo 3, a alta prevalência demonstrada no presente estudo está em conformidade com dados prévios na literatura em pacientes com o diagnóstico de TB (BEYER et al, 2005). A sobreposição de comorbidades clínicas no paciente com o diagnóstico de TB possui provável etiologia multifatorial, sendo apontados como possíveis fatores relacionados: (1) a presença de substratos neurobiológicos envolvendo a desregulação do humor e do apetite. Podem ser apontados circuitos neuronais sobrepostos ligados à regulação do

humor e do comportamento alimentar, como as conexões entre a amígdala e o córtex pré-frontal (LANGAN & MCDONALD, 2009; KILLGORE et al, 2003); (2) a hiperatividade persistente do eixo HPA em pacientes com o diagnóstico de TB relacionando a elevação da pressão arterial, aumento de resistência à insulina e dislipidemia (WHITWORTH et al, 2005; WATSON et al, 2004); (3) o efeito colateral de medicamentos psicotrópicos, particularmente os antipsicóticos atípicos (TEIXEIRA & ROCHA, 2006); (4) a presença de hábitos de vida pouco saudáveis como o consumo excessivo de carboidratos e baixas frequencias de realização de exercícios físicos (BUHAGIAR et al, 2011). Ressalta-se ainda que os pacientes com o diagnóstico de TB apresentaram, em relação à população brasileira, semelhantes taxas de hipertensão arterial sistêmica (V Brazilian Guidelines em Arterial Hypertension, 2007), taxas mais baixas de dislipidemia (DALPINO et al, 2006); e taxas mais elevadas de hipotireoidismo e diabetes mellitus (SICHIERI et al, 2007; PASSOS et al, 2005).

Em relação à presença de comorbidades psiquiátricas, conforme discutido no artigo 3, os dados refletem a prevalência de comorbidades psiquiátricas em pacientes com o diagnóstico de TB na literatura mundial (MERIKANGAS et al, 2011) e brasileira(da SILVA MAGALHÃES et al, 2010; NERY-FERNANDES et al, 2009; NEVES et al, 2009; da SILVA MAGALHÃES et al, 2009; CARDOSO et al, 2008). Os achados refletem a hipótese de que a presença de comorbidades em pacientes bipolares é mais regra do que exceção, entretanto, o motivo para tal coocorrência ainda constitui tema de debate na literatura. De acordo com o artigo 3, o transtorno de ansiedade generalizada e dependência de substâncias foram as comorbidades psiquiátricas mais comuns nos pacientes com o diagnóstico de TB.

Cerca de um terço dos pacientes apresentaram tentativas de suicídio que não se apresentaram associadas com a presença de comorbidades clínicas ou psiquiátricas.Esse dado difere do relatado em demaisestudos que demonstraram o aumento de suicídio no TB associado à presença de comorbidades psiquiátricas (NERY-FERNANDES et al, 2009; CARDOSO et al, 2008; HAWTON et al, 2005). Apesar de não sabermos a real razão para tal discrepância, apontamos como possíveis causas a não inclusão de pacientes com o diagnóstico de TB do tipo II, fato que poderia determinar o aumento da prevalência de tentativas de suicídio (BAEK et al, 2011; NEVES et al, 2009) e por nosso estudo tratar-se de um estudo retrospectivo, apresentando a possibilidade de viés de recordação.

6.2. Avaliação de fatores neurotróficos em pacientes com o diagnóstico de transtorno bipolar em comparação com controles saudáveis

No presente estudo, foram avaliados os 3 principais fatores neurotróficos (BDNF, NGF e GDNF) em pacientes com o diagnóstico de TB em comparação com controles. Foi demonstrado que pacientes com o diagnóstico de TB apresentam aumento dos níveis de BDNF; redução dos níveis de NGF, particularmente em mania; e aumento dos níveis de GDNF especialmente pacientes em eutimia. Aparentemente há um balanço diferenciado entre os diversos fatores neurotróficos no TB.

Conforme avaliado nos artigos 4 e 7,pacientes com o diagnóstico de TB em eutimia, apresentam aumento dos níveis de BDNF em comparação com pacientes com controles.

No artigo 5, pacientes com o diagnóstico de TB apresentam diminuição dos níveis de NGF em comparação com controles. Pacientes em mania apresentam diminuição dos níveis plasmáticos de NGF em comparação a pacientes em eutimia e controles saudáveis. Níveis plasmáticos de NGF estariam correlacionados negativamente com gravidade da mania e positivamente correlacionados com tempo de doença.

O artigo 6 demonstra que pacientes com o diagnóstico de TB em eutimia apresentam aumento dos níveis de GDNF em comparação com pacientes com o diagnóstico de TB em mania e controles. Níveis plasmáticos de GDNF estariam correlacionados negativamente com gravidade da mania e positivamente correlacionados com idade.

O resultado de aumento nos níveis plasmáticos de BDNF em pacientes com o diagnóstico de TB é contrário à diminuição de BDNF demonstrada em pacientes com o diagnóstico de TB, segundo evidências de duas recentes meta-análises (FERNANDES et al, 2011; LIN, 2009). O aumento de BDNF não é completamente inesperado, dado que já foi demonstrado por estudo prévio de nosso grupo (BARBOSA et al, 2010). A principal hipótese é a de que os níveis de BDNF podem apresentar variações ao longo do TB. A elevada concentração de BDNF em pacientes com longo curso de evolução de doença, conforme demonstrado no artigo 4, pode, portanto, representar uma reação cerebral aos mecanismos associados à neuroprogressão e redução dos níveis de BDNF

observado em pacientes com o diagnóstico de TB em estágios mais precoces da doença (BARBOSA et al, 2010). Corrobora com tal hipótese os resultados de pesquisas em demais doenças neuropsiquiátricas de longo tempo de evolução de doença demonstrando elevação dos níveis circulantes de BDNF (REIS et al, 2008; GAMA et al, 2007). Uma hipótese alternativa seria relacionada com o efeito de agentes estabilizadores de humor (*isto é*, lítio e valproato de sódio) em elevar os níveis de BDNF (JORNADA et al, 2010; FREY et al, 2006; YOSHIMURA et al, 2006; PILLAI et al, 2006; EINAT et al, 2003). Portanto, o aumento de BDNF em pacientes com TB, apesar de discordante com a literatura, não é completamente inesperado. Um maior número de estudos em pacientes com o diagnóstico de TB, assim como estudos longitudinais precisam ser realizados para uma melhor compreensão dos achados.

Ademais, pacientes com o diagnóstico de TB apresentam alteração no balanço dos níveis plasmáticos dos demais fatores neurotróficos avaliados. Uma possível hipótese em comum associada aos achados pode estar relacionada ao processo de dano acumulativo associado à fisiopatologia do TB (KAPCZINSKI et al, 2009). Inúmeras evidências demonstram que pacientes com TB apresentam desregulação persistente do eixo HPA (STEEN et al, 2011; WATSON et al, 2004), alteração da imunidade pró-inflamatória (BARBOSA et al, 2011) e de fatores oxidativos (ANDREAZZA et al, 2009). Apesar de as alterações serem descritas principalmente durante as chamadas "fases agudas" da doença, estas ocorreriam mesmo durante os períodos de remissão e /ou eutimia da doença. A ocorrência de repetidos episódios de humor em pacientes com o diagnóstico de TB poderia agir de forma permanente, alterando a atividade neuronal com mudanças progressivas, como as observadas em estudos de neuroimagem (POST, 2007). O longo dano acumulativo ocasionaria e seria refletido, portanto, em anormalidades crônicas demonstradas nos níveis circulantes de fatores neurotróficos e marcadores imunológicos em pacientes com TB (BERK et al, 2011).

6.3. Avaliação de marcadores imunológicos em pacientes com o diagnóstico de transtorno bipolar em comparação com controles saudáveis

No presente estudo diferentes marcadores imunológicos foram avaliados em pacientes com o diagnóstico de TB em comparação com controles. Avaliamos

especificamente três grupos de fatores imunológicos: o grupo de citocinas pró-inflamatórias (TNF-α) e seus receptores solúveis (sTNFR1 e sTNFR2); o grupo de adipocinas, citocinas produzidas por tecido adiposo; e o grupo de quimiocinas, mediadores imunológicos com a propriedade de direcionar o tráfico leucocitário. Pacientes com o diagnóstico de TB em comparação com controles apresentaram aumento dos níveis plasmáticos de citocinas pró-inflamatórias, adipocinas (especialmente adiponectina e leptina), e aumento dos níveis plasmáticos de CCL11, CCL24, CXCL10 e diminuição dos níveis plasmáticos de CXCL8.

Conforme avaliado no artigo 7, pacientes com o diagnóstico de TB em eutimia apresentam aumento da atividade pró-inflamatória, assim como de mediadores imunológicos produzidos pelo tecido adiposo.

Resultados apresentados no artigo 8 apontam que os pacientes diagnóstico de TB apresentam aumento dos níveis plasmáticos de CCL11, CCL24, CXCL10 e diminuição dos níveis plasmáticos de CXCL8. A alteração de mediadores do tráfego leucocitário não diferiu em relação a fase de humor do TB (*isto é*, mania e eutimia), sugerindo que tais marcadores se relacionam propriamente a presença do diagnóstico de TB. Ademais, níveis plasmáticos de CCL2 e CCL24 foram positivamente correlacionados com tempo de evolução do TB.

O aumento da atividade pró-inflamatória em pacientes com o diagnóstico de TB é evidenciado pelo aumento dos níveis plasmáticos de sTNFR1, apesar de não ser propriamente observado o aumento nos níveis circulantes de TNF-α. Os achados confirmam dados previamente publicados por nosso grupo (BARBOSA et al., 2011). O TNF-α é uma molécula pró-inflamatória prototípica que se liga a dois receptores, TNFR1 e TNFR2, que são os responsáveis pelos efeitos biológicos do TNF-α. O TNF-α caracteriza-se por ser uma molécula menos estável que seus receptores solúveis, sendo degradada tão logo liberada pelos tecidos periféricos (KRONFOL & REMICK,2000). Portanto, os níveis dos receptores de TNF-α refletem sua atividade, mesmo que não haja a detecção de níveis circulantes de TNF-α (COELHO et al, 2008).Conforme discutido no artigo 7, o aumento dos níveis plasmáticos de sTNFR1, seria um reflexo dos níveis plasmáticos de TNF-α. A hipótese relacionada ao aumento dos níveis de TNF-α e TNFR1 seria a estimulação das vias apoptóticas e de mecanismos de morte celular e neuronal, que, ocasionariamas mudanças progressivas no SNC de indivíduos com o diagnóstico de TB, fato ocasionando a chamada neuroprogressão do TB (BERK et al,

2011; BRIETZKE & KAPCZINSKI, 2008). O aumento de estímulos pró-inflamatórios seria exacerbado durante as fases de humor do TB, ocasionando os efeitos tóxicos exacerbados ao SNC durante tais fases (KAPCZINSKI et al, 2010).

O aumento da atividade pró-inflamatória em pacientes com TB também é observado em outros mecanismos inflamatórios além dos ligados TNF-α. O aumento do nível circulante de adipocinas, particularmente pelo aumento de leptina, conforme demonstrado no artigo 7, contribui para a manutenção do estado pró-inflamatório ligado ao TB. A leptina é considerada uma citocina pró-inflamatória, produzida principalmente na obesidade, que contribui para a atividade inflamatória crônica e para a disfunção metabólica associada à obesidade (OUCHI et al, 2011). Há uma relação de aumento dos níveis de leptina em resposta ao aumento de estímulos pró-inflamatórios, principalmente TNF-α e de citocinas com perfil Th1 (OUCHI et al, 2011; GRUNFELD et al, 1996). O aumento de leptina em pacientes com o diagnóstico de TB reforça a hipótese de hiperativação de TNF-α e de um status pró-inflamatório no TB.

Conforme os achados, as disfunções imunológicas relacionadas ao TB não são apenas relacionadas a um perfil pró-inflamatório, mas também a uma alteração no perfil Th1 e Th2 (BRIETZKE et al, 2009). Com o objetivo de avaliar tal hipótese, foi realizado o estudo de níveis plasmáticos de quimiocinas em pacientes com o diagnóstico de TB, conforme descrito no artigo 8. Pacientes com o diagnóstico de TB apresentam um aumento da atividade no perfil Th1, refletida por um aumento dos níveis plasmáticos de CXCL10. Tal achado foi previamente apontado na literatura (BRIETZKE et al, 2009). Além da hiperativação Th1 observada no TB, há ainda uma hiperatividade Th2, representada por aumento plasmático de CCL11 e CCL24 em pacientes com o diagnóstico de TB comparação com controles.

A presença de comorbidades no TB e alterações nos fatores neurotróficos e marcadores imunológicos estão associadas à neuroprogressão do TB. Um dos objetivos dos artigos 7 e 9 foi avaliar tais interrelações. No artigo 9, a presença de disfunção executiva nos pacientes com TB foi estudada por ser considerado uma marcação de estado no TB e estar presente desde os estágios iniciais da doença (HARVERY et al,2010; YATHAM et al, 2010). Demonstramos, conforme esperado, que os pacientes com o diagnóstico de TB em eutimia apresentavam um pior desempenho em teste de função executiva (FAB), particularmente em testes que avaliaram a capacidade de sensibilidade a interferência e de controle inibitório. Os níveis plasmáticos de citocinas

pró-inflamatórias (TNF-α) e se correlacionaram com os parâmetros cognitivos, apesar de níveis plasmáticos de BDNF não. Nossa hipótese seria a de que parâmetros cognitivos executivos dependem de uma complexa rede envolvendo circuitos neuronais, neurotransmissores e demais fatores que somente uma única molécula ou biomarcador (REICHARDT, 2006; SANDI, 2004). No artigo 7, avaliamos pacientes com o diagnóstico de TB, com sobrepeso e comparamos com controles com sobrepeso com o objetivo de controlar o efeito do aumento de peso nos parâmetros imunes (LUMENG & SALTIEL, 2011). O aumento plasmático de adipocinas e de sTNFR1 no presente estudo ratifica a hipótese de que alterações imunológicas ligadas ao TB, independente das alteração do peso.

6.4. Avaliação de diferentes populações leucocitárias em células mononucleares do sangue periférico em pacientes com o diagnóstico de transtorno bipolar em comparação com controles saudáveis

O aumento da frequência de monócitos (CD14+) em pacientes com TB em relação a controles retoma a antiga visão de Smith de que transtornos de humor se associariam a alterações em células macrofágicas (SMITH, 1991). Os monócitos fazem parte do sistema mononuclear de fagocitose (composto também por células dendríticas e macrófagos) que apresentam como funções principais induzir a tolerância a auto-antígenos e serem efetores da primeira linha de resposta imune contra diversos insultos (DREXHAGE et al, 2010). O achado de aumento da frequência de monócitos no TB, no presente estudo, contribui para as hipóteses de um estado pró-inflamatório ligado ao TB, e de uma maior ativação de resposta inflamatória em monócitos de pacientes com o diagnóstico de TB (PADMOS et al, 2008). De nota,o perfil demaior ativação monocitária também foi demonstrado em filhos de pacientes com o diagnóstico de TB, especialmente em crianças que desenvolveram transtornos de humor (PADMOS et al, 2008). Há algumas hipóteses que tentam explicar asassociações entre o TB e um estado de hiperativação dos monócitos: (i) O TB seria o indutor deste estadode hiperativação dos monócitos (por exemplo, o estado de estresse da doença pode induzir a ativação de monócitos); (ii) o estado de hiperativação dos monócitos poderia causar perturbações do humor (como apontado na teoria macrofágica da depressão); (iii) háum fator subjacente

que influenciaria independentemente o TB e o estado de hiperativação dos monócitos (por exemplo, pacientes e seus familiares estão presentesem um ambiente contagiante e/ou estressante que afeta tantoos seus sistemas de monócitos, bem como o SNC); (iv) é possível que existam dois fatores subjacentes, não relacionados, compartilhados no mesmo ambiente, levando a uma ativação de monócitos e ao TB (PADMOS et al, 2009). Estudos aprofundados são necessários para clarificar tais hipóteses.

Além dos monócitos, os linfócitosT também são contribuintes para a resposta imune. Evidências na literatura sobre a avaliação dos linfócitos T no TB ainda são escassas. No presente estudo, demonstrou-se que pacientes com o diagnóstico de TB em comparação com controles saudáveis apresentaram tendência a umaredução de os linfócitos T reguladores (CD4+CD25+FOXp3+). Os linfócitos T reguladores têm sido associadas à funçãode prevenir a autoimunidade e induzir a tolerância. A população de linfócitos T reguladores que expressam o fator de transcrição foxp3 (CD4+CD25+FOXp3+) são de grande interesse por suas propriedades de suprimirem a proliferação de outros linfócitos T*in vitro*, assim como de inibirem o desenvolvimentode doenças autoimunes*in vivo* (SAKAGUCHI et al, 2006). A redução relativa da frequência dos linfócitos T reguladoras (CD4+CD25+FOXp3+) em pacientes com o diagnóstico de TB já foi previamente demonstrado (DREXHAGE et al, 2011) e pode estar associada a maior associação com doenças autoimunes e autoanticorpos observados no TB (BARBOSA et al, 2009). A diminuição dos linfócitos T citotóxicas (CD3+CD8+) em pacientes com TB em comparação a controles demonstrada pode estar relacionada com a hiperatividade persistente do eixo HPA em pacientes com o diagnóstico de TB (WHITWORTH et al, 2005; WATSON et al, 2004). Portanto a hiperativação do eixo HPA estimularia em sua cascata final a liberação de glucocorticóides que modulariam o padrão das células T, resultando em um efeito imunossupressor de linfócitos T (JURURENA et al, 2004). A relevância clínica da diminuição de células linfócitos T (CD3+CD8+) em pacientes com TB ainda não está clara.

Em conclusão, as nossas observações combinadas com os dados da literatura, assim, sugerem que tanto o sistema de monócitos-macrófagoseo sistema de células T estão alterados em pacientes com o diagnóstico de TB.

6.5. Avaliação de vias sinalizadoras intracelulares desencadeadas por citocinas pró-inflamatórias e fatores neurotróficos em pacientes com o diagnóstico de TB em comparação a controles

Alterações nos níveis de fatores neurotróficos, principalmente BDNF, e de um estado pró-inflamatório exacerbado, principalmente associado a alteração dos níveis de TNF-α, interagem intracelularmente por vias em comum (FURUNO & NAKANISHI, 2006). A principal via intracelular em comum mediada por estímulos dos níveis de TNF-α e BDNF é a do NF-kB. NF-kB é um fator de transcrição presente em todos os tipos celulares, incluindo neurônios e células gliais, e tem uma função importante na promoção da sobrevivência celular. O NF-kB, em sua forma fosforilada, tem a capacidade de translocar para o núcleo celular e induzir a transcrição de genes que impedem a apoptose celular (SUN et al., 2001).O aumento da frequência de NF-kB/p65 em pacientes com o diagnóstico de TB pode estar relacionado com a contenção dos danos celulares frente às elevações dos níveis de TNF-α e estímulos pró-inflamatórios associados os TB (BARBOSA et al, 2010). A hipótese do estado de equilíbrio entre estímulos pro e anti-apoptóticos em pacientes com o diagnóstico de TB em períodos de eutimia é reforçado pelos achados do aumento de fosforilação de proteínas ligadas a via das proteínas quinases ativadas por mitógenos (as MAPKs). De nota, a via do p38 é uma das principais MAPKs associadas a estímulos apoptóticos, e a via da ERK1/2 é uma das principais MAPKs associadas a estímulos de sobrevivência intracelular (KIM & CHOI, 2010). Destaca-se, entretanto, que tal mecanismo intracelular de balanço apoptótico e anti-apoptótico pode se encontrar em desequilíbrio quando frente a estímulos tais como novos episódios de humor, que são caracterizados por seu papel tóxico so SNC (KAPCZINSKI et al, 2010).

7. CONCLUSÕES

- Pacientes com o diagnóstico de TB apresentam elevadas taxas de comorbidades psiquiátricas, principalmente relacionadas a transtornos de ansiedade e dependência de substâncias.

- Pacientes com o diagnóstico de TB apresentam aumento dos níveis de BDNF.

- Pacientes com o diagnóstico de TB apresentam redução dos níveis de NGF, particularmente em mania.

- Pacientes com o diagnóstico de TB apresentam aumento dos níveis de GDNF particularmente em estado de eutimia.

- Pacientes com o diagnóstico de TB apresentaram aumento dos níveis plasmáticos de citocinas pró-inflamatórias e adipocinas (especialmente adiponectina e leptina).

- A presença de sobrepeso não é condição suficiente para explicar as alterações imunológicas apresentadas por pacientes com o diagnóstico de TB.

- Pacientes com o diagnóstico de TB apresentam aumento da frequência de monócitos (CD14+), e redução da frequência de células T citotóxicas (CD3+CD8+).

- Vias de sinalização intracelular anti e pro-apoptóticas encontram-se em desequilíbrio em pacientes com o diagnóstico de TB em eutimia.

8. PROPOSIÇÃO E PERSPECTIVAS

O mecanismo fisiopatológico associado ao transtorno bipolar assim como a suas mudanças de estado de humor ainda permanece desconhecido. Os achados apresentados no presente trabalho apontam para o envolvimento dos fatores neurotróficos e marcadores imunológicos na fisiopatologia do TB, entretanto, a descoberta do mecanismo fisiopatológico ainda apresenta um longo caminho a ser percorrido. Algumas limitações do presente trabalho, como a inclusão de pacientes com o diagnóstico de TB majoritariamente com longo período de evolução de doença e por estudo tratar-se de estudo transversal, impedem a extrapolação dos achados para pacientes com o diagnóstico de TB em estágio inicial, assim como anão podemos inferir relações de causa/efeito. Nesse sentido, a continuidade do trabalho com a coleta de dados de pacientes em estágios variados de tempo de evolução de doença e diferentes estados de humor, estudos de neuroimagem, avaliações neuropsiquiátricas e a aplicação o desenvolvimento de técnicas de avaliação de resposta celular frente a diferentes estímulos e avaliação genética em tais pacientes poderiam contribuir para a melhor compreensão do transtorno bipolar.

9.0.REFERÊNCIAS BIBLIOGRÁFICAS

1. AIRAKSINEN M, SAARMA M. The GDNF family: signaling, biological functions and therapeutic value. **Nat Rev Neurosci**. 2002; 3: 383-94.

2. ALDERSON RF, ALTERMAN AL, BARDE YA, LINDSAY RM.Brain-derived neurotrophic factor increases survival and differentiated functions of rat septal cholinergic neurons in culture.**Neuron.** 1990;5(3):297-306.

3. ALTAMURA AC, SERATI M, ALBANO A, PAOLI RA, GLICK ID, DELL'OSSO B. An epidemiologic and clinical overview of medical and psychopathological comorbidities in major psychoses. **Eur Arch Psychiatry Clin Neurosci**. 2011;261(7):489-508.

4. AMORIM P. Mini International Neuropsychiatric Interview (MINI): validação de entrevista breve para diagnóstico de transtornos mentais. **Rev Bras Psiquiatr**. 2000; 22(3): 106-15.

5. ANDREAZZA AC, KAPCZINSKI F, KAUER-SANT'ANNA M, WALZ JC, BOND DJ, GONÇALVES CA, YOUNG LT, YATHAM LN. 3-Nitrotyrosine and glutathione antioxidant system in patients in the early and late stages of bipolar disorder. **J Psychiatry Neurosci.** 2009; 34(4): 263-71.

6. ANGST J, GAMMA A, SELLARO R, LAVORI PW, ZHANG H. Recurrence of bipolar disorders and major depression. A life-long perspective. **Eur Arch Psychiatry Clin Neurosci.** 2003; 253(5): 236-40.

7. BAEK JH, PARK DY, CHOI J, KIM JS, CHOI JS, HA K, KWON JS, LEE D, HONG KS. Differences between bipolar I and bipolar II disorders in clinical features, comorbidity, and family history. **J Affect Disord**. 2011; 131(1-3): 59-67.

8. BARBOSA IG, HUGUET RB, NEVES FS, BAUER ME, TEIXEIRA AL.Imunologia do transtorno bipolar. **J Bras Psiquiatr**. 2009;58(1):52-9.

9. BARBOSA IG, HUGUET RB, MENDONÇA VA, NEVES FS, REIS HJ, BAUER ME, JANKA Z, PALOTÁS A, TEIXEIRA AL.Increased plasma levels of brain-derived neurotrophic factor in patients with long-term bipolar disorder.**Neurosci Lett**. 2010;475(2):95-8.

10. BARBOSA IG, HUGUET RB, MENDONÇA VA, SOUSA LP, NEVES FS, BAUER ME, TEIXEIRA AL.Increased plasma levels of soluble TNF receptor I in patients with bipolar disorder.**Eur Arch Psychiatry Clin Neurosci**. 2011;261(2):139-43.

11. BARBOSA IG, HUGUET RB, NEVES FS, REIS HJ, BAUER ME, JANKA Z, PALOTÁS A, TEIXEIRA AL.Impaired nerve growth factor homeostasis in patients with bipolar disorder.**World J Biol Psychiatry**. 2011;12(3):228-32.

12. BARBOSA IG, HUGUET RB, SOUSA LP, ABREU MN, ROCHA NP, BAUER ME, CARVALHO LA, TEIXEIRA AL.Circulating levels of GDNF in bipolar disorder.**Neurosci Lett**. 2011;502(2):103-6.

13. BARBOSA IG, FERREIRA SA, HUGUET RB, ROCHA FL, SALGADO JV, TEIXEIRA AL. Comorbidades clínicas e psiquiátricas empacientes com transtorno bipolar do tipo I. **J Bras Psiquiatr**. 2011;60(4):271-6.

14. BARBOSA IG, ROCHA NP, DE MIRANDA AS, MAGALHÃES PV, HUGUET RB, DE SOUZA LP, KAPCZINSKI F, TEIXEIRA AL.Increased levels of adipokines in bipolar disorder.**J Psychiatr Res**. 2012;46(3):389-93.

15. BARBOSA IG, ROCHA NP, HUGUET RB, FERREIRA RA, SALGADO JV, CARVALHO LA, PARIANTE CM, TEIXEIRA AL.Executive dysfunction in euthymic bipolar disorder patients and its association with plasma biomarkers.**J Affect Disord**. 2012;137(1-3):151-5.

16. BEATO RG, NITRINI R, FORMIGONI AP, CARAMELLI P. Brazilian version of the Frontal Assessment Batery (FAB): preliminary data on administration to healthy elderly. **Dementia & Neuropsychologia.** 2007; 1: 59–65.

17. BELMAKER RH. Bipolar disorder. **N Engl J Med.** 2004; 351(5): 476-86.

18. BERRIOS GE. Melancholia and depression during the 19th century: a conceptual history. **Br J Psychiatry**. 1988;153:298-304.

19. BERK M, KAPCZINSKI F, ANDREAZZA AC, DEAN OM, GIORLANDO F, MAES M, YÜCEL M, GAMA CS, DODD S, DEAN B, MAGALHÃES PV, AMMINGER P, MCGORRY P, MALHI GS. Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. **Neurosci Biobehav Rev**. 2011;35(3):804-17.

20. BERTOLUCCI PH, BRUCKI SM, CAMPACCI SR, JULIANO Y. The Mini-Mental State Examination in a general population: impact of educational status. **Arq Neuropsiquiatr.** 1994; 52(1): 1-7.

21. BEYER J, KUCHIBHATLA M, GERSING K, KRISHNAN KR. Medical comorbidity in a bipolar outpatient clinical population. **Neuropsychopharmacology**. 2005; 30(2): 401-4.

22. BOYD JG, GORDON T. Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury. **Mol Neurobiol**. 2003;27(3):277-324.

23. V BRAZILIAN GUIDELINES IN ARTERIAL HYPERTENSION. Sociedade Brasileira de Cardiologia-SBC, Sociedade Brasileira de Hipertensão-SBH, Sociedade Brasileira de Nefrologia-SBN. **Arq Bras Cardiol**. 2007; 89(3) e24-79.

24. BRIETZKE E, KAPCZINSKI F. TNF-alpha as a molecular target in bipolar disorder. **Prog Neuropsychopharmacol Biol Psychiatry**. 2008;32(6):1355-61.

25. BRIETZKE E, STERTZ L, FERNANDES BS, KAUER-SANT'ANNA M, MASCARENHAS M, ESCOSTEGUY VARGAS A, CHIES JA, KAPCZINSKI F.Comparison of cytokine levels in depressed, manic and euthymic patients with bipolar disorder. **J Affect Disord.** 2009;116(3):214-7.

26. BRIETZKE E, KAUER-SANT'ANNA M, TEIXEIRA AL, KAPCZINSKI F Abnormalities in serum chemokine levels in euthymic patients with bipolar disorder. **Brain Behav Immun**. 2009; 23: 1079-82.

27. BRUCKI SM, NITRINI R, CARAMELLI P, BERTOLUCCI PH, OKAMOTO IH.Suggestions for utilization of the mini-mental state examination in Brazil. **Arq Neuropsiquiatr**. 2003; 61(3B): 777-81.

28. BUHAGIAR K, PARSONAGE L, OSBORN DP. Physical health behaviours and health locus of control in people with schizophrenia-spectrum disorder and bipolar disorder: a cross-sectional comparative study with people with non-psychotic mental illness. **BMC Psychiatry.** 2011;11:104.

29. CARDOSO BM, KAUER SANT'ANNA M, DIAS VV, ANDREAZZA AC, CERESÉR CM, KAPCZINSKI F. The impact of co-morbid alcohol use disorder in bipolar patients. **Alcohol**. 2008; 42: 451-457.

30. COELHO FM, REIS HJ, NICOLATO R, ROMANO-SILVA MA, TEIXEIRA MM, BAUER ME, TEIXEIRA AL. Increased serum levels of in!ammatorymarkers in chronic institutionalizedpatients with schizophrenia. **Neuroimmunomodulation.** 2008;15:140e4.

31. COUNTS SE, MUFSON EJ. The role of nerve growth factor receptors in cholinergic basal forebrain degeneration in prodromal Alzheimer disease. **J Neuropathol Exp Neurol**. 2005; 64(4): 263-72.

32. CUELLO AC, BRUNO MA, ALLARD S, LEON W, IULITA MF. Cholinergic involvement in Alzheimer's disease. A link with NGF maturation and degradation. **J Mol Neurosci**. 2010; 40(1-2): 230-5.

33. DALPINO FB, SODRE FL, DE FARIA EC. The use of a hospital laboratory cohort to estimate the prevalence of dyslipidemia in an adult Brazilian population. **Clin Chim Acta**. 2006; 367 (1-2): 189-91.

34. DEL PORTO JA. Bipolar disorder: evolution of the concept and current controversies **Rev Bras Psiquiatr**. 2004; 26: 3 (Suppl 3) 3-6.

35. DEPP CA, MAUSBACH BT, BOWIE C, WOLYNIEC P, THORNQUIST MH, LUKE JR, MCGRATH JA, PULVER AE, HARVEY PD, PATTERSON TL. Determinants of occupational and residential functioning in bipolar disorder.**J Affect Disord**. 2012;136(3):812-8.

36. DIAGNOSTIC AND STATISTICAL MANUAL OF MENTAL DISORDERS DSM-IV-TR (TEXT REVISION). American psychiatry Association press, 2004.

37. DOYLE I. **Nosologia Psiquiátrica**. Rio de Janeiro. Encadernadora Universitária, 1956.

38. DREXHAGE RC, KNIJFF EM, PADMOS RC, HEUL-NIEUWENHUIJZEN L, BEUMER W, VERSNEL MA, DREXHAGE HA. The mononuclear phagocyte system and its cytokine inflammatory networks in schizophrenia and bipolar disorder. **Expert Rev Neurother.** 2010;10(1):59-76.

39. DREXHAGE RC, HOOGENBOEZEM TH, VERSNEL MA, BERGHOUT A, NOLEN WA, DREXHAGE HA.The activation of monocyte and T cell networks in patients with bipolar disorder.**Brain Behav Immun**. 2011;25(6):1206-13.

40. DUBOIS B, SLACHEVSKY A, LITVAN I, PILLON B. The FAB: a Frontal Assessment Battery at bedside. **Neurology**. 2000; 55(11): 1621-6.

41. DUCRAY A, KREBS SH, SCHALLER B, SEILER RW, MEYER M, WIDMER HR. GDNF-family ligands display distinct action profiles on cultured GABAergic and serotonergic neurons of rat ventral mesencephalon. **Brain Res**. 2006; 1069: 104-112.

42. DUMAN RS, MONTEGGIA LM. A neurotrophic model for stress-related mood disorders. **Biol Psychiatry.** 2006;59(12):1116-27.

43. DUTTA R, BOYDELL J, KENNEDY N, VAN OS J, FEARON P, MURRAY RM. Suicide and other causes of mortality in bipolar disorder: a longitudinal study. **Psychol Med.** 2007;37(6):839-47.

44. EINAT H, YUAN P, GOULD TD, LI J, DU J, ZHANG L, MANJI HK, CHEN G.The role of the extracellular signal-regulated kinase signaling pathway in mood modulation. **J Neurosci.** 2003;23(19):7311-6.

45. FAJUTRAO L, LOCKLEAR J, PRIAULX J, HEYES A. A systematic review of the evidence of the burden of bipolar disorder in Europe. **Clin Pract Epidemiol Ment Health**. 2009; 23(5-3): 1-8.

46. FERNANDES BS, GAMA CS, CERESÉR KM, YATHAM LN, FRIES GR, COLPO G, DE LUCENA D, KUNZ M, GOMES FA, KAPCZINSKI F. Brain-derived neurotrophic factor as a state-marker of mood episodes in bipolar disorders: a systematic review and meta-regression analysis.**J Psychiatr Res**. 2011;45(8):995-1004.

47. FIGIEL I. Pro-inflammatory cytokine TNF-alpha as a neuroprotective agent in the brain. **Acta Neurobiol Exp (Wars)**. 2008; 68(4): 526-34.

48. FREY BN, DA FONSECA MMR, MACHADO-VIEIRA R, SOARES J, KAPCZINSKI F. Neuropatological and neurochemical abnormalities in bipolar disorder. **Rev Bras Psiquiatr**. 2004; 26 (3): 180-8.

49. FREY BN, ANDREAZZA AC, CERESÉR KM, MARTINS MR, VALVASSORI SS, RÉUS GZ, QUEVEDO J, KAPCZINSKI F. Effects of mood stabilizers on hippocampus BDNF levels in an animal model of mania.**Life Sci.** 2006;79(3):281-6.

50. FURUNO T, NAKANISHI M. Neurotrophic factors increase tumor necrosis factor-alpha-induced nuclear translocation of NF-kappaB in rat PC12 cells. **Neurosci Lett.** 2006;392(3):240-4.

51. GAMA CS, ANDREAZZA AC, KUNZ M, BERK M, BELMONTE-DE-ABREU PS, KAPCZINSKI F. Serum levels of brain-derived neurotrophic factor in patients with schizophrenia and bipolar disorder. **Neurosci. Lett**. 2007; 420: 45–48.

52. GERSHON ES, ALLIEY-RODRIGUEZ N, LIU C. After GWAS: searching for genetic risk for schizophrenia and bipolar disorder. **Am J Psychiatry**. 2011; 168(3): 253-6.

53. GRUNFELD C, ZHAO C, FULLER J, POLLACK A, MOSER A, FRIEDMAN J, FEINGOLD KR. Endotoxin and cytokines induce expression of leptin, the ob gene product, in hamsters. **J Clin Invest**. 1996;97(9):2152-7.

54. HAMILTON M. A rating scale for depression. **J Neurol Neurosurg Psychiatry**. 1960; 23:56-62.

55. HAWTON K, SUTTON L, HAW C, SINCLAIR J, HARRISS L. Suicide and attempted suicide in bipolar disorder: a systematic review of risk factors. **J Clin Psychiatry**. 2005; 66(6): 693-704.

56. HELLWEG R, VON RICHTHOFEN S, ANDERS D, BAETHGE C, RÖPKE S, HARTUNG HD, GERICKE CA. The time course of nerve growth factor content in different neuropsychiatric diseases--a unifying hypothesis. **J Neural Transm.** 1998; 105(8-9): 871-903.

57. HOPE S, DIESET I, AGARTZ I, STEEN NE, UELAND T, MELLE I, AUKRUST P, ANDREASSEN OA.Affective symptoms are associated with markers of inflammation and immune activation in bipolar disorders but not in schizophrenia.**J Psychiatr Res**. 2011;45(12):1608-16.

58. HUXLEY N, BALDESSARINI RJ.Disability and its treatment in bipolar disorder patients. **Bipolar Disord**. 2007;9(1-2):183-96.

59. HYMAN C, HOFER M, BARDE YA, JUHASZ M, YANCOPOULOS GD, SQUINTO SP, LINDSAY RM. BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra.**Nature**. 1991;350(6315):230-2.

60. JORNADA LK, MORETTI M, VALVASSORI SS, FERREIRA CL, PADILHA PT, ARENT CO, FRIES GR, KAPCZINSKI F, QUEVEDO J.Effects of mood stabilizers on hippocampus and amygdala BDNF levels in an animal model of mania induced by ouabain.**J Psychiatr Res**. 2010;44(8):506-10.

61. JUDD LL, SCHETTLER PJ, AKISKAL HS, MASER J, CORYELL W, SOLOMON D, ENDICOTT J, KELLER M. Long-term symptomatic status of bipolar I vs. bipolar II disorders. **Int J Neuropsychopharmacol**. 2003; 6(2):127-37.

62. JURUENA MF, CLEARE AJ, PARIANTE CM.The hypothalamic pituitary adrenal axis, glucocorticoid receptor function and relevance to depression. **Rev Bras Psiquiatr**. 2004;26(3):189-201.

63. KAPCZINSKI F, VIETA E, ANDREAZZA AC, FREY BN, GOMES FA, TRAMONTINA J, KAUER-SANT'ANNA M, GRASSI-OLIVEIRA R, POST RM.Allostatic load in bipolar disorder: implications for pathophysiology and treatment.**Neurosci Biobehav Rev**. 2008;32(4):675-92.

64. KAPCZINSKI F, DAL-PIZZOL F, TEIXEIRA AL, MAGALHAES PV, KAUER-SANT'ANNA M, KLAMT F, PASQUALI MA, QUEVEDO J, GAMA CS, POST R. A systemic toxicity index developed to assess peripheral changes in mood episodes.**Mol Psychiatry**. 2010;15(8):784-6.

65. KAPCZINSKI F, DAL-PIZZOL F, TEIXEIRA AL, MAGALHAES PV, KAUER-SANT'ANNA M, KLAMT F, MOREIRA JC, DE BITTENCOURT

PASQUALI MA, FRIES GR, QUEVEDO J, GAMA CS, POST R. Peripheral biomarkers and illness activity in bipolar disorder. **J Psychiatr Res**. 2011;45(2):156-61.

66. KAUER-SANT'ANNA M, KAPCZINSKI F, ANDREAZZA AC, BOND DJ, LAM RW, YOUNG LT, YATHAM LN.Brain-derivedneurotrophic factor and inflammatory markers in patients with early- vs.late-stage bipolar disorder. **Int J Neuropsychopharmacol.** 2008;4:1-12.

67. KESSLER RC, MERIKANGAS KR, WANG PS. Prevalence, comorbidity, and service utilization for mood disorders in the United States at the beginning of the twenty-first century. **Annu Rev Clin Psychol.** 2007; 3:137-58.

68. KILLGORE WD, YOUNG AD, FEMIA LA, BOGORODZKI P, ROGOWSKA J, YURGELUN-TODD DA. Cortical and limbic activation during viewing of high- versus low-calorie foods. **Neuroimage**. 2003; 19(4): 1381-94.

69. KIM EK, CHOI EJ. Pathological roles of MAPK signaling pathways in human diseases. **Biochim Biophys Acta**. 2010;1802(4):396-405.

70. KIM YK, MYINT AM, LEE BH, HAN CS, LEE SW, LEONARD BE, STEINBUSCH HW. T-helper types 1, 2, and 3 cytokine interactions in symptomatic manic patients.**Psychiatry Res.** 2004;129(3):267-72.

71. KNIJFF EM, BREUNIS MN, KUPKA RW, DE WIT HJ, RUWHOF C, AKKERHUIS GW, NOLEN WA, DREXHAGE HA.An imbalance in the production of IL-1beta and IL-6 by monocytes of bipolar patients: restoration by lithium treatment.**Bipolar Disord.** 2007;9(7):743-53.

72. KOJIMA M, TAKEI N, NUMAKAWA T, ISHIKAWA Y, SUZUKI S, MATSUMOTO T, KATOH-SEMBA R, NAWA H, HATANAKA H.Biological characterization and optical imaging of brain-derived neurotrophic factor-green fluorescent protein suggest an activity-dependent local release of brain-derived neurotrophic factor in neurites of cultured hippocampal neurons.**J Neurosci Res**. 2001;64(1):1-10.

73. KONARSKI JZ, MCINTYRE RS, KENNEDY SH, RAFI-TARI S, SOCYNSKA JK, KETTER TA. Volumetric neuroimaging investigators in mood disorders: bipolar disorder versus major depressive disorder. **Bipolar disorder**. 2008; 10 (1): 1-37.

74. KRISHNAN KR. Psychiatric and medical comorbitidies of bipolar disorder. **Psychosom Med**. 2005; 67(1):1-8.

75. KRONFOL Z, REMICK DG. Cytokines and the brain: implications for clinical psychiatry.**Am J Psychiatry.** 2000;157:683e94.

76. KUPFER DJ, FRANK E, GROCHOCINSKI VJ, CLUSS PA, HOUCK PR, STAPF DA. Demographic and clinical characteristics of individuals in a bipolar disorder case registry. **J Clin Psychiatry**. 2002; 63(2):120-5.

77. KUPFERSCHMIDT DA, ZAKZANIS KK. Toward a functional neuroanatomical signature of bipolar disorder: quantitative evidence from the neuroimaging literature. **Psychiatry Res**. 2011; 193(2): 71-9

78. LANGAN C, MCDONALD C. Neurobiological trait abnormalities in bipolar disorder. **Mol Psychiatry**. 2009; 14(9): 833-46.

79. LEE KW, WOON PS, TEO YY, SIM K. Genome wide association studies (GWAS) and copy number variation (CNV) studies of the major psychoses: what have we learnt?**Neurosci Biobehav Rev**. 2012;36(1):556-71.

80. LIN PY. State-dependent decrease in levels of brain-derived neurotrophic factor in bipolar disorder: a meta-analytic study. **Neurosci Lett**. 2009; 466(3): 139-43.

81. LINDHOLM D, CARROLL P, TZIMAGIOGIS G, THOENEN H. Autocrine-paracrine regulation of hippocampal neuron survival by IGF-1 and the neurotrophins BDNF, NT-3 and NT-4.**Eur J Neurosci.** 1996;8(7):1452-60.

82. LUMENG CN, SALTIEL AR. Inflammatory links between obesity and metabolic disease. **J Clin Invest**. 2011;121(6):2111-7.

83. MCADAMS C, LEONARD BE. Neutrophil and monocyte phagocytosis in depressed patients.**Prog Neuropsychopharmacol Biol Psychiatry.** 1993;17(6):971-84.

84. MANJI HK, MOORE GJ, RAJKOWSKA G, CHEN G. Neuroplasticity and cellular resilience in mood disorders. **Mol Psychiatry**. 2000; 5(6): 578-93.

85. MANJI HK, QUIROZ JA, PAYNE JL, SINGH J, LOPES BP,VIEGAS JS, ZARATE CA. The underlying neurobiology of bipolar disorder. **World Psychiatry**. 2003; 2 (3) 136-46.

86. MERIKANGAS K, YU K. Genetic epidemiology of bipolar disorder. **Clin Neurosci Res.** 2002; 2: 127-41.

87. MERIKANGAS KR, JIN R, HE JP, KESSLER RC, LEE S, SAMPSON NA, VIANA MC, ANDRADE LH, HU C, KARAM EG, LADEA M, MEDINA-MORA ME, ONO Y, POSADA-VILLA J, SAGAR R, WELLS JE, ZARKOV Z.Prevalence and correlates of bipolar spectrum disorder in the world mental health survey initiative. **Arch Gen Psychiatry**. 2011; 68(3): 241-51.

88. MILLER AH, MALETIC V, RAISON CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. **Biol Psychiatry**. 2009; 65(9): 732-41.

89. MILLER CA. Stressed and depressed? Check your GDNF for epigenetic repression. **Neuron**. 2011; 69(2): 188-90.

90. NERY-FERNANDES F, QUARANTINI LC, GALVÃO-DE-ALMEIDA A, ROCHA MV, KAPCZINSKI F, MIRANDA-SCIPPA A. Lower rates of comorbidities in euthymic bipolar patients. **World J Biol Psychiatry**. 2009; 10 (4 Pt 2): 474-9.

91. NEVES FS, MALLOY-DINIZ LF, CORRÊA H. Suicidal behavior in Bipolar Disorder: What is the influence of psychiatric comorbidities.**J Clin Psychiatry**. 2009; 70(1): 13-18.

92. NIEWIADOMSKA G, MIETELSKA-POROWSKA A, MAZURKIEWICZ M. The cholinergic system, nerve growth factor and the cytoskeleton. **Behav Brain Res**. 2011; 221(2): 515-26.

93. NOVICK DM, SWARTZ HA, FRANK E. Suicide attempts in bipolar I and bipolar II disorder: a review and meta-analysis of the evidence. **Bipolar Disord.** 2010;12(1):1-9.

94. O'BRIEN SM, SCULLY P, SCOTT LV, DINAN TG. Cytokine profiles in bipolar affective disorder: focuson acutely ill patients. **J Affect Disord**. 2006;90(2-3):263-7.

95. ORTIZ-DOMÍNGUEZ A, HERNÁNDEZ ME, BERLANGA C, GUTIÉRREZ-MORA D, MORENO J, HEINZE G, PAVÓN L.Immune variations in bipolar disorder: phasic differences. **Bipolar Disord.** 2007;9(6):596-602.

96. OUCHI N, PARKER JL, LUGUS JJ, WALSH K. Adipokines in in!ammation and metabolicdisease. **Nat Rev Immunol**. 2011;11:85-97.

97. PADMOS RC, HILLEGERS MH, KNIJFF EM, VONK R, BOUVY A, STAAL FJ, DE RIDDER D, KUPKA RW, NOLEN WA, DREXHAGE HA. A discriminating messenger RNA signature for bipolar disorder formed by an aberrant expression of inflammatory genes in monocytes. **Arch Gen Psychiatry**. 2008; 65(4): 395-407.

98. PADMOS RC, VAN BAAL GC, VONK R, WIJKHUIJS AJ, KAHN RS, NOLEN WA, DREXHAGE HA. Genetic and environmental influences on pro-inflammatory monocytes in bipolar disorder: a twin study. **Arch Gen Psychiatry**. 2009;66(9):957-65.

99. PASSOS VM, BARRETO SM, DINIZ LM, LIMA-COSTA MF. Type 2 diabetes: prevalence and associated factors in a Brazilian community—the Bambui health and aging study. **Sao Paulo Med J**. 2005; 123 (2): 66-71.

100. PHILLIPS ML, FRANK E. Redefining bipolar disorder: toward DSM-V. **Am J Psychiatry**. 2006;163(7):1135-6.

101. PILLAI A, TERRY AV JR, MAHADIK SP. Differential effects of long-term treatment with typical and atypical antipsychotics on NGF and BDNF levels in rat striatum and hippocampus. **Schizophr Res.** 2006;82(1):95-106.

102. POO MM. Neurotrophins as synaptic modulators. **Nat Rev Neurosci**. 2001; 2: 24-32.

103. POST RM. Kindling and sensitization as models for affective episode recurrence, cyclicity, and tolerance phenomena. **Neurosci Biobehav Rev**. 2007; 31: 858–873.

104. RAO JS, HARRY GJ, RAPOPORT SI, KIM HW. Increased excitotoxicity and neuroinflammatory markers in postmortem frontal cortex from bipolar disorder patients. **Mol Psychiatry.** 2010;15(4):384-92.

105. REICHARDT LF. Neurotrophin-regulated signalling pathways. **Philos Trans R Soc Lond B Biol Sci**. 2006; 361(1473): 1545-64.

106. REIS HJ, NICOLATO R, BARBOSA IG, TEIXEIRA DO PRADO PH, ROMANO-SILVA MA, TEIXEIRA AL. Increased serum levels of brain-derived neurotrophic factor in chronic institutionalized patients with schizophrenia. **Neurosci Lett**. 2008; 439(2): 157-9.

107. ROSA AR, FREY BN, ANDREAZZA AC, CERESÉR KM, CUNHA AB, QUEVEDO J, SANTIN A, GOTTFRIED C, GONÇALVES CA, VIETA E, KAPCZINSKI F.Increased serum glial cell line-derived neurotrophic factor immunocontent during manic and depressive episodes in individuals with bipolar disorder.**Neurosci Lett.**2006;407(2):146-50.

108. SAAVEDRA A, BALTAZAR G, DUARTE EP.Driving GDNF expression: the green and the red traffic lights. **Prog Neurobiol**. 2008; 86(3): 186-215.

109. SADOCK BJ, SADOCK VA, editors. **Kaplan & Sadock´s Synopsis of Psychiatry: behavioral sciences / clinical psychiatry.** Philadelphia. Lippincott Williams & Wilkins, a Wolters Klumer Bussiness Press; 2007.

110. SAKAGUCHI S, ONO M, SETOGUCHI R, YAGI H, HORI S, FEHERVARI Z, SHIMIZU J, TAKAHASHI T, NOMURA T. Foxp3+ CD25+ CD4+ natural regulatory T cells in dominant self-tolerance and autoimmune disease. **Immunol Rev**. 2006;212:8-27.

111. SANCHES M, KESHAVAN MS, BRAMBILLA P, SOARES JC. Neurodevelopmental basis of bipolar disorder: a critical appraisal. **Prog Neuropsychopharmacol Biol Psychiatry**. 2008; 32(7): 1617-27.

112. SANDI C. Stress, cognitive impairment and cell adhesion molecules. **Nat Rev Neurosci**. 2004;5(12):917-30.

113. SARABI A, HOFFER BJ, OLSON L, MORALES M. GFR alpha-1 is expressed in parvalbumin GABAergic neurons in the hippocampus. **Brain Res**. 2000; 877: 262–270.

114. SAVITZ J, DREVETS WC. Bipolar and major depressive disorder: neuroimaging the developmental-degenerative divide. **Neurosci Biobehav Rev.** 2009; 33(5): 699-771.

115. SCHLOESSER RJ, HUANG J, KLEIN PS, MANJI HK. Cellular plasticity cascades in the pathophysiology and treatment of bipolar disorder. **Neuropsychopharmacology**. 2008; 33(1): 110-33.

116. SHEEHAN DV, LECRUBIER Y, SHEEHAN KH, AMORIM P, JANAVS J, WEILLER E, HERGUETA T, BAKER R, DUNBAR GC.The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. **J Clin Psychiatry**. 1998; 59 (Suppl 20): 22-33; quiz 4-57.

117. SICHIERI R, BAIMA J, MARANTE T, DE VASCONCELLOS MT, MOURA AS, VAISMAN M. Low prevalence of hypothyroidsm among Black and Mulatto people in a population-based study of Brazilian women. **Clin Endocrinol (Oxf)**. 2007; 66 (6): 803-7.

118. DA SILVA MAGALHÃES PV, GOMES FA, KUNZ M, KAPCZINSKI F. Birth cohort and dual diagnosis effects on age-at-onset in Brazilian patients with bipolar I disorder. **Acta Psychiatr Scand.** 2009; 120: 492–495.

119. DA SILVA MAGALHÃES PV, KAPCZINSKI NS, KAPCZINSKI F. Correlates and impact of obsessive-compulsive comorbidity in bipolar disorder. **Compr Psychiatry.** 2010; 51: 353–356.

120. SMITH RS. The macrophage theory of depression. **Med Hypotheses.** 1991;35(4):298-306.

121. SÖDERLUND J, OLSSON SK, SAMUELSSON M, WALTHER-JALLOW L, JOHANSSON C, ERHARDT S, LANDÉN M, ENGBERG G.Elevation of cerebrospinal fluid interleukin-1ß in bipolar disorder. **J Psychiatry Neurosci.** 2011;36(2):114-8.

122. SOLOMON DA, LEON AC, CORYELL WH, ENDICOTT J, LI C, FIEDOROWICZ JG, BOYKEN L, KELLER MB. Longitudinal course of bipolar I disorder: duration of mood episodes. **Arch Gen Psychiatry**. 2010; 67(4): 339-47.

123. SONG G, OUYANG G, BAO S. The activation of Akt/PKB signaling pathway and cell survival. **J Cell Mol Med.**2005; 9:59-71.

124. SOUERY D, ZANINOTTO L, CALATI R, LINOTTE S, SENTISSI O, AMITAL D, MOSER U, KASPER S, ZOHAR J, MENDLEWICZ J, SERRETTI A. Phenomenology of psychotic mood disorders: lifetime and major depressive episode features. **J Affect Disord**. 2011;135(1-3):241-50.

125. SOUSA LP, SILVA BM, BRASIL BS, NOGUEIRA SV, FERREIRA PC, KROON EG, KATO K, BONJARDIM CA.Plasminogen/plasmin regulates alpha-enolase expression through the MEK/ERK pathway. **Biochem Biophys Res Commun**. 2005;337(4):1065-71.

126. SOUSA LP, CARMO AF, REZENDE BM, LOPES F, SILVA DM, ALESSANDRI AL, BONJARDIM CA, ROSSI AG, TEIXEIRA MM, PINHO V. Cyclic AMP enhances resolution of allergic pleurisy by promoting inflammatory cell apoptosis via inhibition of PI3K/Akt and NF-kappaB. **Biochem Pharmacol**. 2009;78(4):396-405.

127. SOUSA LP, LOPES F, SILVA DM, TAVARES LP, VIEIRA AT, REZENDE BM, CARMO AF, RUSSO RC, GARCIA CC, BONJARDIM CA, ALESSANDRI AL, ROSSI AG, PINHO V, TEIXEIRA MM. PDE4 inhibition drives resolution of neutrophilic inflammation by inducing apoptosis in a PKA-PI3K/Akt-dependent and NF-kappaB-independent manner. **J Leukoc Biol**. 2010; 87(5): 895-904.

128. STEEN NE, METHLIE P, LORENTZEN S, HOPE S, BARRETT EA, LARSSON S, MORK E, ALMÅS B, LØVÅS K, AGARTZ I, MELLE I, BERG JP, ANDREASSEN OA. Increased systemic cortisol metabolism in patients with schizophrenia and bipolar disorder: a mechanism for increased stress vulnerability? **J Clin Psychiatry**. 2011;72(11):1515-21.

129. SUN Y, ZHANG L, JOHNSTON NL, TORREY EF, YOLKEN RH. Serial analysis of gene expression in the frontal cortex of patients with bipolar disorder. **Br J Psychiatry**. 2001; Suppl (41):s137-41.

130. TAKEBAYASHI M, HISAOKA K, NISHIDA A, TSUCHIOKA M, MIYOSHI I, KOZURU T, HIKASA S, OKAMOTO Y, SHINNO H, MORINOBU S, YAMAWAKI S.Decreased levels of whole blood glial cell line-derived neurotrophic factor (GDNF) in remitted patients with mood disorders**. Int J Neuropsychopharmacol.** 2006;9(5):607-12.

131. TEIXEIRA PJR, ROCHA FL. Efeitos adversos metabólicos de antipsicóticos e estabilizadores de humor. **Rev Psiquiatr RS**. 2006; 28(2): 186-196.

132. TEIXEIRA AL, BARBOSA IG, DINIZ BS, KUMMER A. Circulating levels of brain-derived neurotrophic factor: correlation with mood, cognition and motor function.**Biomark Med**. 2010;4(6):871-87.

133. TORRES KC, SOUZA BR, MIRANDA DM, NICOLATO R, NEVES FS, BARROS AG, DUTRA WO, GOLLOB KJ, CORREA H, ROMANO-SILVA MA.The leukocytes expressing DARPP-32 are reduced in patients with

schizophrenia and bipolar disorder.**Prog Neuropsychopharmacol Biol Psychiatry**. 2009;33(2):214-9.

134. VALENZUELA MJ, BREAKSPEAR M, SACHDEV P. Complex mental activity and the aging brain: molecular, cellular and cortical network mechanisms. **Brain Res Rev**. 2007; 56(1): 198-213.

135. VALVASSORI SS, ELIAS G, DE SOUZA B, PETRONILHO F, DAL-PIZZOL F, KAPCZINSKI F, TRZESNIAK C, TUMAS V, DURSUN S, CHAGAS MH, HALLAK JE, ZUARDI AW, QUEVEDO J, CRIPPA JA.Effects of cannabidiol on amphetamine-induced oxidative stress generation in an animal model of mania.**J Psychopharmacol**. 2011;25(2):274-80.

136. YATHAM LN, TORRES IJ, MALHI GS, FRANGOU S, GLAHN DC, BEARDEN CE, BURDICK KE, MARTÍNEZ-ARÁN A, DITTMANN S, GOLDBERG JF, OZERDEM A, AYDEMIR O, CHENGAPPA KN. The International Society for Bipolar Disorders — Battery for Assessment of Neurocognition (ISBD-BANC). **Bipolar Disord**. 2010;12(4):351-63.

137. YE RD. Regulation of nuclear factor kappaB activation by G-protein-coupled receptors. **J Leukoc Biol**. 2001;70(6):839-48.

138. YOSHIMURA R, NAKANO Y, HORI H, IKENOUCHI A, UEDA N, NAKAMURA J.Effect of risperidone on plasma catecholamine metabolites and brain-derived neurotrophic factor in patients with bipolar disorders. **Hum Psychopharmacol**. 2006;21(7):433-8.

139. YOUNG RC, BIGGS JT, ZIEGLER VE, MEYER DA. A rating scale for mania: reliability, validity and sensitivity. **Br J Psychiatry.** 1978; 133: 429–435.

140. WATSON S, GALLAGHER P, RITCHIE JC, FERRIER IN, YOUNG AH. Hypothalamic-pituitary-adrenal axis function in patients with bipolar disorder. **Br J Psychiatry**. 2004; 184: 496-502.

141. WHITWORTH JA, WILLIAMSON PM, MANGOS G, KELLY JJ. Cardiovascular consequences of cortisol excess. **Vasc Health Risk Manag**. 2005; 1(4): 291-9.

142. ZARATE CA, SINGH J, MANJI HK. Cellular Plasticity Cascades: Targets for the Development of Novel Therapeutics for Bipolar Disorder. **Biol Psychiatry**. 2006; 59: 1006–1020.

143. ZHANG X, ZHANG Z, SHA W, XIE C, XI G, ZHOU H, ZHANG Y.Effect of treatment on serum glial cell line-derived neurotrophic factor in bipolar patients.**J Affect Disord**. 2010;126(1-2):326-9.

144. ZIEGENHORN AA, SCHULTE-HERBRÜGGEN O, DANKER-HOPFE H, MALBRANC M, HARTUNG HD, ANDERS D, LANG UE, STEINHAGEN-THIESSEN E, SCHAUB RT, HELLWEG R. Serum neurotrophins--a study on the time course and influencing factors in a large old age sample. **Neurobiol Aging**. 2007; 28(9): 1436-45.

ANEXOS

ANEXO A. Escala de Hamilton para Avaliação de Depressão (HAMD)

Todos os itens devem ser preenchidos. Assinalar o número que melhor caracteriza o paciente na última semana.

1. HUMOR DEPRIMIDO (Tristeza, desesperança, desamparo, inutilidade)

0 ( ) Ausente.

1 ( ) Sentimentos relatados apenas ao ser inquirido.

2 ( ) Sentimentos relatados espontaneamente com palavras.

3 ( ) Comunica os sentimentos não com palavras, isto é, com a expressão facial, a postura, a voz e a tendência ao choro.

4 ( ) Sentimentos deduzidos da comunicação verbal e não-verbal do paciente.

2. SENTIMENTOS DE CULPA

0 ( ) Ausente

1 ( ) Auto-recriminação; sente que decepcionou os outros.

2 ( ) Idéias de culpa ou ruminação sobre erros passados ou más ações.

3 ( ) A doença atual é um castigo.

4 ( ) Ouve vozes de acusação ou denúncia e/ou tem alucinações visuais ameaçadoras.

3. SUICÍDIO

0 ( ) Ausente.

1 ( ) Sente que a vida não vale a pena.

2 ( ) Desejaria estar morto ou pensa na probabilidade de sua própria morte.

3 ( ) Idéias ou gestos suicidas.

4 ( ) Tentativa de suicídio (qualquer tentativa séria, marcar 4).

4. INSÔNIA INICIAL

0 ( ) Sem dificuldades para conciliar o sono.

1 ( ) Queixa-se de dificuldade ocasional para conciliar o sono, isto é, mais de meia hora.

2 ( ) Queixa-se de dificuldade para conciliar o sono todas as noites.

5. INSÔNIA INTERMEDIÁRIA

0 ( ) Sem dificuldades.

1 ( ) O paciente se queixa de inquietude e perturbação durante a noite.

2 ( ) Acorda à noite - qualquer saída da cama marcar 2(exceto p/ urinar).

6. INSÔNIA TARDIA

0 ( ) Sem dificuldades.

1 ( ) Acorda de madrugada, mas volta a dormir

2 ( ) Incapaz de voltar a conciliar o sono se deixar a cama.

7. TRABALHO E ATIVIDADES

0 ( ) Sem dificuldades.

1 ( ) Pensamento e sentimentos de incapacidade, fadiga ou fraqueza relacionada a atividades, trabalho ou passatempos.

2 ( ) Perda de interesse por atividades (passatempos ou trabalho) quer diretamente relatada pelo paciente, quer indiretamente por desatenção, indecisão e vacilação (sente que precisa esforçar-se para o trabalho ou atividade).

3 ( ) Diminuição do tempo gasto em atividades ou queda de produtividade. No hospital, marcar 3 se o paciente não passar ao menos 3 horas por dia em atividades externas (trabalho hospitalar ou passatempo).

4 ( ) Parou de trabalhar devido à doença atual. No hospital, marcar 4 se o paciente não se ocupar com outras atividades, além de pequenas tarefas do leito, ou for incapaz de realizá-las sem ajuda.

8. RETARDO (lentidão de idéias e fala; dificuldade de concentração; atividade motora diminuída)

0 ( ) Pensamento e fala normais.

1 ( ) Leve retardo à entrevista.

2 ( ) Retardo óbvio à entrevista.

3 ( ) Entrevista difícil.

4 ( ) Estupor completo.

9. AGITAÇÃO

0 ( ) Nenhuma.

1 ( ) Inquietude.

2 ( ) Brinca com as mãos, com os cabelos,etc.

3 ( ) Mexe-se, não consegue sentar quieto.

4 ( ) Torce as mãos, rói as unhas, puxa os cabelos, morde os lábios.

10. ANSIEDADE PSÍQUICA

0 ( )Sem dificuldade.

1 ( ) Tensão e irritabilidade subjetivas.

2 ( ) Preocupação com trivialidades.

3 ( ) Atitude apreensiva aparente no rosto ou na fala.

4 ( ) Medos expressos sem serem inquiridos.

11.ANSIEDADE SOMÁTICA (Concomitantes fisiológicos de ansiedade, tais como: Gastrintestinais: boca seca, flatulência, indigestão, diarréia, cólicas, eructação; Cardiovasculares: palpitações, cefaléia; Respiratórios: hiperventilação, suspiros; sudorese; ter que urinar freqüentemente)

0 ( ) Ausente :

1 ( ) Leve

2 ( ) Moderada

3 ( ) Grave

4 ( ) Incapacitante

12. SINTOMAS SOMÁTICOS GASTRINTESTINAIS

0 ( ) Nenhum

1 ( ) Perda de apetite, mas alimenta-se voluntariamente. Sensações de peso no abdomen

2 ( ) Dificuldade de comer se não insistirem. Solicita ou exige laxativos ou medicações para os intestinos ou para sintomas digestivos.

13. SINTOMAS SOMÁTICOS EM GERAL

0 ( ) Nenhum

1 ( ) Peso nos membros, nas costas ou na cabeça. Dores nas costas, cefaléia, mialgias. Perda de energia e cansaço.

2 ( ) Qualquer sintoma bem caracterizado e nítido, marcar 2.

14. SINTOMAS GENITAIS

Sintomas como: perda da libido, distúrbios menstruais

0 ( ) Ausentes

1 ( ) Leves

2 ( ) Intensos

15. HIPOCONDRIA

0 ( ) Ausente

1 ( ) Auto-observação aumentada (com relação ao corpo)

2 ( ) Preocupação com a saúde

3 ( ) Queixas freqüentes, pedidos de ajuda,etc.

4 ( ) Idéias delirantes hipocondríacas.

16. PERDA DE PESO (Marcar A ou B)

A - Quando avaliada pela história clínica

0 ( ) Sem perda de peso.

1 ( ) Provável perda de peso associada à moléstia atual.

2 ( ) Perda de peso definida (de acordo com o paciente)

3 ( ) Não avaliada.

B - Avaliada semanalmente pelo psiquiatra responsável, quando são medidas alterações reais de peso

0 ( ) Menos de 0,5 Kg de perda por semana.

1 ( ) Mais de 0,5 Kg de perda por semana.

2 ( ) Mais de 1 Kg de perda por semana.

3 ( ) Não avaliada.

17. CRÍTICA

0 ( ) Reconhece que está deprimido e doente.

1 ( ) Reconhece a doença mas atribui-lhe a causa à má alimentação, ao clima, ao excesso de trabalho, a vírus, à necessidade de repouso, etc.

2 ( ) Nega estar doente.

ESCORE TOTAL DA HAMD DE 17 ITENS: _____(Faixa de variação: 0-50)

ANEXO B. Escala de Young para Avaliação de Mania (YOUNG)

Todos os itens devem ser preenchidos. Assinalar o número que melhor caracteriza o paciente na última semana.

1. HUMOR E AFETO ELEVADOS

0 ( ) Ausente.

1 ( ) Humor e afeto discreta ou possivelmente aumentados quando questionado.

2 ( ) Relato subjetivo de elevação clara do humor.

3 ( ) Afeto elevado ou inapropriado ao conteúdo do pensamento; jocoso.

4 ( ) Eufórico; risos inadequados, cantando.

2. ATIVIDADE MOTORA - ENERGIA AUMENTADAS

0 ( ) Ausente

1 ( ) Relato subjetivo de aumento da energia ou atividade motoras.

2 ( ) Apresenta-se animado ou com gestos aumentados.

3 ( ) Energia excessiva; as vezes, hiperativo; inquieto (mas pode ser acalmado).

4 ( ) Excitação psicomotora; hiperatividade contínua (não pode ser acalmado)

3. INTERESSE SEXUAL

0 ( ) Normal; sem aumento.

1 ( ) Discreta ou possivelmente aumentado.

2 ( ) Descreve aumento subjetivo; quando questionado

3 ( ) Conteúdo sexual espontâneo; discurso centrado em questões sexuais; auto-relato de hipersexualidade.

4 ( ) Relato confirmado ou observação direta de comportamento explicitamente sexualizado, pelo entrevistador ou por outras pessoas

4. SONO

0 ( ) Não relata diminuição do sono.

1 ( ) Dorme menos que a quantidade normal, cerca de 1 hora a menos que o habitual.

2 ( ) Dorme menos que a quantidade normal, mais que 1 hora a menos que o habitual.

3 ( ) Relata diminuição da necessidade de sono.

4 ( ) Nega necessidade de sono

5. IRRITABILIDADE

0 ( ) Ausente.

2 ( ) Subjetivamente aumentada.

4 ( ) Irritável em alguns momentos da entrevista; episódios recentes (nas últimas 24 horas) de ira ou irritação na enfermaria.

6 ( ) Irritável durante a maior parte da entrevista; ríspido e lacônico o tempo todo.

8 ( ) Hostil; não cooperativo; entrevista impossível.

6. FALA (Velocidade e quantidade)

0 ( ) Sem aumento.

2 ( ) Percebe-se mais falante que o seu habitual

4 ( ) Aumento da velocidade ou da quantidade da fala em alguns momentos; verborréico, as vezes com solicitação, consegue-se interromper a fala.

6 ( ) Quantidade e velocidade constantemente aumentadas; dificuldade para ser interrompido (não atende as solicitações, fala junto com o entrevistador).

8 ( ) Fala pressionada, ininterruptível, contínua (ignora a solicitação do entrevistador).

7. LINGUAGEM – DISTÚRBIO DO PENSAMENTO

0 ( ) Sem alterações.

1 ( ) Circunstancial, pensamentos rápidos.

2 ( ) Perde objetivos do pensamento; muda de assunto frequentemente; pensamentos muito acelerados.

3 ( ) Fuga de idéias; tangencialidade; dificuldades para acompanhar o pensamento; ecolalia consonante.

4 ( ) Incoerência; comunicação impossível.

8. CONTEÚDO

0 ( ) Normal.

2 ( ) Novos interesses e planos compatíveis com a condição sociocultural do paciente, mas questionáveis.

4 ( ) Projetos especiais totalmente incompatíveis com a condição socioeconômica do paciente; hiper-religioso .

6 ( ) Idéias supervalorizadas.

8 ( ) Delírios.

9. COMPORTAMENTO DISRUPTIVO AGRESSIVO

0 ( ) Ausente.

2 ( ) Sarcástico; barulhento, as vezes, desconfiado.

4 ( ) Ameaça o entrevistador, gritando; entrevista dificultada.

6 ( ) Agressivo; destrutivo; entrevista impossível.

10. APARÊNCIA

0 ( ) Arrumado e vestido apropriadamente.

1 ( ) Descuidado minimamente; adornos ou roupas minimamente inadequados ou exagerados.

2 ( ) Precariamente asseado; despenteado moderadamente; vestido com exagero.

3 ( ) Desgrenhado; vestido parcialmente; maquiagem extravagante.

4 ( ) Completamente descuidado; com muitos adornos e adereços; roupas bizarras.

11.INSIGHT (Discernimento)

0 ( ) Insight presente: espontaneamente refere estar doente e concorda com a necessidade de tratamento.

1 ( ) Insight duvidoso: com argumento admite possível doença e necessidade de tratamento.

2 ( ) Insight prejudicado: espontaneamente admite alteração comportamental, mas não relaciona com a doença ou discorda da necessidade de tratamento.

3 ( ) Insight ausente: com argumento admite de forma vaga alteração comportamental, mas não relaciona com a doença ou discorda da necessidade de tratamento.

4 ( ) Insight ausente: nega a doença, qualquer alteração comportamental e necessidade de tratamento.

ANEXO C. Bateria de Avaliação Frontal (BAF)

1) Semelhanças (elaboração de conceitos)	**Respostas**	**Pontuação**
Qual é a semelhança entre: **- Uma laranja e uma banana?** Ajudar o paciente no caso de uma resposta parcial ou nenhuma resposta: "Elas não têm nenhuma semelhança" ou "Elas têm casca" dizendo: "A laranja e a banana são..." Não ajudar o paciente nos itens seguintes. **- Uma mesa e uma cadeira?** **- Uma rosa, uma margarida e uma tulipa?** Apenas as respostas referentes às categorias são consideradas corretas (frutas, móveis, flores).	- 3 respostas corretas - 2 respostas corretas - 1 resposta correta - Nenhuma resposta correta	___3 ___2 ___1 ___0
2) Evocação Lexical (flexibilidade mental)	**Respostas**	**Pontuação**
"Diga o maior número possível de palavras, por exemplo animais, plantas, objetos, começando com a letra S. Não diga nome de pessoas e nomes próprios em geral." Se o paciente não disser nenhuma palavra nos dez primeiros segundos, dar um exemplo: "serpente". Se o paciente interromper a seqüência mais de 30 segundos estimula-lo após cada pausa dizendo "Qualquer palavra começando com a letra S."	- Mais de 9 palavras - De 6 a 9 palavras - De 3 a 5 palavras - Menos de 3 palavras	___3 ___2 ___1 ___0
3) Seqüências motoras (programação)	**Respostas**	**Pontuação**
"Olhe com atenção o que estou fazendo." O examinador se assenta em frente do paciente e executa três vezes a seqüência de Luria: "palma-canto-punho". **"Agora você vai fazer esta seqüência com a mão direita, primeiro ao mesmo tempo que eu e depois sozinho."** O examinador realiza três vezes a seqüência com a mão esquerda ao mesmo tempo que o paciente e depois lhe pede para continuar sozinho.	- O paciente realiza 6 seqüências corretas. - O paciente faz sozinho pelo menos 3 seqüências consecutivas corretas. - O paciente não é capaz de fazer a seqüência sozinho, mas faz 3 seqüências consecutivas. - O paciente não pode realizar 3 seqüências consecutivas corretas mesmo com o examinador.	___3 ___2 ___1 ___0

2) Evocação Lexical (flexibilidade mental)
Palavras:

_____
_____
_____
_____
_____
_____
_____
_____

4) Instruções geradoras de conflito (sensibilidade à interferência)	**Respostas**	**Pontuação**
"Eu bato uma vez e você bate duas vezes." Para certificar-se que o paciente compreendeu a regra, ele deve realizar uma seqüência de três batidas: 1 – 1 – 1 **"Eu bato duas vezes e você bate uma vez."** Para certificar-se que o paciente compreendeu a regra, ele deve realizar uma seqüência de três batidas: 2 – 2 – 2 A seqüência proposta é a seguinte: 1 – 1 – 2 – 1 – 2 – 2 – 2 – 1 – 1 – 2	- Nenhum erro - 1 ou 2 erros - Mais de 2 erros - O paciente bate o mesmo número de vezes que o examinador pelo menos 4 vezes consecutivas	___3 ___2 ___1 ___0
5) Go-No-Go (controle inibidor)	**Respostas**	**Pontuação**
"Eu bato uma vez e você bate uma vez." Para certificar-se que o paciente compreendeu a regra, ele deve realizar uma seqüência de três batidas: 1 –1 – 1 **"Eu bato duas vezes e você não bate".** Para certificar-se que o paciente compreendeu a regra, ele deve realizar uma seqüência de três batidas: 2 – 2 – 2 A seqüência proposta é a seguinte: 1 – 1 – 2 – 1 – 2 – 2 – 2 – 1 – 1 – 2	- Nenhum erro - 1 ou 2 erros - Mais de 2 erros - O paciente bate o mesmo número de vezes que o examinador pelo menos 4 vezes consecutivas	___3 ___2 ___1 ___0
6) Comportamento de preensão (autonomia ambiental)	**Respostas**	**Pontuação**
O examinador se assenta em frente do paciente. Este deixa as mãos sobre os joelhos com a palma virada para cima. O examinador toca as mãos do paciente e observa a sua reação. Se o paciente segura as mãos do examinador, pedir: **"Não segure as minhas mãos."**	- O paciente não segura as mãos do examinador. - O paciente hesita e pergunta o que ele deve fazer. - O paciente segura as mãos sem hesitação. - O paciente segura as mãos mesmo após o pedido para não fazê-lo.	___3 ___2 ___1 ___0

ESCORE:

ANEXO D. Mini Exame do Estado Mental (MEEM)

MINI-EXAME DO ESTADO MENTAL
(Folstein, Folstein & McHugh, 1.975)

Paciente: _____

Data da Avaliação: ____/ ____/ ____ Avaliador: _____

ORIENTAÇÃO
- Dia da semana (1 ponto) ..... ( )
- Dia do mês (1 ponto) ..... ( )
- Mês (1 ponto) ..... ( )
- Ano (1 ponto) ..... ( )
- Hora aproximada (1 ponto) ..... ( )
- Local específico (aposento ou setor) (1 ponto) ..... ( )
- Instituição (residência, hospital, clínica) (1 ponto) ..... ( )
- Bairro ou rua próxima (1 ponto) ..... ( )
- Cidade (1 ponto) ..... ( )
- Estado (1 ponto) ..... ( )

MEMÓRIA IMEDIATA
- Fale 3 palavras não relacionadas. Posteriormente pergunte ao paciente pelas 3 palavras. Dê 1 ponto para cada resposta correta ..... ( )

  Depois repita as palavras e certifique-se de que o paciente as aprendeu, pois mais adiante você irá perguntá-las novamente.

ATENÇÃO E CÁLCULO
- (100 - 7) sucessivos, 5 vezes sucessivamente (1 ponto para cada cálculo correto) ..... ( )

  (alternativamente, soletrar MUNDO de trás para frente)

EVOCAÇÃO
- Pergunte pelas 3 palavras ditas anteriormente (1 ponto por palavra) ..... ( )

LINGUAGEM
- Nomear um relógio e uma caneta (2 pontos) ..... ( )
- Repetir "nem aqui, nem ali, nem lá) (1 ponto) ..... ( )
- Comando: "pegue este papel com a mão direita dobre ao meio e coloque no chão (3 ptos) ..... ( )
- Ler e obedecer: "feche os olhos" (1 ponto) ..... ( )
- Escrever uma frase (1 ponto) ..... ( )
- Copiar um desenho (1 ponto) ..... ( )

ESCORE: (____/30)