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Biomarcadores periféricos no transtorno bipolar

Um estudo de base populacional em adultos jovens

Pedro Vieira da Silva Magalhães

Orientador: Prof. Dr. Flávio Kapczinski

Porto Alegre

2011

PEDRO VIEIRA DA SILVA MAGALHÃES

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Tese apresentada ao curso de pós-graduação em Ciências Médicas: Psiquiatria da Universidade Federal do Rio Grande do Sul como requisito parcial à obtenção do título de Doutor em Psiquiatria

Orientador: Prof. Dr. Flávio Pereira Kapczinski

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Porto Alegre, 2011

Dedicatória

Para Marina, que está perto,

sempre

Ring the bells that still can ring

Forget your perfect offering

There is a crack in everything

That's how the light gets in.

Leonard Cohen

Anthem

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LISTA DE ABREVIATURAS

ASSIST – do inglês, *Alcohol, Smoking and Substance Involvement Screening Test*

BDNF – Fator neurotrófico derivado do cérebro (do inglês, *brain-derived neurotrophic factor*)

CIDI – do inglês, *Composite International Diagnostic Interview*

ECA – do inglês, *Epidemiologic Catchment Area*

IL-2 – Interleucina 2

IL-6 – Interleucina 6

IL-10 – Interleucina 10

MINI – do inglês, *Mini-International Neuropsychiatric Interview*

NCS – do inglês, *National Comorbidity Survey*

NAC – N acetil-cisteína

PCC – Conteúdo da proteína carbonil (do inglês, *protein carbonyl content*)

SCID – do ingles, *Structured Clinical Interview for DSM-IV*

TBARS - Conteúdo de substâncias reativas ao ácido tiobarbitúrico (do inglês, *thiobarbituric acid reactive substances*)

TNF-α – Fator de necrose tumoral alfa (do inglês, *tumor necrosis factor alpha*)

Sumário

PREFÁCIO

Este documento representa um projeto que pode ser entendido como razoavelmente ousado e relativamente simples. O primeiro, porque foi composto de mais de uma fase e envolveu mais de um grupo de pesquisa; exigiu uma grande dose de planejamento e esforço na execução. As conversas e entendimentos foram longos, mas finalmente levaram a um trabalho que aproveitou ao máximo o melhor de cada grupo. O segundo, porque o propósito principal do empreendimento foi verificar uma série de estudos previamente realizados em amostras clínicas. A tentativa foi realizar do início ao fim um projeto translacional, e observar se os dados obtidos de pacientes são observados na população geral.

A epidemiologia e a fisiopatologia são áreas com uma tradição muito rica e extremamente interessantes. Aqui talvez seja útil lembrar que pode haver tensão entre estas disciplinas. Por vezes, há um entendimento que a primeira é necessariamente superficial e abrangente, a segunda profunda e restrita. Embora a genética já se beneficie há um certo tempo de desenhos populacionais, os estudos de fisiopatologia em psiquiatria ainda são em grande parte baseados em casos graves. Isso é bastante compreensível, já que estudos populacionais têm uma série de dificuldades. Na psiquiatria, especialmente, a confirmação de caso é sensível não só ao instrumento utilizado, como também ao entrevistador.

Nossa saída aqui foi planejar um estudo um tanto misto, com uma fase puramente epidemiológica de rastreamento. Em uma segunda fase, os diagnósticos foram confirmados, as amostras biológicas coletadas. Inevitavelmente, houve reclassificações, e talvez isso seja um bom exemplo do tipo de tensão que foi

suportada. O próprio desenho talvez fosse entendido por puristas como arruinando a virtude da epidemiologia. Mesmo assim, pareceu-nos benéfico utilizar um diagnóstico mais confiável em prejuízo de um desenho ideal.

Como não é o propósito confundir o leitor, tratamos aqui de explicar a organização geral da tese. Primeiramente, há uma introdução, onde se procura mostrar como de costume os fatos mais relevantes em relação à epidemiologia e fisiopatologia do transtorno bipolar. Conceitos básicos, como o de prevalência durante a vida, idade de início e busca de serviços foram essenciais no planejamento do estudo. Em última instância eles permitiram a formulação de hipóteses realistas com o tamanho de amostra disponível.

Às introduções, segue uma explanação geral do método empregado. Os principais artigos que vêm do trabalho estão na próxima seção. Os resultados se compõem de quatro artigos. Eles tratam basicamente de diferenças nos mediadores de interesse entre os grupos estudados. Todos têm uma seqüência parecida, com as análises bivariadas usuais seguidas de algum tipo de modelo multivariado que teve o objetivo de controlar vieses esperados. O primeiro artigo dos resultados trata da relação de estresse oxidativo com o diagnóstico. O segundo, de uma neurotrofina, o fator neurotrófico derivado do cérebro. O terceiro, de citocinas. A seção é fechada por dados sobre a relação entre esses mesmos marcadores e os diagnósticos de transtorno de humor.

Após as conclusões e considerações finais, ainda há alguns anexos. Eles são artigos relacionados com o estudo principal. Como a tese é sobre a fisiopatologia sistêmica do transtorno bipolar, uma derivação imediata é a possibilidade de utilizar os mesmos mecanismos de forma a atenuar tais vias. Antioxidantes possivelmente

têm sido mais freqüentemente empregados com esta finalidade. Entre os anexos, encontram-se dois estudos sobre o uso da N-acetil-cisteína em um ensaio clínico randomizado. Finalmente, há dois protocolos desenvolvidos com a Colaboração Cochrane –em andamento – sobre o uso de antioxidantes no transtorno bipolar e na esquizofrenia. O propósito delas será organizar o campo e averiguar qual deve ser o lugar dos antioxidantes na clínica psiquiátrica atual.

MAGALHÃES, Pedro Vieira da Silva. Biomarcadores periféricos no transtorno bipolar: um estudo de base populacional em adultos jovens. 2011. Tese (Programa de Pós-Graduação em Ciências Médicas: Psiquiatria). Universidade Federal do Rio Grande do Sul.

RESUMO

OBJETIVO: Confirmar, em uma amostra de jovens provenientes da população geral, achados recentes em relação à fisiopatologia do transtorno bipolar. Foi escopo desta investigação avaliar diferenças em uma neurotrofina, dois marcadores de dano oxidativo, duas citocinas pró-inflamatórias e uma antiinflamatória entre grupos de participantes com transtorno bipolar, depressão maior e também pessoas sem quaisquer episódios de humor. Nominalmente, foram elas o fator neurotrófico derivado do cérebro (*brain-derived neurotrophic factor*, BDNF), conteúdo de substâncias reativas ao ácido tiobarbitúrico (*thiobarbituric acid reactive* substances, TBARS), o conteúdo de proteína carbonil (*protein carbonyl content*, PCC), o fator de necrose tumoral-alfa (*tumor necrosis factor-alpha,* TNF-α), a interleucina-6 (IL-6) e a interleucina-10 (IL-10). **MÉTODO:** Indivíduos provenientes da população geral, que haviam participado de um estudo transversal (n=1560), com um rastreamento positivo para o transtorno bipolar foram recrutados, bem como dois grupos de controles. O primeiro tinha apenas episódios depressivos e o segundo não tinha história de episódios de humor. Isso levou a uma amostra de 231 participantes que passou por confirmação diagnóstica com a Entrevista Clínica Estruturada para o DSM-IV. Todas as análises incluíram avaliação de associações bivariadas. Um modelo *a priori* que incluía sexo, classe social, estado atual de humor, uso de substâncias e grupo diagnóstico como preditores foi utilizado. **RESULTADOS:** A amostra final foi composta por 55 participantes com transtorno bipolar, 82 com

depressão maior e 95 controles. Uma minoria (9,6%) utilizava medicações psiquiátricas quando da entrevista. O transtorno bipolar foi associado a níveis circulantes elevados de PCC e TNF-α quando comparado com o grupo controle. A depressão maior também foi associada a níveis elevados de PCC quando comparada com o grupo sem episódios de humor. O uso de medicações psiquiátricas se associou com níveis mais baixos de TNF-α. As correlações entre os marcadores não foram tão fortes quanto em amostras clínicas anteriores. **CONCLUSÕES:** Os resultados encontrados apontam para duas conclusões mais amplas. Primeiramente, o transtorno bipolar se associa com um estado pró-oxidante e pró-inflamatório desde fases iniciais. Em segundo lugar, essas alterações parecem mais sutis que as observadas em amostras clínicas compostas por pessoas com doença crônica, o que reforçaria a idéia da ocorrência de algum tipo de progressão da doença. O principal cuidado com esses resultados é que provêm de amostras transversais, não longitudinais. Isso faz com que causalidade não possa ser inferida, e permanece a possibilidade que outros fatores além da doença bipolar sejam responsáveis pela toxicidade sistêmica observada.

Palavras chave: transtorno bipolar, transtornos de humor, fisiopatologia, neurotrofinas, marcadores inflamatórios, estresse oxidativo, população geral, caso-controle.

MAGALHÃES, Pedro Vieira da Silva. Peripheral biomarkers in bipolar disorder: a population-based study in young adults. 2011. Tese (Programa de Pós-Graduação em Ciências Médicas: Psiquiatria). Universidade Federal do Rio Grande do Sul.

ABSTRACT

OBJECTIVE: The aim of this study was to confirm, in a sample of young adults from the general population, recent findings regarding the pathophysiology of bipolar disorder. The focus of this investigation was finding group differences in one neurotrophin, two markers of oxidative damage, two pro-inflammatory cytokines and one anti-inflammatory cytokine in participants with bipolar disorder, major depression and people without any mood episodes. Markers assessed here were brain-derived neurotrophic factor (BDNF), thiobarbituric acid reactive substances (TBARS), protein carbonyl content (PCC), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-10 (IL-10). **METHOD:** Individuals from the general population, previously included in a cross-sectional study (n=1560), with a positive screen for bipolar disorder were recruited, as well as two groups of controls. One had only depressive episodes and the other had no history of mood episodes. This yielded a sample of 231 participants that further underwent diagnostic confirmation with the Structured Clinical Interview for DSM-IV (SCID). All analyses included a check for bivariate associations as well as an *a priori* multivariate model with sex, social class, current mood state, use of substances and SCID diagnoses as predictors. **RESULTS:** The final sample included 55 participants with bipolar disorder, 82 with major depression and 95 healthy controls. Only a minority was using any psychiatric medications (9.6%). Bipolar disorder was associated with higher PCC and TNF-α levels when compared to the control group. Major depression was also associated with higher

PCC levels when compared to the control condition. Use of psychiatric medication was associated with lower TNF-α levels. Correlations between the same markers were not as strong as in clinical samples. **CONCLUSIONS** Two broad conclusions are called for from these results. The first is that early-stage bipolar disorder is already associated with a pro-oxidant, pro-inflammatory state. The second is that these changes appear more subtle than those observed in typical late-stage, chronic patients, supporting the notion that a form of illness progression takes place. The main caveat is that this data is cross-sectional, not longitudinal. This precludes causal inferences as factors other than the bipolar illness can conceivably induce systemic toxicity.

Keywords: bipolar disorder, mood disorders, pathophysiology, neurotrophins, inflammation markers, oxidative stress, general population, case-control.

INTRODUÇÃO

Epidemiologia do transtorno bipolar[1]

Idealmente, evidências epidemiológicas devem fornecer uma medida de magnitude da doença, distribuição na população e uma composição de distintos fatores de risco associados. Além das conseqüências para saúde pública, tais evidências podem ser utilizadas a fim de associar a ocorrência de uma doença com fatores genéticos, psicológicos, sociais e ambientais. Talvez por a história da pesquisa em psiquiatria ser repleta de falhas na replicação de estudos e seus achados, a epidemiologia já foi comparada a um campo minado que exige precisão constante na linguagem e rigor incansável na lógica para ser atravessado (Goodwin e Jamison, 1990). Muitas dessas falhas se devem a desenhos de pesquisa inadequados, executados sem um pensamento epidemiológico cuidadoso. O diagnóstico comunitário das taxas de risco em uma determinada população é dos principais objetivos de estudos epidemiológicos (Regier e Robins, 1991). Essa pesquisa deve ser necessariamente baseada em amostras populacionais, minimizando os vieses presentes ao se estudar apenas aqueles casos que buscaram tratamento (Anthony et al, 1995a). Assim, estudos populacionais, com todas as despesas que acarretam, têm sido cada vez mais atraentes (Anthony et al, 1995b).

Muito embora estudos comunitários tenham sido conduzidos nos Estados Unidos desde o final da segunda guerra mundial, somente após o começo dos anos

[1] Publicado originalmente em Magalhaes PVdS, Pinheiro RT. Epidemiologia do transtorno bipolar. In: Kapczinski F, Quevedo J, eds. Transtorno bipolar: teoria e clínica. Porto Alegre: Artmed; 2009.

80, com a criação de entrevistas estruturadas baseadas no DSM-III, tem sido possível estimar a distribuição de transtornos mentais específicos (Kessler et al, 2007; Lima et al, 2005). Esta “terceira geração” da epidemiologia psiquiátrica combina o inquérito de campo com uma abordagem deliberada em transtornos específicos (Anthony et al 1995b).

Um elemento essencial para a condução de qualquer estudo epidemiológico é um método de definição de caso apropriado para a população alvo. Com amostras pequenas, é possível utilizar métodos muito similares àqueles usados na prática clínica; quando amostras grandes são necessárias, contudo, os métodos clínicos devem ser adaptados para que entrevistadores leigos possam entrevistar os sujeitos da pesquisa em suas residências (Regier e Robins, 1991). Esses entrevistadores necessitam entrevistas altamente estruturadas, já que não se pode esperar que interpretem respostas a perguntas abertas relativas a significância clínica. Como resultado, uma questão importante a avaliar nesses estudos é a validade dos diagnósticos. Mesmo que diagnósticos gerados por profissionais competentes e treinados em entrevistas clínicas semi-estruturadas, como o *Structured Clinical Interview for DSM* (SCID; Spitzer et al, 1992), sejam considerados “padrão ouro” em psiquiatria (Zimmerman, 2003) atualmente, nos grandes estudos de campo é impossível disponibilizar tais recursos. Logo, a comparação da performance do intrumento utilizado pelos entrevistadores nos grandes estudos comunitários com entrevistas clínicas é vital para a compreensão dos resultados.

Apoiada nestes métodos, a epidemiologia do transtorno bipolar tem sido melhor delimitada nas últimas décadas, e tecnologias de ensaios de campo cada vez mais sofisticadas tem ajudado a melhor dimensionar o problema. Obviamente, o estudo da distribuição dos transtornos mentais depende da definição de caso, e esta

variação nos critérios diagnósticos parece ser a maior causa de erro na epidemiologia psiquiátrica. Inconsistências diagnósticas podem advir tanto de baixa confiabilidade das definições de termos diagnósticos, quanto dos limiares utilizados para definição de caso (Goodwin e Jamison, 1990). Enquanto o DSM-IV distingue pacientes com transtorno bipolar tipo I, transtorno bipolar tipo II e transtorno bipolar sem outra especificação, a validade desses grupos tem sido criticada, principalmente quanto à identificação de morbidade subdiagnóstica (Angst et al, 2002). Também é claro que a prevalência na população do transtorno bipolar depende de quão restritivos os critérios diagnósticos adotados são; critérios mais restritivos, como os adotados nos esquemas atuais tenderão a gerar uma proporção maior de casos de depressão, em detrimento ao transtorno bipolar. Como exemplo, a proporção de pacientes com transtorno bipolar entre pacientes com transtornos de humor, dependendo da definição de hipomania, variou entre um quarto e metade no estudo de Zurique (Angst et al, 2002).

Oferecemos aqui uma revisão e uma crítica metodológica aos principais inquéritos epidemiológicos realizados em amostras populacionais representativas (tabela 1).

PREVALÊNCIA

Prevalência se refere à proporção da população afetada em algum intervalo de tempo especificado. As duas estimativas de prevalência mais utilizadas são a prevalência durante a vida (a proporção da população com história de doença até o momento da avaliação) e a prevalência nos últimos 12 meses (a proporção da população afetada nos últimos 12 meses antes da avaliação) (Kessler et al, 2007).

O primeiro estudo comunitário a utilizar critérios do DSM-III para gerar dados relativos a taxas e risco para transtornos afetivos foi o *Epidemiologic Catchment Area Study* (ECA, Weissman et al, 1991). Utilizando uma entrevista diagnóstica totalmente estruturada especialmente desenhada para o estudo, a *Diagnostic Interview Schedule* (DIS), a prevalência durante a vida encontrada neste estudo para o transtorno bipolar tipo I foi de 0,8%, e para o transtorno bipolar tipo II, 0,5%.

Tabela 1. Características do delineamento dos principais inquéritos epidemiológicos de base populacional

Estudo	**País**	**Instrumento**	**Taxa de resposta**	**Tamanho da amostra**	**Subtipos avaliados**
Weissman et al (1991)	EUA	DIS*	68-79%	19.182	Transtorno bipolar tipo I Transtorno bipolar tipo II
Kessler et al (1997)	EUA	CIDI**	82,4%	8.098	Transtorno bipolar tipo I
ten Have et al (2002)	Holanda	CIDI v1.1	Nd	7.076	Transtorno bipolar tipo I Transtorno bipolar SOE
Mitchell et al (2004)	Austrália	CIDI v2.1	78,1%	10.641	Transtorno bipolar tipo I
Moreno et al (2005)	Brasil	CIDI v1.1	65,2%	1.464	Transtorno bipolar tipo I Transtorno bipolar tipo II Hipomania subsindrômica Sintomas maníacos
Schaffer et al (2006)	Canada	CIDI	77%	36.984	Transtorno bipolar tipo I
Merikengas et al (2007)	EUA	CIDI v3.0	70,9%	9.282	Transtorno bipolar tipo I Transtorno bipolar tipo II Transtorno bipolar sublimiar

* Diagnostic Interview Schedule

** Composite International Diagnostic Interview

As reavaliações da *Composite International Daignostic Interview* (CIDI), um instrumento criado pela Organização Mundial de Saúde para gerar diagnósticos de transtornos mentais através de entrevista estruturada aplicada por entrevistadores leigos (Kessler et al, 2006a; Quintana et al, 2007), demonstra o ponto que tanto a definição de caso quanto as características psicométricas do instrumento utilizado para o diagnóstico são cruciais para a estimativa de prevalência. No *National Comorbidity Survey* (NCS) original (Kessler et al, 1994), por exemplo, a alta taxa de falso-positivos gerada pela CIDI aplicado por entrevistadores não clínicos em comparação a diagnósticos clínicos utilizando o SCID levou os autores, em relatos subsequentes (Kessler et al, 1997), a analisarem apenas aqueles casos em que o humor era eufórico (e não irritável), os únicos com validade considerável.

Tabela 2. Prevalência de transtorno bipolar tipo I, tipo II e formas sublimiares, durante a vida e nos últimos 12 meses

Estudo	**Transtorno bipolar tipo I**		**Transtorno bipolar tipo II**		**Transtorno bipolar sublimiar**	
	12m	Vida	12m	Vida	12m	Vida
Weissman et al (1991)	0,7%	0,8%	0,3%	0,5%	-*	-
Kessler et al (1997)	0,37%	0,45%	-	-	-	-
ten Have et al (2002)	-	1,3%	-	0,6%	-	-
Mitchell et al (2004)	0,5%	-	-	-	-	-
Moreno et al (2005)	-	1%	-	0,7%	-	6,6%
Shaffer et al (2006)	-	2,2%	-	-	-	-
Merikengas et al (2007)	0,6%	1%	0,8%	1,1%	1,4%	2,4%

* Não disponível

Desta maneira, estudos que utilizaram versões anteriores da CIDI e a definição de síndrome eufórica acharam prevalências mais baixas de transtorno bipolar, mas com uma taxa menor de falso-positivos. Uma reavaliação do inquérito

holandês (Regeer et al, 2004) também demonstrou este efeito, e apenas 40% dos diagnósticos feitos pela CIDI foram confirmados pelo SCID. Entretanto, nesta reavaliação uma proporção importante de pacientes diagnosticados com transtorno depressivo maior pela CIDI também seria reclassificada como transtorno bipolar; esta versão da CIDI também gera, portanto, excessivos falso-negativos.

Além do NCS, o inquérito populacional australiano (Mitchell et al, 2004) também utilizou esta definição, e os dois estudos chegaram a prevalências muito similares do transtorno bipolar tipo I, 0,5% no primeiro e 0,45% no segundo. O inquérito canadense (Schaffer et al, 2006) ignorou esta característica da CIDI e, com uma definição de mania que não requeria os 7 dias de duração, chegou a uma prevalência durante a vida de 2,2%.

Esta também pode ter sido uma questão no estudo populacional brasileiro (Moreno et al, 2007), que também utilizou uma versão anterior da CIDI, e chegou a prevalências durante a vida de 1% para o transtorno bipolar tipo I, 1,1% para o transtorno bipolar tipo II e 6,6% para o espectro bipolar. Neste estudo, entretanto, os diagnósticos gerados pela CIDI não foram comparados ao de entrevistas clínicas. Uma outra versão (v2.1) foi testada separadamente (Quintana et al, 2004; Quintana et al, 2007), entretanto, e a sensibilidade para o diagnóstico de transtorno bipolar foi bastante baixa (38,9%).

Estas dificuldades na validade da CIDI parecem ter sido, pelo menos parcialmente, superadas em versões subsequentes (Kessler et al, 2006a; Kessler et al, 2007). Na nova versão, utilizada na replicação do *National Comorbidity Survey* (NCS-R), a concordância foi excelente para qualquer transtorno bipolar e para o transtorno bipolar tipo I, embora ainda haja dificuldade em distinguir o transtorno bipolar tipo II de casos sublimiares, definidos aqui como hipomania sublimiar

recorrente na presença ou não de episódio depressivo ou hipomania recorrente, na presença ou não de episódio depressivo sublimiar. Os valores preditivos positivo e negativo para a CIDI em relação ao SCID foram, respectivamente de 88,4% e 100% para qualquer transtorno bipolar. Portanto, o NCS-R fornece provavelmente a melhor estimativa da prevalência do transtorno bipolar como atualmente conceitualizado, além de fornecer dados quanto a uma parcela dos casos sublimiares. Este estudo chegou a uma prevalência durante a vida de 1% para o transtorno bipolar tipo I, 1,1% para o transtorno bipolar tipo II e 2,4% para casos sublimiares.

FATORES ASSOCIADOS

Além de identificar a taxa basal de prevalência da doença, estudos comunitários são essenciais para a identificação de subgrupos de risco na população. Assim, o objetivo final de um estudo epidemiológico é identificar componentes específicos, que possam eventualmente ser passíveis de mudança, na cadeia causal que leva à doença (Regier e Robins, 1991). Enquanto impressões clínicas estimulam a pesquisa em fenomenologia e tratamento, achados epidemiológicos podem apontar abordagens promissoras para o entendimento de processos patológicos (Goodwin e Jamison, 1990).

Sexo

Um achado consistente nos estudos populacionais tem sido prevalências similares entre os sexos, pelo menos para o transtorno bipolar tipo I (Weissman et al, 1991; Kessler et al, 1997; ten Have, 2002; Mitchell et al, 2004; Schaffer et al, 2006). Um menor número de estudos também não achou diferenças na prevalência do

transtorno bipolar tipo II (Moreno et al, 2005; Merikengas et al, 2007), embora no inquérito holandês a categoria que incluía tanto transtorno bipolar tipo II quanto não-especificado tenha sido associada ao sexo feminino (ten Have, 2002). Assim, o transtorno bipolar se diferencia da depressão unipolar, na qual a predominância do sexo feminino é clara (Moreno e Dias, 2002).

Idade

Nos inquéritos epidemiológicos, a prevalência do transtorno bipolar, não apenas durante a vida (Weissman et al, 1991; Kessler et al, 1997; Moreno et al, 2005; Shaffer et al, 2006; Merikengas et al, 2007) mas como nos últimos 12 meses (Weissman et al, 1991; Mitchell et al, 2004) tem sido maior em grupos de menor faixa etária.

Algumas explicações tem sido oferecidas para esta diferença nos grupos etários. Como pessoas com transtorno bipolar têm um risco de morte precoce elevado em relação à população geral, e não apenas por suicídio (Osby et al, 2001), uma possibilidade seria que viés de sobrevivência esteja distorcendo os resultados (Kessler et al, 1997). Uma outra possibilidade é que um fenômeno descrito como antecipação, em que em sucessivas gerações ou a doença aumenta sua gravidade ou dimiunui sua idade de instalação, esteja ocorrendo (McInnis et al, 1993; Parker et al, 2006). Isso constituiria um verdadeiro efeito de coorte, ou seja, a idade de começo do transtorno bipolar vem diminuindo nas novas gerações.

Estado civil

História de divórcio, independentemente do estado civil atual, tem estado associada ao transtorno bipolar (Weissmen et al, 1991; Mitchell et al, 2004; Moreno

et al, 2005; Merikengas et al, 2007). É possível que relações causais recíprocas ocorram neste caso: tanto o episódio afetivo pode ser resultado da separação quanto o estresse causado pelo transtorno bipolar levar ao rompimento.

Nível educacional e sócio-econômico

O transtorno bipolar esteve associado a baixo status sócio-econômico em alguns estudos (Weissman et al, 1991 , Kessler et al, 1997), mas não em todos (Merikengas et al, 2007). A relação com desemprego é mais consistente nos estudos americanos, e indivíduos com transtorno bipolar tem uma maior probabilidade de depender de recursos públicos (Weissman et al, 1991) e estarem desempregados (Merikengas et al, 2007), embora isto não tenha sido verificado em outros estudos (ten Have et al, 2002; Mitchell et al, 2004).

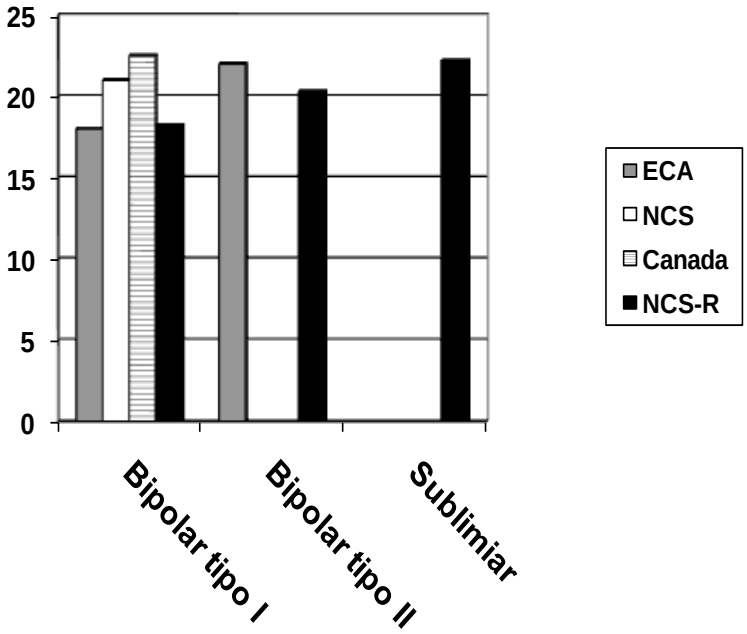
IDADE DE INÍCIO

Em amostras clínicas têm havido interesse em investigar a idade de início do transtorno bipolar e, aparentemente, o início precoce aumenta o risco de piores prognósticos em geral, e particularmente de ciclagem rápida, ideação suicida e comorbidade com transtornos relacionados a substâncias (Bauer & Pfenning, 2005); possivelmente, formas de início precoce sejam subtipos válidos, e já existem modelos de investigação propostos (Leboyer et al, 2005).

Nos estudos comunitários transversais, a principal dificuldade na estimativa da idade de início é o viés de memória, já que a avaliação é realizada retrospectivamente (Lima et al, 2005). Mesmo com esta limitação, estudos comunitários situam a idade de início do transtorno bipolar entre o fim da

adolescência e o começo da idade adulta. No ECA, a idade média de início para o transtorno bipolar tipo I foi de 18 anos e para o transtorno bipolar tipo II, 22 anos (Weissman 1991); no NCS a mediana foi de 21 anos (Kessler 1997). No estudo holandês, a idade média para o primeiro episódio maníaco ou hipomaníaco foi de 26,2 anos; para 40% da amostra, o primeiro episódio foi entre 18 e 24 anos. No estudo canadense a média foi mais baixa, de 22,5 anos, e mais da metade havia desenvolvido a doença antes dos 21 anos. Finalmente, o NCS-R estimou em separado a idade de início para o transtorno bipolar tipo I (18,2 anos), tipo II (20,3 anos) e sublimiar (22,2 anos).

Figura 1. Média de idade de início para as formas clínicas do transtorno bipolar



Como se pode observar na Figura, há uma tendência a um início mais precoce do transtorno bipolar relacionada à gravidade dos sintomas maníacos, isto se observou tanto no ECA quanto no NCS-R. A maior idade de início observada no

inquérito canadense pode estar relacionada à imprecisão do diagnóstico (com, por exemplo, a inclusão errônea de casos de depressão unipolar que normalmente têm idade de início mais tardia), como discutido na seção anterior sobre prevalência.

COMORBIDADE

Uma das consequências do método atual de classificação em psiquiatria, em que diagnósticos categóricos são gerados, é a presença de extensa comorbidade entre casos. Assim, no transtorno bipolar, a extensa maioria dos pacientes é afetada por alguma comorbidade durante a vida, e a presença de multimorbidade é especialmente importante.

Tabela 3. Prevalência de transtornos mentais comórbidos com o transtorno bipolar

Estudo / Comorbidade	NCS (Kessler et al, 1997)*	NCS-R (Merikengas et al, 2007)*	Mitchell et al, 2004
Qualquer transtorno de ansiedade	92,9%	74,9%	52%
Transtorno de ansiedade generalizada	42,6%	29,6%	25,3%
Agorafobia	62,4%	5,7%	6,2%
Fobia simples	66,6%	35,5%	Nd
Fobia social	47,2%	37,8%	19,1%
Transtorno de pânico	33,1%	20,1%	26,3%
Transtorno de estresse pós-traumático	38,8%	24,2%	10,6%
Distimia	49,6%	Nd	7,8%
Qualquer transtorno relacionado ao uso de substância	71%	42,3%	38,9%
Dependência ao álcool	61,1%	23,2%	28,9%
Dependência a drogas	46,1%	14%	26,4%
Multimorbidade (3 ou mais comorbidades)	95,5%	70,1%	Nd

* Comorbidades durante a vida

** Comorbidades em 12 meses

Embora os achados sejam similares para o risco das morbidades descritas abaixo, algumas frequências mostradas na tabela 3 são bastante díspares. É possível que isso possa ser explicado por dois fatores: a definição de caso empregada e o uso de comorbidade durante a vida ou nos últimos 12 meses. No NCS e no inquérito australiano os casos eram de transtorno bipolar tipo I, definidos por mania eufórica, enquanto no NCS-R casos de transtorno bipolar tipo I, tipo II e sublimiares foram incluídos.

Transtornos de ansiedade

No NCS, além de uma notável associação com transtornos de ansiedade, neste estudo episódios maníacos ou depressivos geralmente ocorriam após a instalação do transtorno comórbido (Kessler et al, 1997). Esta associação com transtornos de ansiedade também foi verificada nos inquéritos australiano e canadense, assim como no NCS-R (Mitchell et al, 2004; Schaffer et al, 2006; Merikangas et al, 2007).

Transtornos relacionados a substâncias

No NCS, abuso e dependência de estimulantes foram os únicos diagnósticos com poder para predizer o transtorno bipolar (Kessler et al, 1997). Um efeito similar foi descoberto no inquérito australiano, em que o grupo com transtorno bipolar teve uma probabilidade maior para abuso ou dependência de substâncias ilícitas, mas não de álcool (Mitchell et al, 2004). Já no NCS-R (Merikengas et al, 2007), a comorbidade para abuso ou dependência tanto de drogas ilícitas quanto de álcool

esteve aumentada para todas as subformas, embora a associação tenha sido mais forte para aqueles com transtorno bipolar tipo I.

INCAPACIDADE

Embora uma quantidade relativamente grande de estudos tenha sido dedicada aos custos relacionados à incapacidade nos transtornos de humor, o foco destes tem sido na depressão (Kessler et al, 2007). O transtorno bipolar tem tão grande incapacidade quanto diversas doenças crônicas, e ainda maior incapacidade que a depressão unipolar (Bauer et al, 2005).

Um achado consistente nos estudos populacionais é uma maior quantidade de dias de trabalho perdidos, tanto quando comparado à população geral (ten Have et al, 2002; Mitchell et al, 2004; Kessler et al, 2007), quanto a outros transtornos mentais (ten Have et al, 2002) e à depressão unipolar (Mitchell et al, 2004, ten Have et al, 2002, Kessler et al, 2007). No estudo australiano (Mitchell et al, 2004), aqueles com transtorno bipolar tipo I foram mais afetados que aqueles com transtorno bipolar tipo II ou SOE.

No NCS-R, ainda foi feita uma separação entre absenteísmo (dias de trabalho perdidos) e “presenteísmo” (dias de baixa performance no trabalho). Pacientes com transtornos de humor tanto perdem mais dias de trabalho quanto produzem menos quando presentes; ainda, o impacto do absenteísmo foi menor que do presenteísmo. Ainda, o transtorno bipolar esteve associado a mais perdas que a depressão maior individualmente, embora o prejuízo agregado tenha sido maior para a depressão devido à maior prevalência. Outro achado interessante deste estudo foi que o maior

prejuízo associado ao transtorno bipolar se deve ao fato que nesta condição os episódios depressivos são mais incapacitantes que no transtorno depressivo maior (Kessler et al, 2007).

USO DE SERVIÇOS

O uso de serviços médicos variou bastante conforme a cultura. No NCS original, quase todos os respondentes com transtorno bipolar relataram ter estado em tratamento em alguma ponto de sua vida; no inquérito holandês, a utilização dos cuidados foi menor (72,1%) durante a vida. Um achado importante nos Estados Unidos foi uma maior proporção de tratamento nos últimos 12 meses no NCS-R que no NCS. Conforme esperado, a utilização de serviços, tanto de saúde mental, quanto de qualquer profissional de saúde é muito elevada para aqueles com transtorno bipolar quando comparados à população geral (Moreno et al, 2005; Mitchell et al, 2004). Nos Estados Unidos, o uso de serviços para aqueles com transtorno bipolar foi maior que na depressão maior (Kessler et al, 2007), o que não se repetiu no estudo australiano (Mitchell et al, 2004).

No inquérito holandês (ten Have et al, 2002), aqueles com transtorno bipolar tipo I tiveram uma probabilidade maior de procurar tratamento comparados àqueles com outras formas; ainda, o uso de serviços de saúde mental esteve relacionado a um maior grau de comorbidade, e de maneira especial com transtornos de ansiedade. Embora no inquérito brasileiro os autores afirmem que a procura de serviços é inversamente relacionada à gravidade da sintomatologia maníaca, a sobreposição dos intervalos de confiança para os riscos destes grupos comparados

à população geral sugere que o estudo não tem poder estatístico para tal comparação.

Dado a procura de pacientes com transtorno bipolar por serviços de saúde, uma questão importante é o quão adequado é o tratamento que esses pacientes recebem. Na Holanda, ten Have e cols. (2002) estimam que quase 75% dos pacientes não recebiam tratamento adequado. Novamente, o NCS-R é o estudo que fornece respostas mais detalhadas sobre a adequação do tratamento (Kessler et al, 2007). Embora os pacientes com transtorno bipolar tipo I recebam tratamento mais frequentemente que aqueles com transtorno bipolar tipo II ou formas sublimiares, esta forma também é aquela que mais frequentemente recebe tratamento inadequado. A frequencia de tratamento de manutenção para aqueles sem episódios nos últimos 12 meses também foi muito baixa, principalmente para aqueles casos sublimiares (3,2%), comparado àqueles com transtorno bipolar tipo I (35,3%) ou tipo II (24,5%). Outro achado preocupante foi a baixíssima proporção de casos em clínica geral (9%), os responsáveis pelo tratamento da maioria dos casos, tratados adequadamente; uma proporção maior foi tratada de maneira adequada por especialistas (45%).

CONCLUSÃO[2]

Importantes progressos têm sido alcançados no entendimento da distribuição populacional e consequências do transtorno bipolar na última década. Após os dados gerados pelo ECA e pelo NCS, grandes estudos populacionais em diversos países têm reforçado a consistência de alguns achados e também questionado a validade de dados anteriores.

Talvez o primeiro e mais importante passo seja a avaliação sistemática dos instrumentos utilizados no campo para a definição de caso, já que diagnósticos inválidos não geram dados úteis relacionados à distribuição dos transtornos mentais, causando confusão conceitual e desperdício de recursos de pesquisa. As reavaliações da CIDI demonstram bem este ponto. Especificamente no caso do transtorno bipolar, as versões anteriores geravam tanto diagnósticos falso-positivos quanto falso-negativos, o que foi observado para as versões americana e holandesa (Kessler et al, 1997; Regeer et al, 2004). Assim, as reavaliações destes estudos revisaram de maneira substancial a prevalência de transtorno bipolar tipo I, evitando resultados falso-positivos (mas provavelmente mantendo muitos falso-negativos), e os estudos que utilizaram estes mesmos critérios chegaram a uma prevalência próxima aos 0,5%.

Sem uma avaliação sistemática de tais instrumentos, é provável que uma proporção dos casos seja classificada incorretamente. Assim, esforços para refinar e

[2] Após a publicação deste capítulo, foi publicado um estudo epidemiológico de grande porte bastante relevante (Merikangas, Jin et al., 2011). O *World Mental Health Research Initiative* contou com mais de 60.000 respondentes da população geral de 11 países. Neste, as prevalências agregadas foram algo menores que em estudos anteriores. Durante a vida, as prevalências de transtorno bipolar tipo I, II e sublimiar foram, respectivamente, 0,6%, 0,4% e 1,4%. Mesmo assim, os achados confirmaram uma associação consistente do transtorno bipolar nos diversos países com múltiplas comorbidades e incapacidade. Os autores ainda interpretaram que o fato da gravidade da doença aumentar do tipo I para o tipo II para o sublimiar ainda dá algum suporte epidemiológico à validade do conceito de "espectro".

validar os intrumentos de campo, para que sejam o mais próximos possível do padrão de diagnóstico, assim como realizado por Kessler e cols (2006) ainda são imprescindíveis. O NCS-R, cujos achados para o transtorno bipolar vem sendo recentemente reportados (Kessler et al, 2006a; Kessler et al, 2006b; Merikengas et al, 2007; Kessler et al, 2007), representa um avanço na epidemiologia do transtorno bipolar. Além de apresentar uma validade superior em termos de definição de caso, traz medidas mais sofisticadas em termos de incapacidade, uso de serviços e tratamento. Assim, o estudo americano traz um retrato mais fiel dos desafios relacionados ao transtorno bipolar. Com uma maior incapacidade associada individualmente que a depressão maior e uma impressionante inadequação de tratamento clínico, a epidemiologia moderna revela ser o transtorno bipolar um grande desafio para a psiquiatria em termos de detecção, tratamento e prevenção de incapacidade.

Marcadores periféricos e a fisiopatologia do transtorno bipolar: uma revisão da literatura recente[3]

INTRODUÇÃO

Mesmo que ainda seja lugar comum começarem-se artigos da área por "a fisiopatologia do transtorno bipolar é desconhecida" ou, pior, "um mistério", muito se aprendeu nos últimos anos. A discussão no campo tem ido além – por vezes muito além – dos suspeitos usuais, para incorporar fatores sistêmicos e mecanismos compensatórios, ritmos biológicos e interações entre centro e periferia, estados alostáticos e resiliência celular (Duman, 2004; Kapczinski *et al.*, 2008; Soreca *et al.*, 2009; Berk *et al.*, 2011). Já existem hipóteses abrangentes, mesmo que necessitem de testes formais, sobre como e por que a doença progride (Post, 2007a; b; Kapczinski *et al.*, 2008; Post, 2010; Berk *et al.*, 2011).

O entendimento dos transtornos psiquiátricos graves como doenças sistêmicas não é uma tendência nova. Discussões sobre a bile negra afora, a epidemiologia psiquiátrica moderna deve em muito essa noção ao grupo do professor Angst, em Zurique. Desde a publicação em 2002 de um artigo que já tem status de clássico (Angst *et al.*, 2002), a mortalidade precoce por causas naturais e a carga relacionadas a doenças sistêmicas no transtorno bipolar se encontram em destaque (Kupfer, 2005; Roshanaei-Moghaddam e Katon, 2009). Pelo menos em populações clínicas, mais da metade dos pacientes com transtorno bipolar relata algum tipo de comorbidade sistêmica (Altamura *et al.*, 2011). São proeminentes nesse grupo doenças cardiovasculares, diabetes, obesidade, dislipidemia e

[3] Magalhães PV, Fries GR, Kapczinski F. Submetido para publicação na Revista de Psiquiatria Clínica

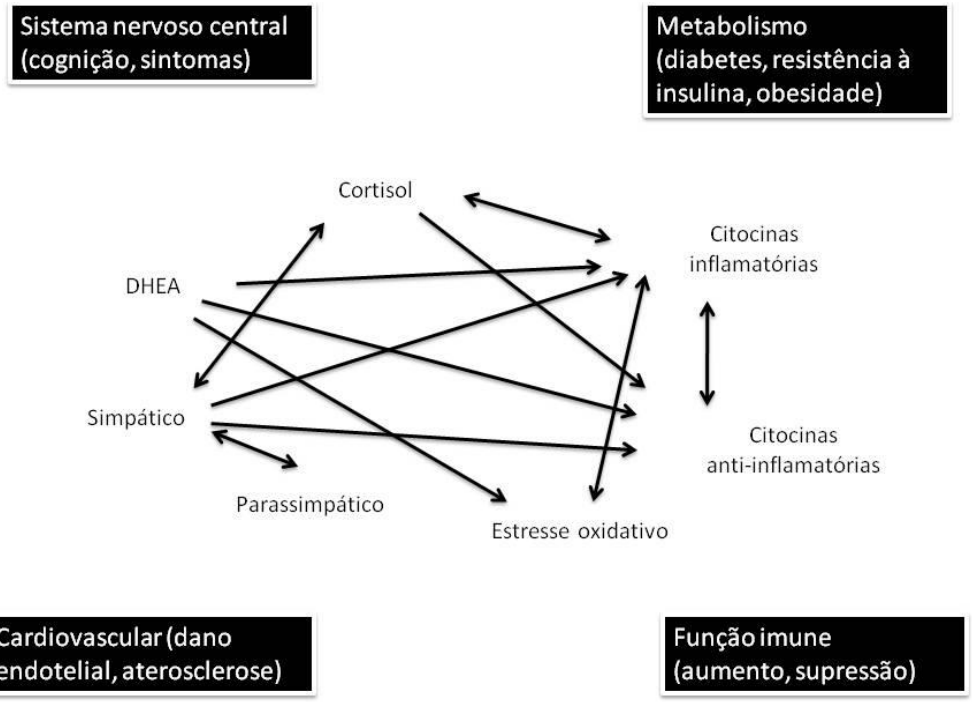
resistência à insulina, componentes da síndrome metabólica (Gomes *et al.*, 2010; Gomes *et al.*, 2010; Altamura *et al.*, 2011). Por um lado, essa comorbidade é responsável por parte significativa da disfunção associada à doença. Mas, fechando o círculo, é possível que a progressão da doença bipolar também se associe a uma maior prevalência de doenças sistêmicas, talvez por uma diátese compartilhada (Pasco *et al.*, 2010). O termo “somatoprogressão” já foi proposto para este fenômeno (Goldstein e*t al.*, 2009).

Ao longo dos últimos anos, essa noção vem ganhando consistência e momento. Assim, o grupo da Universidade de Pittsburgh liderado pelo prof. Kupfer tem se concentrado na carga clínica associada à doença, falando em um “envolvimento multissistêmico” (Kupfer, 2005; Soreca *et al.*, 2009). Os efeitos progressivos no sistema nervoso central (SNC), assim como interações entre centro e periferia, são o enfoque escolhido pelo prof. Berk, na Austrália, com destaque para “neuroproteção” e “neuroprogressão” (Berk *et al.*, 2009; Berk *et al.*, 2011). Isso se alinha a dados recentes que sugerem que alguma versão de estadiamento clínico possa ser útil para uma prescrição individual de tratamento (Berk *et al.*, 2007; Berk, 2009; Berk *et al.*, 2011).

Uma abordagem complementar, que ajuda a atrelar esses conceitos, é a noção de alostase (Kapczinski *et al.*, 2008). Alguns sistemas biológicos são rigidamente controlados e requerem variação mínima, a exemplo do pH. Embora esse tipo de regulação homeostática seja vital, em outros sistemas há vantagem em adaptação através de mudança. Essa regulação alostática permite maior resiliência aos desafios dinâmicos da vida (Juster *et al.*, 2010). Em curto prazo, é um processo benéfico e adaptativo. Se os processos se tornam extremos ou ineficientes, entretanto, falamos em “carga” alostática ou mesmo “sobrecarga” alostática. O

cérebro encontra-se em um ponto chave, pois tanto coordena os processos fisiológicos e comportamentais paralelos, ajustando a mudança interna e externa, como é sensível aos efeitos tóxicos cumulativos resultantes. O que seguiria, nesses casos, seriam o dano sistêmico e a neurodegeneração observados repetidas vezes em sujeitos com transtornos neuropsiquiátricos.

Figura 2. Modelo adaptado da proposta de Juster & McEwen (2010) para entendimento das relações entre mediadores de alostase envolvidos na resposta ao estresse.



O entendimento de como ocorrem essas inter-relações sistêmicas passa pela compreensão de seus mediadores (Juster *et al.*, 2010). O modelo da alostase postula redes complexas e não lineares de múltiplos sistemas de mediadores (Figura 2). Essa busca por fatores intermediários não é incompatível com a proposta recente que biomarcadores individuais não serão suficientes para identificar

transtornos complexos (Singh e Rose, 2009). Embora uma definição mais estrita de biomarcador envolva o poder preditivo de um fator diagnóstico ou prognóstico, os mesmos também são úteis como correlatos da fisiopatologia da doença e mesmo como alvos terapêuticos (Schwarz e Bahn, 2008). O sangue periférico é um fluido corporal facilmente acessível, e determinadas proteínas aí encontradas podem refletir os níveis centrais por trocas através da barreira hematoencefálica (Lakhan e Kramer, 2009). Além disso, a própria natureza de alguns marcadores – de estresse oxidativo e inflamação, por exemplo – torna lógica sua investigação na periferia.

Nosso objetivo aqui é descrever achados recentes em relação à fisiopatologia sistêmica do transtorno bipolar. Será dado um enfoque especial a dados provenientes de uma experiência brasileira de colaboração que vem obtendo resultados progressivamente em relação à toxicidade sistêmica nos últimos anos (Kapczinski *et al.*, 2009; Hallak *et al.*, 2010). Assim, tentaremos articular uma visão coerente do conhecimento atual do campo.

MÉTODO

Foi realizada uma revisão direcionada e narrativa da literatura. Para excelentes revisões sistemáticas recentemente publicadas sugerimos os trabalhos de Grande e colegas (Grande *et al.*, 2010) em relação a neurotrofinas, de Goldstein e colegas (Goldstein *et al.*, 2009) e Drexhage e colegas (Drexhage *et al.*, 2010) para inflamação. Algumas revisões do grupo do Prof. Berk tratam de maneira exemplar a relação entre biologia oxidativa e transtorno bipolar (Ng *et al.*, 2008; Berk *et al.*, 2011; Dean *et al.*, 2011).

Neuroplasticidade

As neurotrofinas são centrais em vários aspectos do funcionamento do sistema nervoso central. No cérebro de mamíferos foram identificados classicamente quatro membros desta família, o fator de crescimento neural (*nerve growth factor*, NGF), o fator neurotrófico derivado do cérebro (*brain-derived neurotrophic factor*, BDNF), a neurotrofina 3 (NT3) e a neurotrofina 4 (NT4). Desde então, mais de 50 fatores de crescimento neurais foram identificados, como o fator de crescimento semelhante à insulina (*insulin-like growth factor*, IGF) e o fator neurotrófico derivado da glia (*glia-derived neurotrophic factor*, GDNF) (Nagahara e Tuszynski, 2011). Estas moléculas agem ao se ligarem a uma de duas classes de receptores transmembrana, o receptor de neurotrofina p75 e a família Trk de receptores. A interação das neurotrofinas maduras com os receptores Trk promove, entre uma série de efeitos, a sobrevivência celular, um aspecto chave no estabelecimento de neurocircuitos funcionais (Lu *et al.*, 2005).

No sistema nervoso em formação, as neurotrofinas são essenciais para o desenvolvimento através de sua capacidade de promover a sobrevivência e estimular o crescimento de neurônios no sistema nervoso central e periférico (Twiss *et al.*, 2006). No cérebro adulto, atuam decisivamente na plasticidade sináptica, um mecanismo utilizado por animais para aprendizado e adaptação ao ambiente (Chessick *et al.*, 2006). O mesmo processo também tem se provado essencial para a resiliência aos efeitos do estresse (Duman e Monteggia, 2006).

O papel do BDNF tem sido intensamente investigado em diversas situações clínicas (Chessick *et al.*, 2006). Como uma regra geral, uma diminuição nos níveis circulantes de neurotrofina é prejudicial (Twiss *et al.*, 2006). Nos transtornos de humor uma variedade de agentes com propriedades antidepressivas são capazes de

elevar a expressão de BDNF hipocampal quando administrados cronicamente, revertendo diminuições causadas por estresse; é possível que tal mudança seja uma via comum nos efeitos destes agentes (Post, 2007b). O lítio é uma das substâncias que tem a propriedade de aumentar o BDNF e promover neuroproteção (Machado-Vieira *et al.*, 2009; De Sousa *et al.*, 2011). Corroborando este mecanismo, há dados clínicos que sugerem que o lítio esteja associado a uma menor chance para doença de Alzheimer (Nunes *et al.*, 2007).

O BDNF não é a única neurotrofina relevante no transtorno bipolar, com estudos também apontando alterações em NT-3, NT-4/5 e GDNF (Rosa *et al.*, 2006; Walz *et al.*, 2007; Walz *et al.*, 2008; Walz *et al.*, 2009). O BDNF é, entretanto, a neurotrofina de maior distribuição e abundância no SNC e também a mais estudada. Há evidência consistente relacionando seus níveis séricos e atividade da doença no transtorno bipolar. Efeitos agudos dos episódios de humor foram observados, com o BDNF diminuído nos dois pólos (Cunha *et al.*, 2006). Estudos subseqüentes confirmaram esta observação em pacientes não medicados (Machado-Vieira *et al.*, 2007; De Oliveira *et al.*, 2009). Embora nem todos os estudos tenham confirmado este efeito (Kapczinski *et al.*, 2010), metanálises mostram um tamanho de efeito bastante robusto (Lin, 2009; Fernandes *et al.*, 2010). Dados longitudinais preliminares ainda indicam que o tratamento bem sucedido da mania se associa com a normalização dos níveis da neurotrofina (Tramontina *et al.*, 2009; De Sousa *et al.*, 2011).

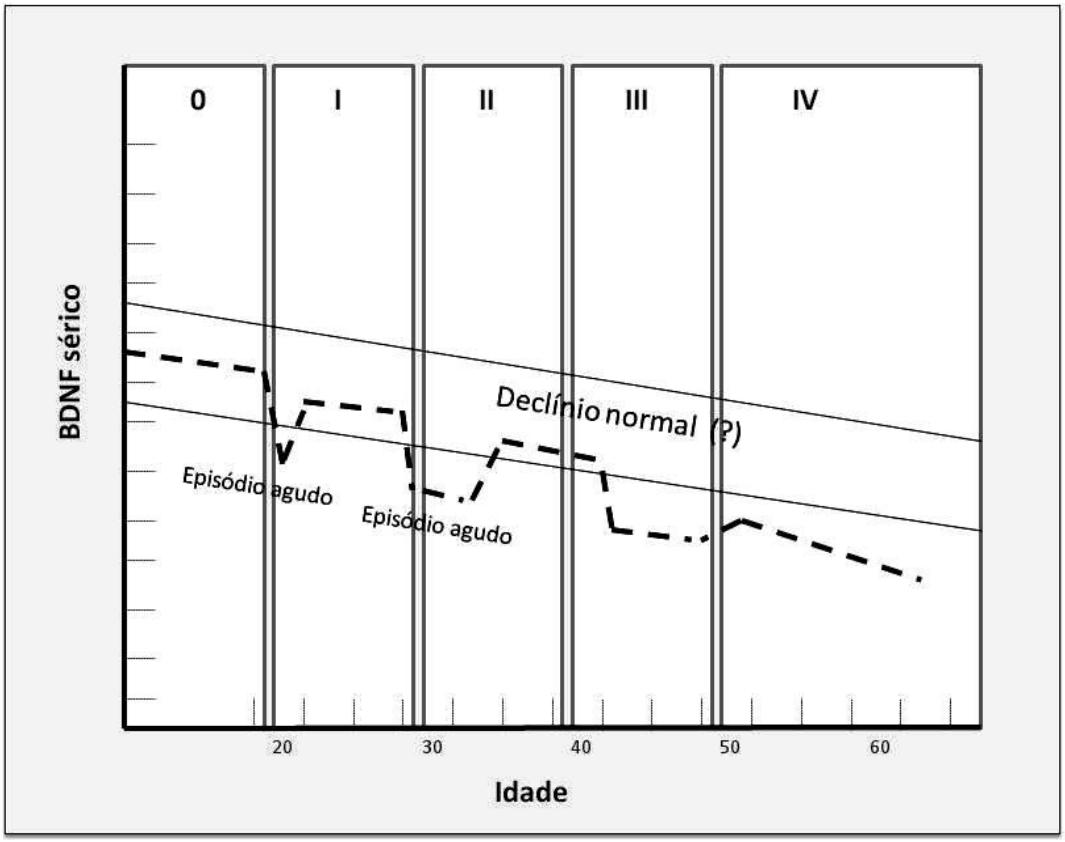
Modelos experimentais ampliam e corroboram muitos destas descobertas em humanos. Mesmo com diferentes graus de validade, modelos animais são relevantes, já que permitem manipulações não factíveis em estudos clínicos (Fries e Magalhaes, 2010). No momento, há modelos animais de mania com validade

suficiente, utilizando a anfetamina e a ouabaína (Machado-Vieira 2004; Frey *et al.*, 2006; Jornada *et al.*, 2010). Ambos os modelos estão associados a uma diminuição do BDNF hipocampal, que é revertida com lítio. Estes achados no sistema nervoso central convergem com os achados em pacientes em episódios agudos. Isso também reforça a validade da avaliação de níveis periféricos de BDNF. Provavelmente o estudo que melhor demonstrou essa associação foi o de Lang (Lang *et al.*, 2007), no qual os níveis séricos de BDNF se correlacionaram com um marcador *in vivo* de integridade neuronal no hipocampo. Outra frente interessante é a de estudos que mostram os efeitos comportamentais em modelos animais de manipulação do BDNF sérico, demonstrando a relevância da neurotrofina na periferia (Schmidt e Duman, 2010).

Uma discussão que ocorre no momento é se o BDNF é apenas relacionado a estados agudos ou é um traço da doença. Este entendimento é dificultado pela ausência de estudos longitudinais. Dito isto, há uma possibilidade que esta neurotrofina tenha características tanto de estado como de traço (Kapczinski *et al.*, 2009). Um estudo recente comparou uma amostra brasileira com doença bipolar crônica com uma amostra canadense de primeiro episódio; todos os pacientes encontravam-se eutímicos (Kauer-Sant'anna *et al.*, 2009; Yatham *et al.*, 2009). Este estudo mostrou uma diminuição no BDNF em relação a controles apenas nos pacientes crônicos. Além disso, um maior tamanho de efeito para a correlação entre o BDNF e a idade foi encontrado nos pacientes com transtorno bipolar que nos controles. Se confirmado em estudos longitudinais, esse achado corroboraria a hipótese que o BDNF atua como um transdutor do estresse psico-social. Inicialmente, estaria baixo em episódios agudos. Mais tardiamente, os níveis de BDNF estariam cronicamente baixos (Figura 2). Isso estaria de acordo com a noção

de neuroprogressão, e seria uma explicação, ao menos parcial, para os déficits associados à doença quando crônica.

Figura 3. Modelo teórico para entendimento da relação entre níveis séricos de BDNF e progressão da doença bipolar. Os numerais romanos acima representam os estágios como propostos por Kapczinski (2009)



O conjunto desses achados levou à introdução de uma proposta de estadiamento do transtorno bipolar, parcialmente baseada em biomarcadores (Berk *et al.*, 2009; Kapczinski *et al.*, 2009). Assim, o BDNF sérico estaria normal nos estágios iniciais durante eutimia. A toxicidade estaria mais ligada ao número de episódios que à idade, e a cada episódio haveria mais prejuízo cognitivo e disfunção

ao retornar à eutimia, com níveis mais baixos de BDNF. Embora o modelo ainda precise ser refinado e formalmente testado, a idéia é que intervenção em estágios iniciais possa ser benéfica, oferecendo um tipo de “neuroproteção” (Berk *et al.*, 2011).

O mecanismo exato desta redução no BDNF sérico não está completamente claro no momento. Ele não parece se dever, pelo menos exclusivamente, a um polimorfismo do gene *BDNF* (Kauer-Sant'anna *et al.*, 2008). Mais do que isso, parece que a sua transcrição é modulada epigeneticamente, sendo influenciada pelo estado de metilação dos promotores do gene *BDNF* e pela ligação de diferentes fatores de transcrição nucleares (Martinowich *et al.*, 2003). O BDNF e outros marcadores de toxicidade sistêmica, como estresse oxidativo e citocinas, freqüentemente se correlacionam em pacientes com transtorno bipolar (Kapczinski *et al.*, 2010). Várias fontes de evidência apontam, por exemplo, que alterações no status redox celular alteram a expressão de BDNF. Uma das hipóteses atuais, portanto, é que a diminuição do BDNF sérico seja em parte causada por um estado de toxicidade sistêmica (Kapczinski *et al.*, 2008).

Estresse oxidativo

As reações chamadas *redox* são base de inúmeras vias e integram a biologia e regulação celular. De uma forma geral, oxidação é o processo em que ocorre uma perda de elétrons; em termos bioquímicos, uma substância que pode receber elétrons é pró-oxidante, e aquela que doa elétrons é um antioxidante. Substâncias pró-oxidantes derivadas do oxigênio e do nitrogênio, conhecidas como espécies reativas, podem causar danos a alvos celulares como lipídeos, DNA e proteínas. Por outro lado, os sistemas de defesa celular incluem enzimas e equivalentes

antioxidantes (Nyska e Kohen, 2002). O estado de estresse oxidativo é resultante de um desequilíbrio entre as moléculas pró-oxidantes e antioxidantes, comumente associado a danos celulares.

Refletindo o interesse no estresse oxidativo, o volume de trabalhos publicados sobre este tema em psiquiatria tem crescido exponencialmente (Ng *et al.*, 2008). Isso provavelmente se deve à ênfase dada nos últimos anos na neuropsiquiatria na sensibilidade cerebral ao dano oxidativo. O cérebro utiliza uma taxa alta de oxigênio e possui defesas antioxidantes modestas, com sua constituição rica em lipídeos também favorecendo o dano (Ng *et al.*, 2008). No transtorno bipolar, a hipótese prevalente é que uma maior carga de estresse oxidativo seja gerada por um distúrbio fundamental na função mitocondrial (Berk *et al.*, 2011). Mais recentemente, estudos *post-mortem* apoiaram esta noção. Tanto alterações no complexo I quanto reduções nos níveis de glutationa foram detectadas no transtorno bipolar (Andreazza *et al.*, 2010; Gawryluk *et al.*, 2011).

Em relação aos antioxidantes, também tem havido interesse em melhor compreender seu papel nos transtornos neuropsiquiátricos. A noção de suplementar o tratamento convencional com substâncias que aliviem a sobrecarga oxidativa no transtorno bipolar é interessante e já foi investigada em alguns ensaios clínicos (Berk *et al.*, 2008; Machado-Vieira *et al.*, 2008; Magalhaes *et al.*, 2011b). Há uma revisão sistemática em andamento na colaboração *Cochrane* que deverá esclarecer o papel atual dessas substâncias como tratamentos adjuvantes (Magalhães *et al.*, 2011).

Os modelos animais de mania efetivamente mostram desbalanço na biologia oxidativa (Frey *et al.*, 2006; Frey *et al.*, 2006; Frey *et al.*, 2006a; b; Andreazza *et al.*, 2008; Valvassori *et al.*, 2008). Estudos clínicos indicam alto dano oxidativo sistêmico

em pacientes com transtorno bipolar (Andreazza *et al.*, 2007; Andreazza *et al.*, 2007; Frey *et al.*, 2007; Kunz *et al.*, 2008; Kapczinski *et al.*, 2011). Além disso, os sistemas antioxidantes também parecem estar freqüentemente alterados, com aumentos significativos nos sistemas da glutationa e da superóxido dismutase (Andreazza *et al.*, 2007; Kunz *et al.*, 2008; Andreazza *et al.*, 2009), provavelmente decorrentes de mecanismos compensatórios ao estado pró-oxidativo existente. Em uma meta-análise recente, os níveis de óxido nítrico e dano oxidativo a lipídeos foram identificados como os marcadores sistêmicos mais consistentemente presentes em pacientes com transtorno bipolar (Andreazza *et al.*, 2008).

É possível que alguma forma de dano oxidativo já acompanhe o início da doença. Isso foi verificado através de níveis aumentados de 3-nitrotirosina em um grupo de pacientes em estágio inicial, o que se verificou também em estágios mais adiantados (Andreazza *et al.*, 2009). De uma forma geral, esses achados sustentam a hipótese de um papel do estresse oxidativo na neuroprogressão do transtorno bipolar, justificando sua importância na pesquisa de novos alvos terapêuticos.

Mediadores inflamatórios

A inflamação é outro componente que associa vias disfuncionais e mortalidade precoce ao transtorno bipolar (Brietzke, 2008; Goldstein *et al.*, 2009; Drexhage *et al.*, 2010). A neuro-inflamação é outro dos mecanismos implicados na progressão da doença bipolar (Berk, 2009; Berk *et al.*, 2011); algumas citocinas provenientes de células residentes e provenientes da periferia têm a capacidade de causar toxicidade e apoptose em neurônios e células da glia (Kraft *et al.*, 2009; Witte *et al.*, 2010). Mecanismos inflamatórios já haviam sido relacionados com a depressão maior, conectando altos níveis de citocinas pró-inflamatórias (por

exemplo, a interleucina 1 (IL-1), interleucina 6 (IL-6) e o fator de necrose tumoral alfa – TNF-α) ao episódio depressivo (Dinan *et al.*, 2009). No entanto, até recentemente, ainda havia poucos estudos sobre o papel da inflamação no transtorno bipolar. Isso vem mudando justamente pelo entendimento do papel da inflamação na articulação dos fatores neuroimunes, neuroendócrinos e neuroquímicos (Goldstein *et al.*, 2009).

Um dos alvos de pesquisa primários tem sido o TNF- α (Brietzke e Kapczinski, 2008). Um dos principais mediadores pró-inflamatórios, o TNF- α age em vias de neuroplasticidade, resiliência e sobrevivência celular, podendo induzir morte celular por apoptose. Seus efeitos são influenciados por outras citocinas (pró- e antiinflamatórias), que orquestram uma série de reações que podem levar a um estado agudo de inflamação. Junto com a interleucina-1β, eles são mediadores inflamatórios primários que ativam a produção de outras citocinas, incluindo a interleucina-6 (IL-6), interleucina-8 (IL-8) e o interferon-gama.

De modo geral, os episódios de humor têm sido bem caracterizados como estados pró-inflamatórios. Os achados mais consistentes sugerem um aumento nos níveis de TNF- α e IL-6 nos episódios de mania e depressão, em comparação com eutímicos ou controles saudáveis (Maes *et al.*, 1995; O'brien *et al.*, 2006; Hung *et al.*, 2007; Ortiz-Dominguez *et al.*, 2007). Estes achados são especialmente evidentes quando pacientes com doença crônica são comparados com voluntários saudáveis (Brietzke *et al.*, 2009; Drexhage *et al.*, 2010; Kapczinski *et al.*, 2011). De maneira bastante interessante, controlando-se para estágio da doença em pacientes eutímicos, observou-se que tanto o TNF- α quanto a IL-6 (outra citocina pró-inflamatória) encontravam-se elevados independentemente do estágio (Kauer-Sant'anna *et al.*, 2009). A interleucina-10, uma citocina antiinflamatória, encontrou-se elevada somente no estágio precoce.

Outros achados relevantes sugerem aumento nos níveis de anticorpos circulantes associação a infecções virais, como o herpes, Borna e o parvovírus B19 (Dickerson *et al.*, 2004; Dietrich *et al.*, 2008; Barbosa *et al.*, 2009; Brietzke *et al.*, 2009). Esses dados, de uma maneira ou outra, evidenciam a grande ligação entre o sistema imunológico e as vias patofisiológicas do transtorno bipolar.

Relações entre os marcadores e toxicidade sistêmica

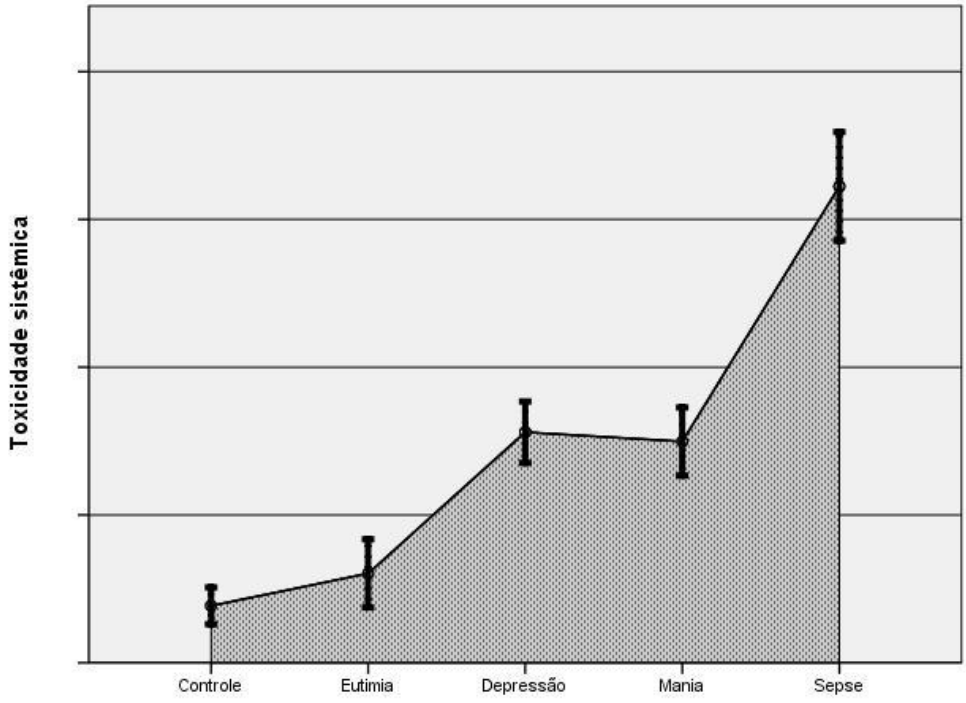
Dadas todas essas observações de estudos pré-clínicos e clínicos, o próximo passo lógico foi avaliar esses marcadores em conjunto. Para tanto, foram recrutados pacientes com transtorno bipolar tanto eutímicos quanto em episódio agudo. Além disso, para ressaltar a direção e relevância das alterações, os marcadores foram avaliados também em um pequeno grupo de pacientes com sepse (Kapczinski *et al.*, 2010; Kapczinski *et al.*, 2011). O estudo resultante permitiu a avaliação de inter-relações entre os marcadores e uma melhor compreensão da fisiopatologia do transtorno bipolar.

Os resultados realmente demonstraram correlações importantes entre a maioria dos marcadores, embora não todos. Utilizando análise de componentes principais, foi extraída uma variável indicando a variância compartilhada pelos biomarcadores. Esta variável assim construída deve ser entendida como um constructo latente de toxicidade sistêmica. A Figura 4 mostra como os episódios de humor são mais bem entendidos como eventos agudamente nocivos e sistemicamente tóxicos.

Individualmente, em sua maioria, os marcadores separaram os indivíduos com transtorno bipolar dos controles normais. O estudo revelou que além de dano oxidativo a lipídeos, a quantidade de dano oxidativo a proteínas a que os pacientes

em episódios agudos estão sujeitos é impressionante, com tamanhos de efeito similares aos de pacientes em sepse.

Figura 4. Toxicidade sistêmica nas diversas fases do transtorno bipolar, em controles saudáveis e na sepse



Direções futuras

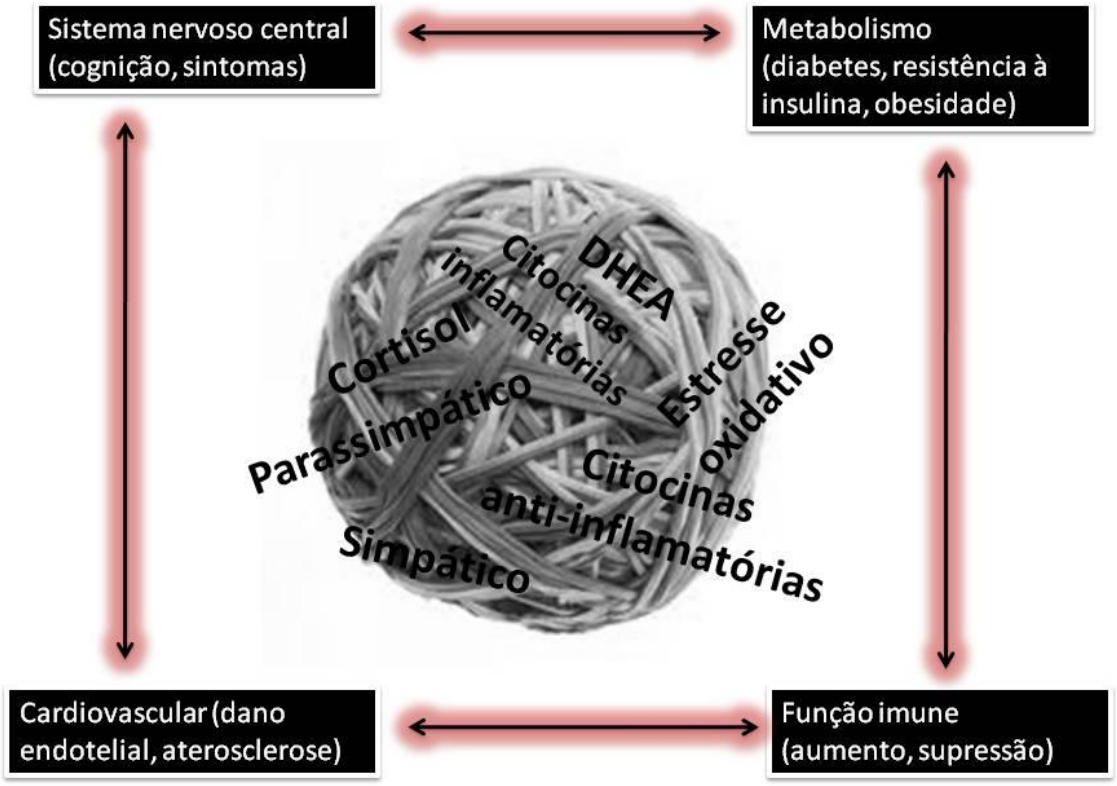
Se ainda não é possível traçar relações de causa-efeito, a literatura recente retrata os episódios agudos de humor como tóxicos. Essa toxicidade sistêmica se associa a características progressivas e incapacitantes ligadas ao transtorno, notoriamente disfunção cognitiva, comorbidades médicas crônicas e mortalidade prematura. Mediadores que ligam o transtorno bipolar a esses desfechos já começam a serem traçados, e as relações entre eles reforçam a hipótese de sobrecarga alostática. Os achados recentes descritos, mesmo que individualmente preliminares, indicam que a exposição repetida a episódios de humor gera

toxicidade tanto na forma de dano quanto na diminuição de defesa. Esses seriam possíveis elementos causadores do dado cognitivo e disfunção progressiva.

Alguns desses fatores, como dano oxidativo e inflamação, também são associados com condições crônicas como diabetes e doença cardiovascular. Esses fatores em conjunto aumentam a vulnerabilidade a novos episódios, levando a um ciclo vicioso. Este conjunto de alças patológicas se retroalimenta, e talvez devido a isso o transtorno bipolar seja uma das principais causas de incapacidade individual (Alonso *et al.*, 2010).

Até o momento, a grande maioria dos achados é válida para pacientes com doença crônica. Realmente, um passo necessário é aumentar a representatividade dos achados a todo o espectro da doença bipolar. Uma maneira seria através de desenhos baseados em amostras populacionais, o que teria a vantagem de se evitar vieses, como o viés de Berkson. A avaliação de adolescentes e adultos jovens com transtorno bipolar é uma outra alternativa interessante. A maioria dos casos de transtorno bipolar tem início até o final da adolescência e estes casos são fenotipicamente similares àqueles vistos em adultos, facilitando sua identificação (Lewinsohn *et al.*, 2003). A avaliação de biomarcadores periféricos em indivíduos jovens daria também uma perspectiva de neurodesenvolvimento (Blumberg *et al.*, 2004) a esta discussão.

Figura 5. Correlações sistêmicas tardias no transtorno bipolar. Neste modelo, a sobrecarga alostática crônica obscurece e distorce as relações complexas anteriores entre as alças de vias de fisiopatologia. O resultado é a associação entre desfechos negativos no sistema nervoso central (“neuroprogressão”) e condições sistêmicas (“somatoprogressão”)



Outra dimensão necessária para esclarecer e firmar o papel destas proteínas é a de desenhos longitudinais. Um exemplo interessante que poderá permitir também uma avaliação do modelo de estadiamento é a coorte de incepção conduzida na Universidade da Columbia Britânica. Esse estudo vem recrutando pacientes após um primeiro episódio maníaco para avaliar preditores de desfecho e curso (Yatham *et al.*, 2009). Especificamente sobre biomarcadores, resultados

preliminares já indicam que a IL-6 possa indicar uma maior chance de recaída e dias com humor deprimido (Kunz *et al.*, 2010).

Finalmente, estudos de intervenção têm o potencial de comprovar a utilidade da avaliação desses mediadores. Há estudos interessantes nesse sentido, por exemplo, avaliando a N-acetil-cisteína adjuntiva (Berk *et al.*, 2008; Magalhaes *et al.*, 2011a; b). Uma ligação mais definitiva tanto com o desfecho clínico quanto com o mediador ainda é essencial. Como recentemente descrito, biomarcadores podem ser utilizados em ensaios clínicos para auxiliar na compreensão dos desfechos (Perlis, 2011). Tais estudos podem ser “enriquecidos” e apenas recrutar pacientes com alta toxicidade sistêmica ou estratificar pela presença de diferentes marcadores, por exemplo. Um desenho assim tem a vantagem adicional de levar em consideração uma possível heterogeneidade no transtorno bipolar (Leboyer e Kupfer, 2010).

A impressão que fica dos estudos até o momento é que a doença bipolar crônica está associada a um emaranhamento de marcadores de patologia. Se na concepção de Juster & McEwen as interações entre biomarcadores são complexas e não lineares (Juster *et al.*, 2010), pode-se imaginar que nos estágios crônicos do transtorno bipolar haja tal intersecção entre estes que apenas um efeito conjunto seja vislumbrado (Figura 5). Nessa disputa crônica entre mecanismos regulatórios e contra-regulatórios, fica evidente a inter-relação entre desfechos patológicos e mediadores sistêmicos da doença. Em longo prazo, o efeito cumulativo dos episódios agudos, comorbidades clínicas e abuso de substâncias se somam para criar estados de grande sobrecarga alostática. É possível que estes mesmos mecanismos também contribuam para originar resistência ao tratamento convencional nos estágios mais avançados. Para o futuro mais imediato, uma alternativa intermediária interessante é testar terapias adjuvantes que abordem

especificamente mecanismos fisiopatológicos relevantes, como os mencionados nesta revisão.

DESCRIÇÃO GERAL DO PROJETO, COM JUSTIFICATIVA, OBJETIVOS E MÉTODO

Justificativa e objetivos

Biomarcadores periféricos, entre eles as neurotrofinas e marcadores inflamatórios e de dano oxidativo, vem se mostrando cada vez mais relevantes no entendimento da fisiopatologia dos transtornos de humor. Os resultados encontrados até o momento, mesmo que expressivos, baseiam-se em amostras clínicas, em pacientes com doenças altamente recorrentes e expostos a múltiplas medicações. Esperou-se que um estudo com jovens, portanto com menor exposição prévia aos fatores mencionados acima, poderia contornar em alguma medida essas preocupações. A adição de um grupo controle com apenas episódios depressivos também permite a observação de efeitos específicos do transtorno bipolar em relação e sua diferenciação de efeitos de transtornos psiquiátricos em geral. O fato de a amostra ser comunitária também aumenta a representatividade dos achados e sua relevância ao entendimento da neurobiologia do transtorno bipolar.

OBJETIVOS

Geral

Avaliar a fisiopatologia periférica precoce do transtorno bipolar em jovens indivíduos da comunidade.

Específicos

- Confirmar a presença de dano oxidativo precoce no transtorno bipolar. Foi medido dano oxidativo a proteína e a lipídeos, através, respectivamente, do conteúdo de proteína carbonil (*protein carbonyl content*; PCC) e da presença

de substâncias reativas do ácido tiobarbitúrico (*thiobarbituric acid reactive substances*; TBARS)

- Verificar alterações precoces no fator neurotrófico derivado do cérebro (*brain-derived neurotrophic factor*; BDNF).
- Investigar a presença precoce de estado pró-inflamatório no transtorno bipolar. Isso se fez através da mensuração de duas citocinas pró-inflamatórias, a interleucina-6 (IL-6) e do fator de necrose tumoral-alfa (*tumor necrosis factor alpha*; TNF-α). Também foi dosada a citocina antiinflamatória interleucina-10 (IL-10).
- Averiguar como se correlacionam na fase precoce do transtorno bipolar os fatores mencionados acima.

Método

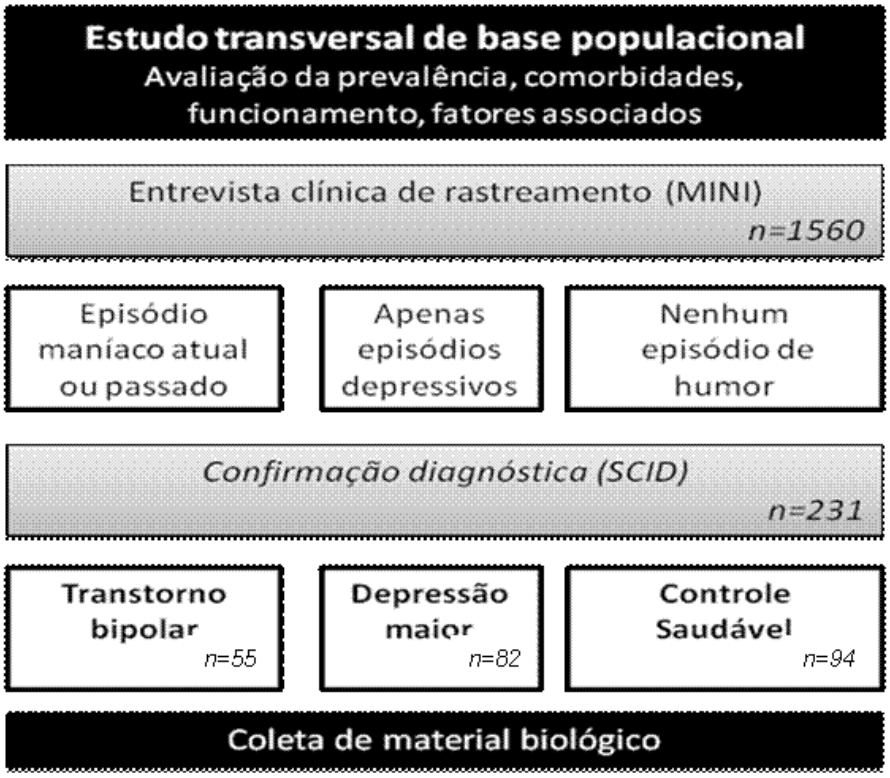
Participantes

Inicialmente, os jovens entre 18 e 24 anos de idade foram captados por um estudo transversal de base populacional residentes na zona urbana de Pelotas (RS), que contou com 1560 indivíduos. Para tal, dos 448 setores censitários da cidade de Pelotas, 89 foram selecionados aleatoriamente. O tamanho da amostra original foi calculado tendo em vistas um estudo de fatores de risco para ideação suicida nesta faixa etária e se incluiu mais 30% para compensar as perdas e recusas, assim como para controlar fatores confundidores. Todos os indivíduos incluídos nesta etapa responderam ao *Mini-International Neuropsychiatric Interview*. Fatores relacionados à psicopatologia nesta amostra encontram-se publicados em Jansen ET AL (2011).

Foram selecionados para a coleta de material biológico todos os indivíduos com diagnóstico atual ou passado de episódio maníaco/ hipomaníaco.

Procedimento

Cada jovem entre 18 e 24 anos de idade localizado nestas visitas respondeu ao instrumento de pesquisa acima descrito logo após o preenchimento do consentimento informado. Aqueles indivíduos que preencheram critérios para episódio maníaco, atual ou passado, assim como o próximo sujeito do mesmo sexo e setor (controles) constituíram a amostra proposta (Fluxograma).



Fluxograma 1. Processamento de participantes desde a inclusão no estudo transversal até a coleta de material biológico

Na segunda fase, o diagnóstico de episódio maníaco ou hipomaníaco, atual ou passado, foi confirmado por profissionais de saúde mental, ou psiquiatras ou psicólogos com nível de mestrado. Para esta confirmação, empregou-se uma entrevista clínica semi-estruturada (a *Structured Clinical Interview for DSM-IV)*, que atualmente é o padrão ouro para o diagnóstico psiquiátrico. Estes avaliadores estiveram cegos ao diagnóstico da primeira fase.

Coleta das amostras

As amostras foram colhidas após o devido consentimento do paciente por um técnico especializado. Foram coletados 10 ml de sangue total em tubos com EDTA. O material biológico foi centrifugado e congelado a –20ºC e, posteriormente, transferido para armazenagem a –80ºC. Foram analisados os níveis do fator

neurotrófico derivado do cérebro, interleucinas 6 e 10, fator de necrose tumoral alfa, proteína carbonil e substâncias reativas ao ácido tiobarbitúrico. Os níveis séricos dos biomarcadores foram mensurados com kits comerciais de acordo com as instruções dos fabricantes.

Análise de dados

Os dados foram analisados principalmente de acordo com as distribuições dos marcadores. Assim, para o BDNF e os marcadores de dano oxidativo utilizou-se um modelo de regressão linear. O mesmo não pode ser feito em relação às citocinas, e utilizou-se restrição como método de verificar associações.

Um modelo de possíveis confundidores foi decidido *a priori*. Em todas as análises ele inclui sexo e classe social e abuso / dependência de substâncias ilícitas, álcool ou tabaco, estado de humor atual e doença clínica auto-relatada. Os diagnósticos derivados do SCID foram entrados no modelo como múltiplas variáveis dicotômicas.

Aspectos éticos

O estudo recebeu a aprovação do comitê de ética e pesquisa da Universidade Católica de Pelotas, órgão vinculado ao CONEP. Foram incluídos no estudo apenas os indivíduos que ofereceram sua concordância por escrito após a leitura do termo de consentimento informado. Este foi elaborado de acordo com as normas da resolução N° 196 do CONEP - Ministério da Saúde. Além disto, foi oferecida a

possibilidade de tratamento a todos os sujeitos do estudo que apresentaram transtornos psiquiátricos.

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RESULTADOS

Peripheral oxidative damage in early-stage mood disorders: a nested population-based case-control study[4]

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ABSTRACT

Systemic toxicity is a relevant dimension of pathophysiology in bipolar disorder, and oxidative damage is one potential link between central and peripheral pathology. Although there is mounting evidence that chronic bipolar disorder is associated with oxidative stress, studies in the early stages of bipolar disorder are scarce, and heavily reliant on clinical in lieu of population studies. The objective of the current study was to confirm leading hypotheses about the role of oxidative damage in bipolar disorder. To that end, we nested a case-control study in a population-based study of young adults aged 18 to 24 years old. After an initial psychopathology screen, all people with a lifetime history of (hypo)mania and matched controls underwent a structured diagnostic interview. This yielded a sample of 231 participants, in whom we measured serum protein carbonyl content (PCC) and thiobarbituric acid reactive substances (TBARS). People with bipolar disorder had higher PCC levels than healthy subjects. Those with major depression were not different from control subjects in either PCC or TBARS levels. Both bipolar disorder and major depression were associated with PCC levels in the *a priori* regression model controlling for possible confounders. These findings indicate that protein oxidative damage is present from early stages and can be seen as a sign of early illness activity in mood disorders.

Keywords: bipolar disorder, major depression, population-based study, oxidative stress, protein carbonyl content, thiobarbituric acid reactive substances.

INTRODUCTION

There is growing interest in systemic pathophysiology as a relevant dimension of bipolar disorder (Kapczinski et al., 2008; Kupfer, 2005). This dimension is thought of as mediating the frequently ensuing illness progression, resulting in medical comorbidity, cognitive deficits, functional impairment and, ultimately, premature mortality (Berk, 2009; Berk et al., 2010; Kapczinski et al., 2008). Recent research has indeed shown that several systemic markers are altered in patients with established bipolar disorder (Kapczinski et al., 2011; Kapczinski et al., 2010). Oxidative stress biomarkers are prominent among these (Andreazza et al., 2008; Berk et al., 2011; Ng et al., 2008).

Allostasis has been a relevant paradigm for understanding how illness progression can be related to poor outcomes in mood disorders (Kapczinski et al., 2008; McEwen, 2003). Although allostasis promotes adaptation, when its mediators are not turned off or are overused by excessive challenge, the cumulative load leads to wear and tear of body and brain (McEwen and Gianaros, 2011). As such, several peripheral markers have been implicated in bipolar disorder as mediators of allostasis (Berk et al., 2011; Juster et al., 2010; Kapczinski et al., 2008).

Oxidative imbalance and damage have been repeatedly demonstrated in patients with bipolar disorder (Andreazza et al., 2008; Berk et al., 2011; Ng et al., 2008). Available evidence points to extensive lipid, protein (Kapczinski et al., 2011) and DNA damage (Andreazza et al., 2007) in such patients when compared to healthy control subjects. This is highly relevant, since pro-oxidant states may link central and peripheral pathophysiology (Gigante et al., 2010). Possibly by altering the permeability of the blood-brain barrier, peripheral oxidative stress has been

demonstrated to effect significant brain toxicity (Chaudhary and Rao, 2010; Gilgun-Sherki et al., 2001).

Thus far, however, research has usually focused on chronic patients treated in tertiary centers. With a high cumulative illness burden, several biomarkers tend to be altered in a highly correlated manner (Kapczinski et al., 2010). This is concordant with the view of complexity in allostatic systems. Accordingly, studies in late-stage samples often reveal complex multivariate associations between disparate markers (Kapczinski et al., 2011; Kapczinski et al., 2010). Although these studies confirm that late-stage is associated with systemic toxicity and neuroprogression (Berk et al., 2011), primary and secondary pathology cannot be teased apart. As a corollary, early disease would be a more developmentally appropriate period to understand primary illness changes (Berk et al., 2009). Furthermore, current biomarker discovery in bipolar disorder relies heavily on clinical samples. Community samples avoid the selection bias that is inherent to studies in individuals who seek treatment (McDade et al., 2007).

The objective of the current study was to confirm leading hypotheses about the role of oxidative stress in bipolar disorder. To that end, we nested a case-control study in a population-based study of young adults aged 18 to 24 years old. Every individual with a positive screen for bipolar disorder was invited to participate, as well as matched controls with only depressive episodes and without mood episodes; serum markers of oxidative damage to proteins and lipids were collected. In this manner, we could test whether oxidative damage is present since early disease stages in mood disorders with the advantage of a representative population-based sample.

METHODS

This is a case-control study nested in a population-based cross-sectional study. Full details on the original study have been published elsewhere (Jansen et al., 2011). Briefly, the sample was consistent of 1560 participants from 18 to 24 years old living in urban Pelotas, Brazil. Sample selection was performed by clusters, in the period of August 2007 to December 2008, considering a population of 39,667 people in that age range in the current census of 448 sectors in the city. From these, 89 census-based sectors were systematically drawn. Individuals provided written informed consent and answered a questionnaire on socio-demographic data, drug misuse, and a diagnostic interview. The study was approved by the Ethics Committee of the Catholic University of Pelotas (UCPel).

As an initial psychopathology screen, the whole population underwent the Mini-International Neuropsychiatric Interview (Sheehan et al., 1998). For the purposes of the current study, cases were those with a past or current history of a manic episode from the population-based study. Ninety-three individuals met this criterion. Additionally, two groups of control subjects were recruited. People without any history of affective disorder were randomly selected and matched for sex, age and socioeconomic situation – i.e., a healthy control sample. Importantly, we did not exclude people from this control group on account of any other mental disorders or clinical morbidity. We also recruited a second control group, those with a current depression but no past history of (hypo)mania. This was, thus, an active control group. From these, 231 individuals provided informed consent (83% of the intended sample; see Fig. 1).

The whole case-control sample further underwent the Structured Clinical Interview for DSM-IV (SCID). This was performed to confirm diagnoses and improve

reliability, and is the group-defining criterion for this study. The SCID-interviews were undertaken by two master's level psychologists at the laboratory of the Health and Behavior Post-Graduate Program in Pelotas. They had intensive training in the specialist outpatient facilities at the Hospital de Clínicas de Porto Alegre under supervision of one of the senior investigators (F.K). After SCID diagnoses, the final sample for the case-control study consisted of 94 control subjects, 82 participants with major depression and 55 with bipolar disorder (33 type I and 22 type II).

Serum samples were assayed by laboratory technicians blinded to clinical characteristics of participants. The levels of lipid peroxidation were measured using the thiobarbituric acid reactive substances (TBARS) method as previously described (Wills, 1966), and data is expressed as nmol/mg of protein. Oxidative damage to proteins was measured by the determination of carbonyl groups (protein carbonyl content; PCC) based on the reaction with dinitrophenylhidrazine (DNPH) (Levine et al., 1990). These are traditional markers of oxidative damage that have been repeatedly employed in patients with mood disorders (Andreazza et al., 2009; Andreazza et al., 2008; Giustarini et al., 2009; Kapczinski et al., 2011). Of the 231 participants for whom a serum sample was available, in eleven samples TBARS levels could not be analysed and in 58 samples (19 control, 32 major depression and 7 bipolar disorder samples) the same was true for PCC levels.

Socio-economic status was evaluated with the Brazilian Association of Research Companies (ABEP, 2008) classification, which is based on the total of material goods and the householder's schooling. It was further dichotomized into high (classes A, B or C) and low (classes D or E). Clinical illness was self-reported. Information on drug misuse was obtained with the Alcohol, Smoking and Substance

Involvement Screening Test (ASSIST), validated to Portuguese (Henrique et al., 2004).

Statistical analysis

One-way analysis of variance was used to test for between-group differences in continuous variables and chi-squared tests were used for differences between proportions.

We constructed full-fledged *a priori* multivariate models to test for differences in PCC and TBARS. We preferred using a theoretical instead of a data driven approach since it avoids overfitting (Babyak, 2004; Harrell et al., 1996). These should be seen as the main results of this study. In the models, we control for features of theoretical relevance or empirically associated with serum biomarker levels in previous studies. Specifically, in addition to diagnostic status, the model included sex and social class (Gianaros and Manuck, 2010; Hackman et al., 2010; Ortona et al., 2008), self-reported clinical illness (Kapczinski et al., 2008), smoking, alcohol or illicit substance abuse (Ng et al., 2008), current depression and mania (Kapczinski et al., 2011; Kapczinski et al., 2010). Bipolar I and II disorder were placed in the same category mainly because of lack of power to investigate them separately. Diagnoses were entered in the model as “dummy variables”, with the control category as reference.

Linear regression with bias-corrected accelerated bootstrapping with 2000 resamples was used with the predictors mentioned above for the two models. Bootstrapping in this case has the advantage of being more robust when handling data for which the population distribution in unclear (Henderson, 2005) and of testing the validity of the model employed (Harrell et al., 1996).

RESULTS

Subjects (n=231) were comparable regarding age and years of education, but women were underrepresented in the controls. Current use of psychiatric medications was very low, with only 9.6% of the sample reporting current use of any medications. Table 1 shows demographic and clinical information according to diagnosis.

PCC and TBARS serum levels were correlated in the sample (rho=0.19, p=0.017). Those with bipolar disorder had higher PCC levels than healthy subjects (F=3.95, p=0.049). TBARS levels, however, did not significantly differ (F=0.72, p=0.397). Those with major depression were not different from control subjects in either PCC (F=2.44, p=0.121) or TBARS levels (F=0.12, p=0.725). Finally, the two mood disorder groups could not be differentiated by PCC (F=0.18, p=0.669) or TBARS (F=1.43, p=0.235) levels.

Serum PCC levels were further associated with a current manic episode (F=4.43, p=0.036), but not with a current depressive episode (F=0.57, p=0.451). Serum TBARS levels were not associated with mania (F=0.18, p=0.671) or depression (F=0.61, p=0.434). Current use of medication was not associated with PCC (F=0.47, p=0.493) or TBARS levels (F=0.32, p=0.570).

The *a priori* regression model kept both bipolar disorder (β=0.199, bias=-0.005, SE=0.080, p=0.014) and major depression (β=0.200, bias=-0.004, SE=0.083, p=0.012) as predictors of higher PCC levels. None of the variables in the model, however, was able to predict TBARS levels (Table 2).

DISCUSSION

This study indicates that oxidative protein damage is present from the early stages in mood disorders. Young adults with bipolar disorder had higher serum levels of a marker of protein damage than participants free of mood disorders. The multivariate model also pointed to significantly increased damage to proteins in major depression. These changes were independent of current mood state.

One attractive hypothesis that links neuroplasticity and oxidative stress to neuroprogression and medical burden is that of mitochondrial dysfunction (Chen et al., 2010; Kato and Kato, 2000). Aberrations in the mitochondrial electron chain have been demonstrated in bipolar disorder, centrally and in the periphery, in patients and animal models (Andreazza et al., 2010; Cataldo et al., 2010; Frey et al., 2006; Valvassori et al., 2010). In one study, bipolar disorder was associated with decreased complex I activity in the prefrontal cortex (Andreazza et al., 2010). This was, in turn, correlated with protein carbonylation, providing a basis for the protein damage observed in bipolar disorder.

Protein carbonyl derivatives are broad markers of oxidation. They are usually considered markers of protein dysfunction, not only oxidative stress (Dalle-Donne et al., 2003). Proteins damage can have a myriad of downstream consequences, from loss of functional properties to apoptosis and necrosis (Aldini et al., 2007). Elevated protein carbonyl levels have been demonstrated to predict adverse clinical outcomes in diverse samples. This includes persistence of illness activity in lupus, an association with colorectal cancer and a greater risk of mortality in elderly women (Morgan et al., 2009; Semba et al., 2007; Yeh et al., 2010). In the central nervous system, protein damage is likely one of the allostatic mechanisms leading to

cognitive dysfunction and illness progression (Berk et al., 2011; Kapczinski et al., 2008).

It is unclear at this time why we detected protein but not lipid damage. Damage to lipids has been extensively shown in mood disorders (Andreazza et al., 2009; Maes et al., 2010b). One possibility is that protein carbonyl derivatives are more sensitive to early oxidative damage because they circulate for longer periods than lipid peroxidation products (Dalle-Donne et al., 2003). Again, most previous studies are heavily weighted towards late-stage patients. This might suggest that lipid damage is characteristic of neuroprogression, but a comparative design is necessary to establish that. In the one previous comparative study, systemic changes in early-stage patients were indeed more subtle than those a late-stage (Andreazza et al., 2009; Kauer-Sant'Anna et al., 2009).

In spite of the obvious advantages of population data of young adults, this is a report on cross-sectional data. So if it is possible to assert that oxidative damage is associated with early stage mood disorders, longitudinal research is necessary to establish causality. This study may also have been underpowered to detect subtle state-related changes in biomarkers of oxidative damage. As this study was designed to detect oxidative damage in bipolar disorder, it is harder to evaluate the meaning of protein damage in major depression. Hypotheses regarding the role of oxidative stress in this condition have been put forward, but far fewer studies are available (Maes et al., 2010a).

These differences in the level of protein damage can be seen as a sign of early illness activity in mood disorders. The findings here reinforce an already very consistent body of work indicating that oxidative imbalance is a prominent node in the chain events leading to disease progression in bipolar disorder.

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Statement of interest

None

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Table 1. Sample demographical, clinical and treatment characteristics according to SCID diagnosis

Characteristic	**Healthy control (n=94)**	**Major depression (n=82)**	**Bipolar disorder (n=55)**
Age	22.4 ± 2.7	21.8 ± 2.0	21.7 ± 2.2
Female sex *	58%	77%	74%
Years of education	9.7 ± 3.1	8.9 ± 2.8	8.8 ± 3.6
Lower social class (D or E)	14%	22%	15%
Self-reported clinical illness	28%	33%	46%
Current medication			
Mood stabilizers	0	3%	4%
Antipsychotics	0	0	4%
Antidepressants	1%	3%	6%
Benzodiazepines	0	1%	4%
Any*	2%	12%	19%
Current depressive episode	0%	77%	76%
Current manic episode	0%	0%	20%
Age at onset	n/a	16.9 ± 3.8	15.3 ± 4.5
Lifetime tobacco misuse*	25%	38%	46%
Lifetime alcohol misuse	27%	38%	38%
Lifetime illicit substance misuse	10%	23%	16%
Previous hospitalizations	1%	6%	9%

* $p<0.05$; Some patients did not recall the medication they were taking, hence "any medication" figure is higher than sum of individual medications

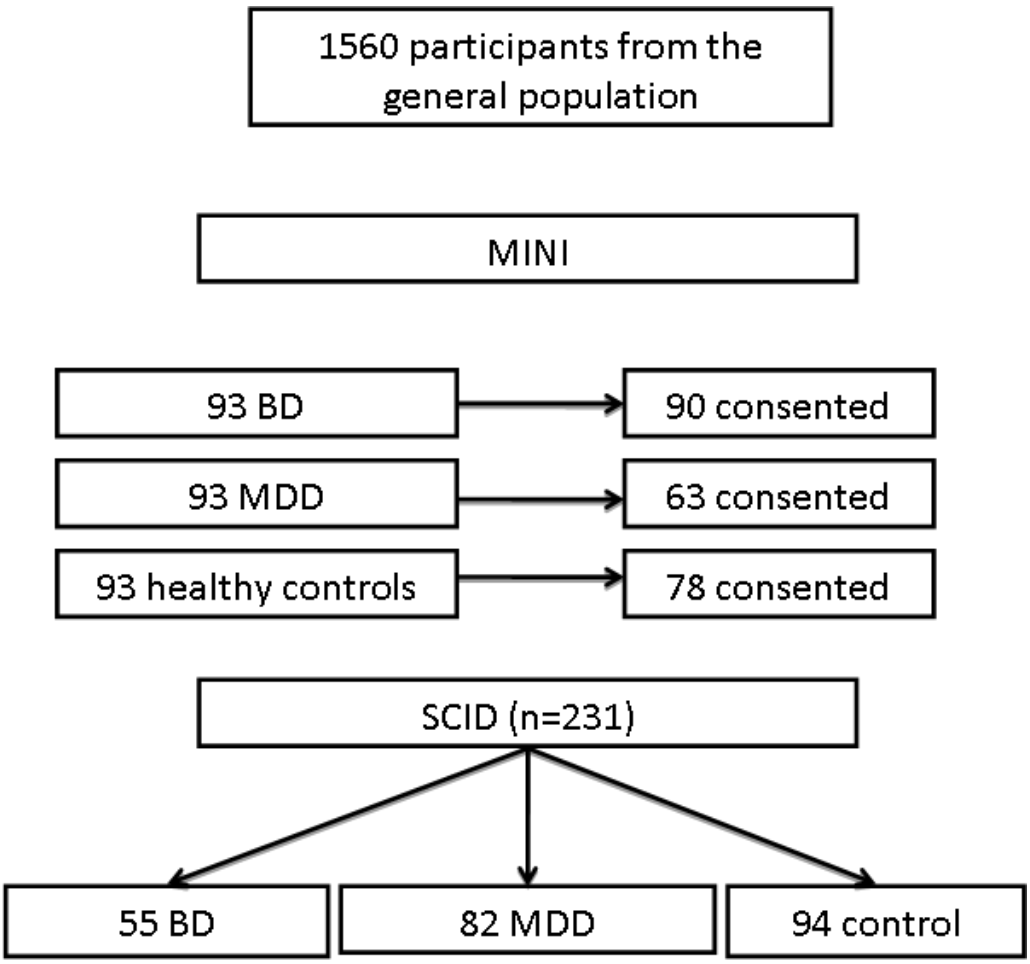
Table 2. Multilevel model predicting protein carbonyl content (PCC) and thiobarbituric acid reactive substances (TBARS) serum levels

Variable	**PCC**		**TBARS**	
	B	95% CI	B	95% CI
Sex	0.120*	0.04 – 0.20	-0.005	-0.011 – 0.001
Lower social class	0.070	-0.05 – 0.19	-0.002	-0.006 – 0.003
Smoking	-0.057	-0.15 – 0.03	0.003	-0.003 – 0.009
Alcohol abuse	0.028	-0.09 – 0.16	0.007	-0.003 – 0.013
Abuse of Illicit drugs	-0.137*	-0.25 – -0.03	-0.002	-0.009 – 0.007
Clinical illness	0.024	-0.07 – 0.13	-0.002	-0.007 – 0.002
Current depression	-0.169*	-0.32 – 0.01	0.001	-0.011 – 0.010
Current mania	0.203	-0.07 – 0.47	0.001	-0.014 – 0.018
Major depression	0.200*	0.04 – 0.34	-0.003	-0.012 – 0.008
Bipolar disorder	0.199*	0.06 – 0.35	0.001	-0.009 – 0.013

Linear regression with bias-corrected accelerated bootstrapping

*p<0.05

Figure 1. Flow chart showing patient inclusion in the case-control study



Serum brain-derived neurotrophic factor in early-stage mood disorders: a nested population-based case-control study[5]

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[5] Manuscrito em avaliação no *Journal of Affective Disorders*

ABSTRACT

Background Brain-derived neurotrophic factor (BDNF) is the most widespread neurotrophin in the central nervous system; it has been associated with bipolar disorder in clinical and preclinical studies. Illness stage, however, may influence results, and most studies have focused on chronic patients treated in tertiary centers.

Aim In the current study, we investigated if serum BDNF was decreased in a sample of young adults 18 to 24 years old from the general population.

Methods We employed a case-control design nested in a population-based study. After an initial psychopathology screen, all people with a lifetime history of mania and matched controls underwent a structured diagnostic interview. Two control groups were recruited, one with major depression and the other without any history of mood episodes. This yielded a sample of 231 participants (223 with serum BDNF levels).

Results We found no between-group differences in the *a priori* regression analysis. The secondary analyses comparing mood disorder subgroups with the control condition revealed only lower BDNF levels for the euthymic participants with bipolar disorder.

Limitations Some comparisons may have suffered from limited statistical power. Longitudinal designs are necessary to understand how BDNF changes in the course of the disorder.

Conclusion Although it may be difficult to compare results from clinical and population-based samples, these findings suggest that a change in BDNF levels during acute episodes is a feature of later stages of mood disorders.

Keywords: bipolar disorder, major depression, population-based study, brain derived neurotrophic factor, staging, neuroprogression.

INTRODUCTION

Bipolar disorder is increasingly seen as a condition associated with neurodegeneration (Berk et al., 2011; Goodwin et al., 2008). As the illness progresses through successive stages, a cycle of increasing cognitive impairment and disability takes place (Berk et al., 2009). Brain-derived neurotrophic factor (BDNF) is the most widespread neurotrophin in the central nervous system, with vital functions in promoting cell resilience and survival. As such, it has been one likely suspect of being involved with the progressive deterioration that often occurs in the mood disorders (Post, 2007a, b, 2010).

The neurobiology of BDNF and its associations with neurodegenerative conditions have led to different versions of a neurotrophic model for mood disorders (Duman, 2004; Duman and Monteggia, 2006; Manji et al., 2000; Martinowich et al., 2007; Zuccato and Cattaneo, 2009). At their core is the idea that decreased BDNF expression leads to decreased cell resilience and synaptic plasticity. The association of this neurotrophin with bipolar disorder has been consistent in empirical studies. This is equally true for animal models of mania and patients treated for bipolar disorder (Cunha et al., 2006; de Oliveira et al., 2009; Fernandes et al., 2010; Frey et al., 2006; Jornada et al., 2010; Lin, 2009; Machado-Vieira et al., 2004). In the latter, mood episodes are associated with lower BDNF levels even in drug-free patients, indicating that this finding is not primarily due to the effect of psychotropic drugs. Furthermore, treatment of acute episodes has been preliminarily shown to increase BDNF levels (de Sousa RT et al., 2011; Tramontina et al., 2009).

While there is strong evidence for an association of BDNF levels with acute mood episodes, its role in illness progression is less well understood. During euthymia, for instance, it has been difficult to demonstrate differences from healthy

control subjects (Lin, 2009). One possible explanation for these discrepancies is heterogeneity in available studies regarding illness stage (Fernandes et al., 2010). In this sense, it is relevant that in the only comparative study conducted thus far, only patients in a late illness stage had lower BDNF levels than controls (Kauer-Sant'Anna et al., 2009; Yatham et al., 2009). This fits well with the idea of neuroprogression (Berk et al., 2011). In the earliest stages, central and peripheral changes would be reversible; this has been one of the arguments for early intervention in psychiatry (Berk et al., 2009; McGorry et al., 2006).

Thus far, however, research has usually focused on chronic patients; early disease may be a more developmentally appropriate period to understand primary illness changes (Berk et al., 2009). Furthermore, current biomarker discovery in bipolar disorder relies heavily on clinical samples. Community samples avoid the selection bias that is inherent to studies in individuals who seek treatment (McDade et al., 2007).

The objective of the current study was to investigate whether BDNF serum levels are already decreased in the early stages of bipolar disorder. To that end, we nested a case-control study in a population-based study of young adults aged 18 to 24 years old. We aimed at comparing a group of people with bipolar disorder with two control groups, one with only episodes of depression and the other without any history of mood episodes.

METHODS

This is a case-control study nested in a population-based cross-sectional study. Participants were 18 to 24 years old. Full details on the original study have been published elsewhere (Jansen et al., 2011). Briefly, the sample was consistent of

1560 participants from 18 to 24 year's old living in urban Pelotas, Brazil. Sample selection was performed by clusters, in the period of August 2007 to December 2008, considering a population of 39,667 people in that age range in the current census of 448 sectors in the city. From these, 89 census-based sectors were systematically drawn. Individuals provided written informed consent and answered a questionnaire that collected socio-demographic data, drug misuse, as well as a structured diagnostic interview. The study was approved by the Ethics Committee of the Catholic University of Pelotas.

As an initial psychopathology screen, the whole population underwent the Mini-International Neuropstchiatric Interview (Sheehan et al., 1998). For the purposes of the current study, we invited every person with a past or current history of a manic or hypomanic episode – the target sample – from the population-based study. Ninety-three individuals met this criterion. Additionally, two groups of control subjects were recruited. People without any history of affective disorder were randomly selected and matched for sex, age and socioeconomic situation – i.e., a healthy control sample. Importantly, we did not exclude people from this control group on account of any other mental disorders or clinical morbidity. We also recruited a second control group, those with a current depression but no past history of (hypo)mania. This was, thus, an active control group. Of these, we were able to obtain data on 231 subjects (83% of the original sample; see Figure 1).

We further used the Structured Clinical Interview for DSM-IV (SCID) to confirm diagnosis and improve reliability in the case-control sample. This was the group-defining criterion for this study. The SCID-interviews were undertaken by two master's level psychologists after intensive training in the specialist outpatient facilities at the Hospital de Clínicas de Porto Alegre under supervision of one of the

senior investigators (F.K) at the laboratory of the Health and Behavior Post-Graduate Program in Pelotas. After reclassifications, the sample for the case-control study consisted of 94 control subjects, 82 participants with major depression and 55 with bipolar disorder (33 type I and 22 type II). Serum samples for BDNF levels were available for 223 participants.

Socio-economic status was evaluated with the Brazilian Association of Research Companies (ABEP, 2008) classification, which is based on some material goods in the household and the householder's schooling. It was further dichotomized into high (classes A, B or C) and low (classes D or E). Clinical illness was self-reported. Information on drug misuse was obtained with the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST), validated to Portuguese (Henrique et al., 2004).

BDNF serum levels were measured with sandwich-ELISA, using a commercial kit according to the manufacturer's instructions (Chemicon, USA), as previously described (Kauer-Sant'Anna et al., 2009).

Statistical analysis

BFDNF levels were log-transformed for parametric analyses. We used analysis of variance to test for between-group differences. The primary analysis was the regression described below. As secondary analyses, we tested for BDNF differences between groups based on mood state and the control condition.

We constructed an *a priori* multivariate model to test for differences in BDNF serum levels. We preferred using a theoretical instead of a data driven approach since it avoids overfitting (Babyak, 2004; Harrell et al., 1996). These should be seen as the main results of this study. We included in the model features of theoretical

relevance or empirically associated with BDNF in previous studies. In addition to diagnostic status, the model included sex and social class {Bus 2011; Gianaros), self-reported clinical illness (Kapczinski et al., 2008), smoking, alcohol or illicit substance abuse {D'Souza 2009; Bus 2011}, current depression and mania (Kapczinski et al., 2011; Kapczinski et al., 2010). Bipolar I and II disorder were placed in the same category mainly because of lack of power to investigate them separately.

Linear regression with bias-corrected accelerated bootstrapping with 2000 resamples was used with every predictor. Bootstrapping in this case has the advantage of being more robust when handling data for which the population distribution in unclear (Henderson, 2005) and of testing the validity of the model employed (Harrell et al., 1996).

RESULTS

Subjects (n=223) were comparable regarding age and years of education, but women were underrepresented in controls. Only 9.6% of the sample reported the current use of any psychiatric medications. Table 1 shows demographic and clinical information according to diagnosis.

There was no association between BDNF levels and mood disorder group (F_2=0.22, p=0.398) or a current depressive (F_1=0.06, p=0.801) or manic episode (F_1=0.99, p=0.321). Current use of medication was not associated with BDNF levels (F_1=1.02, p=0.314).

The multivariable model indicated only trends for the association of BDNF levels with bipolar disorder (B=-0.101, 95% CI -0.207 – 0.006, p=0.064) and mania

(B=0.113, 95%CI -0.009 – 0.236, p=0.073). Full model results can be seen in Table 2.

The secondary analyses comparing mood disorder subgroups with the control condition (Figure 2) revealed only lower BDNF levels for the euthymic participants with BD (F_1=4.87, p=0.030). There was no difference between the control group and the euthymic MDD group (F_1=0.86, p=0.356), the currently depressed MDD (F_1=0.17, p=0.681) or BD (F_1=0.80, p=0.372) groups or the currently manic/ mixed group (F_1=0.59, p=0.446).

DISCUSSION

According to the *a priori* hypothesis tested in this study, there was no difference in BDNF levels between young adults with bipolar disorder or major depression and population controls. A secondary, exploratory, analysis suggested lower BDNF levels in bipolar disorder during euthymia.

The results here can be understood in light of the concepts of staging and neuroprogression in bipolar disorder (Berk, 2009; Berk et al., 2011). Although longitudinal data are not available, serum BDNF is consistently low in late stage patients (Grande et al., 2010). In the only comparative study so far, BDNF was only low in late-stage patients with multiple episodes, but not after the first manic episode (Kauer-Sant'Anna et al., 2009). Recently, an animal model was specifically developed to test this possibility (Fries, 2011). Animals received either 7 or 35 days of saline or amphetamine, mimicking early and late stages. After memory tasks, the late model showed changes in BDNF protein levels in prefrontal cortex and mRNA in hippocampus compared to the early model.

As a consequence, it has been suggested that BDNF may have both state and trait characteristics in bipolar disorder (Kapczinski et al., 2009). Possibly, in addition to reductions related to illness activity, there is a slower decline related to illness progression, when counter-regulatory processes fail to prevent a widespread drop in this trophic factor (Kapczinski et al., 2008; Post, 2007a). In this fashion, BDNF is one of the putative biological underpinnings of late stage related cognitive impairment and treatment resistance (Berk et al., 2011; Post, 2010).

One caveat in trying to reconcile this and previous studies is that this is originally a population-based, not a clinical sample. The latter may have special characteristics such as greater severity and chronicity, more frequent comorbidity and more overall disability (i.e., a form of Berkson's bias). A longitudinal design is necessary to demonstrate a how BDNF changes in the course of the disorder and its relations with other systemic changes. Another issue is the comparisons here might have suffered from limited power, leading only to marginal findings. Bipolar disorder, however, is a relatively low prevalence condition and the current study was constrained by this logistical limitation.

The precise nature of the relation between central and serum BDNF levels is incompletely understood. Peripheral levels, however, have been associated with markers of cortical integrity in neuroimage studies (Lang et al., 2007). Recently, it has been suggested that peripheral levels might not just be correlated with central levels, but also have particular consequential effects (Schmidt and Duman, 2010). Additionally, BDNF is not the only relevant neurotrophin for the mood disorders. Clinical studies have identified NT-3, NT-4/5 and GDNF to be different in bipolar disorder (Rosa et al., 2006; Walz et al., 2007; Walz et al., 2009). Understanding the

relations between these molecules may be necessary for a more refined and realistic conception of this pathway.

Ultimately, the fact that BDNF levels are not consistently altered in young adults with bipolar disorder supports the idea of neuroprogression. It also strengthens the rationale for early intervention before illness toxicity builds up, and neurotrophin pathways as possible targets for neuroprotective strategies.

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Table 1. Demographical, clinical and treatment characteristics of the case-control sample according to SCID diagnosis

Characteristic	**Healthy control (n=94)**	**Major depression (n=82)**	**Bipolar disorder (n=55)**
Age	22.4 ± 2.7	21.8 ± 2.0	21.7 ± 2.2
Female sex *	58%	77%	74%
Years of education	9.7 ± 3.1	8.9 ± 2.8	8.8 ± 3.6
Lower social class (D or E)	14%	22%	15%
Self-reported clinical illness	28%	33%	46%
Current medication			
Mood stabilizers	0	3%	4%
Antipsychotics	0	0	4%
Antidepressants	1%	3%	6%
Benzodiazepines	1%	1%	4%
Any*ǂ	2%	12%	19%
Current depressive episode	0%	77%	76%
Current manic episode	0%	0%	20%
Childhood onset (before 13)	n/a	11%	22%
Lifetime tobacco misuse*	25%	38%	46%
Lifetime alcohol misuse	27%	38%	38%
Lifetime illicit substance misuse	10%	23%	16%
Previous hospitalizations	1%	6%	9%

* p<0.05 for difference between groups

ǂ Some patients did not recall the medication they were taking, hence “any medication” figure is higher than sum of individual medications

Table 2. Multivariable model predicting brain-derived neurotrophic factor (BDNF) serum levels

	Brain-derived neurotrophic factor		
	B	95% CI	P value
Sex	0.04	-0.02 – 0.10	0.240
Lower social class	0.00	-0.08 – 0.08	0.990
Smoking	0.03	-0.03 – 0.10	0.278
Alcohol abuse	0.06	-0.02 – 0.13	0.122
Illicit drugs abuse	-0.07	-0.16 – 0.03	0.182
Clinical illness	0.05	-0.01 – 0.11	0.118
Current depression	0.03	-0.07 – 0.13	0.558
Current mania	0.11	-0.01 – 0.24	0.073
Major depression	-0.02	-0.12 – 0.09	0.738
Bipolar disorder	-0.10	-0.21 – 0.01	0.064

Linear regression with bias-corrected accelerated bootstrapping

Figure 1. Flow chart showing patient inclusion in the case-control study

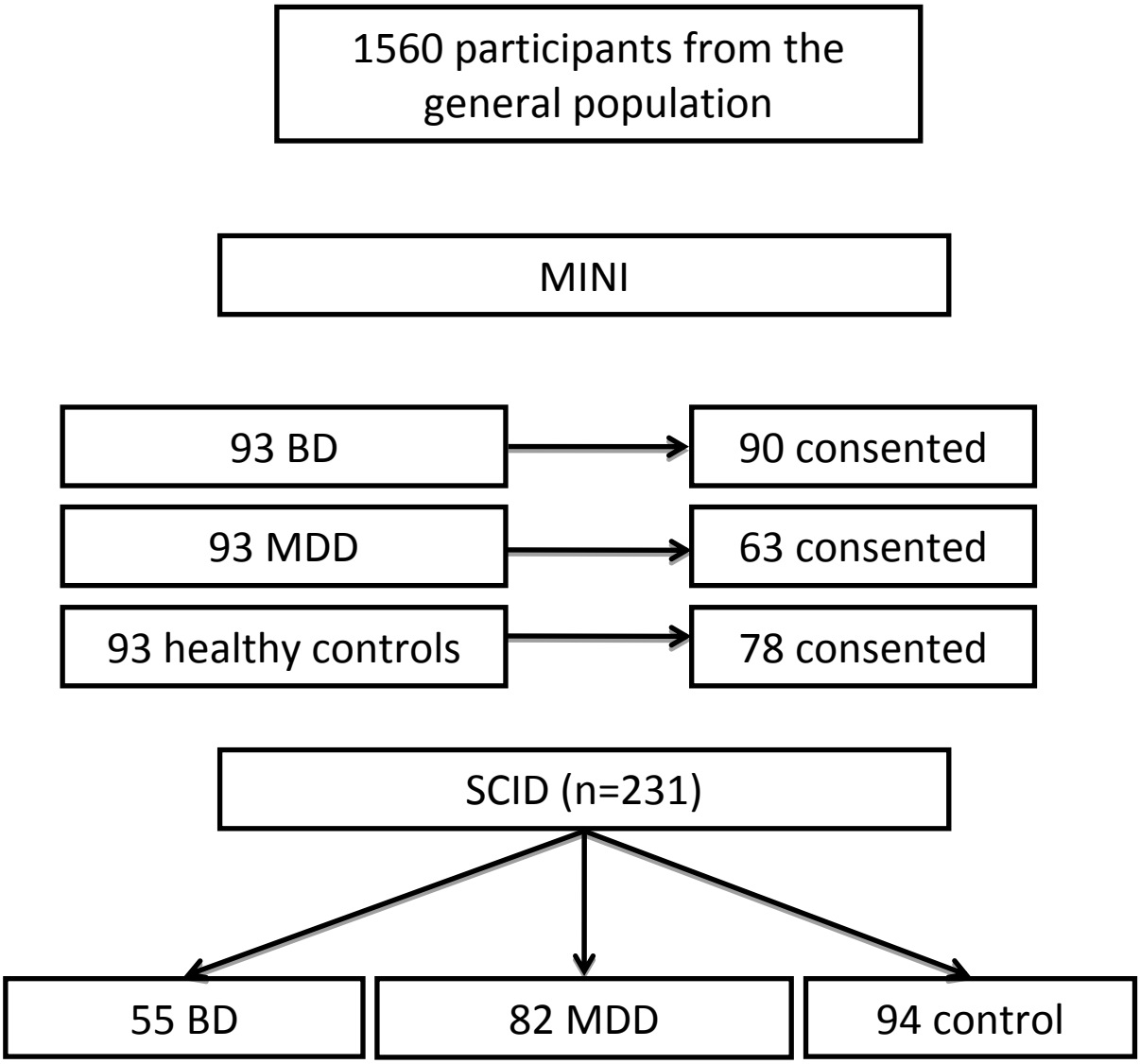
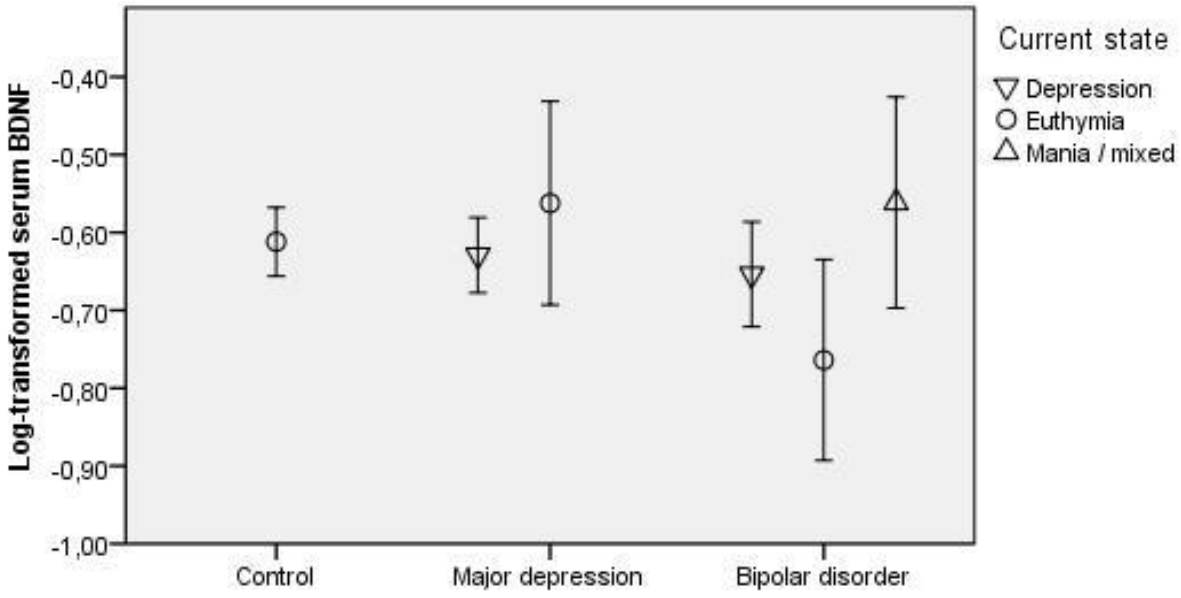


Figure 2. Error bars showing serum levels of brain-derived neurotrophic factor according to diagnostic group and current mood state



A nested population-based case-control study on inflammation markers in early-stage mood disorders[6]

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ABSTRACT

Objectives Previous studies in clinical samples suggest that bipolar disorder is associated with a high inflammation set point, even in the early stages. Here, we sought to confirm these findings in a case-control study nested in a population based sample of young adults aged 18-24 years old.

Methods Individuals from the general population with a positive screen for bipolar disorder were recruited, as well as two groups of controls. One had only depressive episodes and the other had no history of mood episodes. This yielded a sample of 231 participants. Two pro-inflammatory cytokines, interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) and an anti-inflammatory cytokine, interleukin-10 (IL-10), were measured.

Results IL-6 levels were not associated with any of the predictors and IL-10 levels were associated only with social class. TNF-α levels were higher in those who used illicit drugs and lower in those who used any psychiatric medications. Sensitivity analyses restricting to those who did not use any illicit drugs of medications revealed higher TNF-α serum levels in bipolar disorder. Excluding extreme results, TNF-α serum levels were also higher in bipolar disorder than in major depression.

Conclusions This study confirms an early, if subtle, pro-inflammatory state in bipolar disorder.

Keywords: bipolar disorder, major depression, population-based study, tumor necrosis factor alpha, interleukin-6, interleukin-10, early-stage, inflammation, neuroprogression.

INTRODUCTION

Bipolar disorder is increasingly conceptualized as a chronic multisystem illness (Kapczinski and others 2008b; Leboyer and Kupfer 2010; Soreca and others 2009). Several lines of evidence point in this direction. Patients with this mood disorder have a lower life expectancy and high levels of disability that are substantially associated with an extremely high medical burden (Altamura and others 2011; Kupfer 2005; Roshanaei-Moghaddam and Katon 2009). This has led to research looking for mediators of illness-related systemic toxicity (Kapczinski and others 2010).

What has emerged from studies using clinical samples of patients with chronic bipolar disorder is a dysfunction in several regulatory systems (Andreazza and others 2007; Andreazza and others 2009; Andreazza and others 2008; Kapczinski and others 2011; Kapczinski and others 2010; Kapczinski and others 2008a; Kauer-Sant'Anna and others 2009; Simon and others 2006). The relation of these mediators with illness activity is likely to be intricate and, in late-stages it may be even harder to untangle such complex interactions (Kapczinski and others 2008b). This supports a view of bipolar disorder as a progressive illness (Berk and others 2011; Berk and others 2009).

Inflammation has been seen as a key component in these dysfunctional pathways (Brietzke and Kapczinski 2008; Drexhage and others 2010a; Goldstein and others 2009). As the evidence accumulates, studies suggest on the whole the relevance of cytokine inflammatory networks related to the mononuclear phagocyte system (Drexhage and others 2010b). The pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) have been more consistently associated with bipolar disorder (Brietzke and others 2009; Kauer-Sant'Anna and others 2009; O'Brien and others 2006; Ortiz-Dominguez and others 2007). As argued

previously, however, it is difficult drawing more definitive conclusions from the existing literature, since it is mostly based on highly chronic clinical samples (Goldstein and others 2009). The immune system may change with aging, especially after prolonged exposure to symptom burden and pharmacologic treatment (Drexhage and others 2010b).

The objective of the current study was to confirm previous findings on inflammation in bipolar disorder. We assess differences here in the two pro-inflammatory cytokines mentioned above, IL-6 and TNF-α. Additionally, we report on an anti-inflammatory cytokine, interleukin-10 (IL-10), which has been previously shown to be specifically elevated in patients after the first manic episode (Kauer-Sant'Anna and others 2009). To that end, we nested a case-control study in a population-based study of young adults aged 18 to 24 years old (Jansen and others 2011). Individuals from the general population with a positive screen for bipolar disorder were invited to participate, as well as matched controls with only depressive episodes and without mood episodes. Unlike most previous studies, only a minority of participants were in treatment. This allowed us to test the hypothesis that bipolar disorder in young adults is associated with a pro-inflammatory state.

METHODS

This is a case-control study nested in a population-based cross-sectional study of participants 18 to 24 years old. Details on the original study are published elsewhere (Jansen and others 2011). Briefly, the sample was consistent of 1560 participants living in urban Pelotas, Brazil. Sample selection was performed by clusters, from August 2007 to December 2008, considering a population of 39,667 people in that age range and 448 sectors in the city. From these, 89 sectors were

systematically drawn. Individuals provided written informed consent and answered a questionnaire that collected socio-demographic data, drug misuse, as well as a diagnostic interview. The study was approved by the Ethics Committee of the Catholic University of Pelotas.

The whole population underwent the Mini-International Neuropsychiatric Interview (Sheehan and others 1998) as an initial psychopathology screen. For the current study, we invited every person with a past or current history of a manic episode from the population-based study. Ninety-three individuals met this criterion. Additionally, two groups of control subjects were recruited. People without any history of affective disorder were randomly selected and matched for sex, age and socioeconomic situation – i.e., a healthy control sample. We did not exclude people from the study on account of any other mental disorders or clinical morbidity. We also recruited a second group with current depression but no past history of mania. This was, thus, an active control group, recruited to probe pathophysiology specific to bipolar disorder. Of these, we were able to obtain consent from 231 subjects (83% of the intended sample; see Figure 1).

We further used the Structured Clinical Interview for DSM-IV (SCID) to confirm diagnoses. This was the group-defining criterion for this study. The SCID-interviews were undertaken by two master's level psychologists at the laboratory of the Health and Behavior Post-Graduate Program in Pelotas. They had intensive training in the specialist outpatient facilities at the Hospital de Clínicas de Porto Alegre under supervision of one of the senior investigators (F.K). After reclassifications, the sample for the case-control study consisted of 94 control subjects, 82 participants with major depression and 55 with bipolar disorder (33 type I and 22 type II).

Serum samples were assayed by laboratory technicians blinded to clinical characteristics of participants. Cytokines (IL-6, IL-10 and TNF-α) were measured according to the procedures supplied by the manufacturer using highly sensitive sandwich ELISA kits for TNF-α, IL-6 and IL-10 (Quantikine, R&D Systems, Minneapolis, Minn., USA). All samples were assayed in duplicates.

Socio-economic status was evaluated with the Brazilian Association of Research Companies (ABEP, 2008) classification. This is based on material goods in the household and the householder's schooling. It was further dichotomized into high (classes A, B or C) and low (classes D or E). Clinical illness was self-reported. Information on drug misuse was obtained with the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST), validated to Portuguese (Henrique and others 2004).

Statistical analyses

There were two additional losses in TNF-α and IL-10 and eight losses in IL-10 results. Non-parametric tests were used to compare differences in markers across groups. We use restriction to control for possible confounders. Since interleukin and TNF-α levels tend to be highly skewed and bipolar disorder is usually associated with intermediate raises in these markers – at least in serum – (Drexhage and others 2010a; Kapczinski and others 2011; Kapczinski and others 2010), we excluded the top decile in each category as a sensitivity analysis.

RESULTS

Participants were comparable regarding age and socioeconomic status, but women were underrepresented in controls. Current use of psychotropics was low (9.6% of the sample used any medications). Table 1 shows demographic and clinical

information according to diagnosis. IL-6 and TNF-α levels were significantly correlated (rho=0.40, p<0.001), as were IL-6 and IL-10 levels (rho=0.15, p=0.030). IL-10 and TNF-α levels did not correlate (rho=0.07, p=0.297).

IL-6 levels were not associated with any of the predictors and IL-10 levels were associated only with social class (p=0.003). TNF-α levels were higher in those who used illicit drugs (p=0.004) and lower in those who used any psychiatric medications (p=0.004; Table 2). This was due to a difference in the group with bipolar disorder (p=0.002).

Restricting the analysis to those in the upper social classes did not change significantly the association of diagnostic group and IL-10 levels (n=190, p=0.823). When restricted to those who did not use any illicit drugs of psychiatric medications, there was a trend for an association of TNF-α levels and diagnostic group (n=174, p=0.086). TNF-α levels were higher in those with bipolar disorder than in the control group (n=119, Z=2.34, p=0.019). There were no differences between the major depression and the control group (n=138, Z=0.34, p=0.732) or the major depression and the bipolar disorder group (n=91, Z=1.45, p=0.146).

IL-6 and IL-10 results were unchanged (Table 2) in the sensitivity analyses excluding the top decile (outliers). TNF-α levels remained associated with use of medications (Z=3.36. p<0.001). Significant group differences in TNF-α levels were revealed in this analysis (Figure 2; p=0.016). Again, restricting to those who did not use any medications or illicit drugs, those with bipolar disorder had higher TNF-α levels than the control group (n=110, Z=2.91, p=0.004). The bipolar disorder group also had higher TNF levels than the major depression group (n=83, Z=1.16, p=0.031). There were no differences between the major depression and the control group (n=125, Z=0.15, p=0.879).

DISCUSSION

This study confirms in a population-based sample an early-stage increase in tumor necrosis factor-alpha in bipolar disorder. Furthermore, the difference was only apparent in those not using any medications. When only intermediate raises were considered, TNF-α serum levels were also higher in bipolar disorder than in major depression.

This supports an association of bipolar disorder with a high inflammatory set point (Drexhage and others 2010b). This early association may be specific to bipolar disorder, since TNF-α levels in major depression were not different from those of people without any mood disorder. In a series of elegant experiments, Padmos and colleagues (Drexhage and others 2010b; Padmos and others 2008; Padmos and others 2009) demonstrated recently that bipolar disorder is associated with a distinctive inflammatory gene expression signature. In patients with bipolar disorder, circulating monocytes displayed a clearly aberrant expression of genes involved in inflammation. They also showed that protein levels do not rise in the same proportion. This might be pertinent to the results here, since only subtle changes in TNF-α were detected; possibly, a full-blown inflammatory state is a feature of late-stage bipolar disorder (Berk and others 2011; Kapczinski and others 2009; Kapczinski and others 2008b).

A previous comparative study had shown a pro-oxidant state after only one manic episode (Kauer-Sant'Anna and others 2009). Although IL-6 and TNF-α share some properties, there is some data suggesting the former may be more dependent of state, and the latter a more enduring change (O'Brien and others 2006). It appears from the available literature that the results here are more subtle than those found in clinical samples. As noted by Berk et al (Berk and others 2011), an increasing effect

size from early to late stage is supportive of neuroprogression. One caveat in trying to reconcile these results with previous studies is that this is originally a population-based, not a clinical sample. The latter may have special characteristics such as greater severity, chronicity and more frequent comorbidity (i.e., a form of Berkson's bias).

An early high inflammation set point is consequential because it may carry systemic and central nervous system effects. Neuroinflammation has been one of the hypothesized mechanisms responsible for neuroprogression (Berk 2009; Berk and others 2011). In this context, TNF-α is relevant since it induces glial and neuronal toxicicity and apoptosis (Kraft and others 2009). This process is carried out both by resident and infiltrating blood-borne immune cells (Witte and others 2010). It should be mentioned, nevertheless, that TNF-α was not among a host of neuroinflammatory markers demonstrated to be increased in a recent postmortem study (Rao and others 2010). Pro-inflammatory states may also be a relevant connection between bipolar disorder and chronic medical comorbidity (Leboyer and Kupfer 2010). From an early intervention perspective, this is highly relevant since it might be feasible to prevent or attenuate the neuroprogression and "somatoprogression" often seen in bipolar disorder (Berk and others 2007b; Berk and others 2009; Goldstein and others 2009).

There has been interest in testing agents with further specific antagonistic action, since TNF-α has been shown to be modulated by standard treatment to some extent (Soczynska and others 2009). TNF-α serum levels were highly associated with medication use in this sample. Because of the cross-section nature of this study, certainly no causal effect can be ascribed. It is possible that individuals in early treatment have an inherently better prognosis and this, in turn, is associated with less

inflammation. Notwithstanding this design limitation, this finding is relevant given that most previous studies were conducted in highly treated samples. Medications used, especially in euthymic patients, may have been important confounders. In this context, the findings here reinforce the warning that psychotropic medications may introduce significant heterogeneity in the analysis of biological markers (Drexhage and others 2010b; Padmos and others 2008).

Other factors may affect cytokine levels and are by themselves associated with bipolar disorder. Notably among these are obesity, lifestyle, general fitness and sleep. Their absence from this study is a limitation; it would be interesting to test further mediational hypotheses. We were able to control, however, for other possible confounders, such as smoking, alcohol and other substance use disorders. Illicit drugs, such as stimulants, opioids and cannabis, have been shown to present neurotoxic effects in experimental models and conditions and they have been expected to be associated with inflammation (Goldstein and others 2009). A longitudinal design would also be necessary to investigate how inflammation associates with illness progression. Another issue is that some comparisons here may have suffered from limited power. Bipolar disorder is a relatively low prevalence condition and the current study was constrained by this logistical limitation.

This study further reinforces that bipolar disorder is associated with significant systemic toxicity. The confirmation of an early pro-inflammatory state highlights the necessity of stage specific treatments (Berk and others 2007a; Kapczinski and others 2009). Longitudinal studies building upon such recent onset samples are the logical next step towards understanding through which pathophysiological cascades the illness progresses. In the meanwhile, novel, pathology-directed interventions are needed to reduce the burden associated with bipolar disorder. Clinical trials

examining adjunctive compounds that may further attenuate the pro-inflammatory response may be short term feasible goals in this context.

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Statement of interest

None

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Table 1. Sample demographical, clinical and treatment characteristics according to SCID diagnosis

Characteristic	**Healthy control (n=94)**	**Major depression (n=82)**	**Bipolar disorder (n=55)**
Age	22.4 ± 2.7	21.8 ± 2.0	21.7 ± 2.2
Female sex *	58%	77%	74%
Years of education	9.7 ± 3.1	8.9 ± 2.8	8.8 ± 3.6
Lower social class (D or E)	14%	22%	15%
Self-reported clinical illness	28%	33%	46%
Current medication			
Mood stabilizers	0	3%	4%
Antipsychotics	0	0	4%
Antidepressants	1%	3%	6%
Benzodiazepines	1%	1%	4%
Any*	2%	12%	19%
Current depressive episode	0%	77%	76%
Current manic episode	0%	0%	20%
Childhood onset (before 13)	n/a	11%	22%
Lifetime tobacco misuse*	25%	38%	46%
Lifetime alcohol misuse	27%	38%	38%
Lifetime illicit substance misuse	10%	23%	16%
Previous hospitalizations	1%	6%	9%

* $p<0.05$

Table 2. Significance levels for bivariate associations between inflammation markers and demographic and clinical predictors in the whole sample and excluding values in the top decile for each outcome

	Interleukin 6		Interleukin 10		Tumor necrosis factor	
	Whole sample	Top decile excluded	Whole sample	Top decile excluded	Whole sample	Top decile excluded
Sex	0.376	0.300	0.674	0.976	0.990	0.488
Lower social class	0.094	0.117	0.003*	0.006*	0.340	0.458
Smoking	0.568	0.099	0.317	0.181	0.754	0.177
Alcohol abuse	0.191	0.442	0.589	0.780	0.431	0.609
Illicit drugs abuse	0.817	0.321	0.321	0.811	0.004*	0.029*
Clinical illness	0.792	0.452	0.649	0.541	0.894	0.883
Current depression	0.501	0.392	0.495	0.430	0.492	0.620
Current mania	0.336	0.647	0.473	0.841	0.589	0.232
Diagnostic group	0.618	0.359	0.470	0.529	0.408	0.259
Psychiatric medications	0.313	0.290	0.625	0.060	0.004	<0.001*

* p values reported are either derived from Mann-Whitney's U test or from Kruskal-Wallis one-way analysis of variance by ranks

Figure 1. Flow chart showing participant inclusion in the case-control study

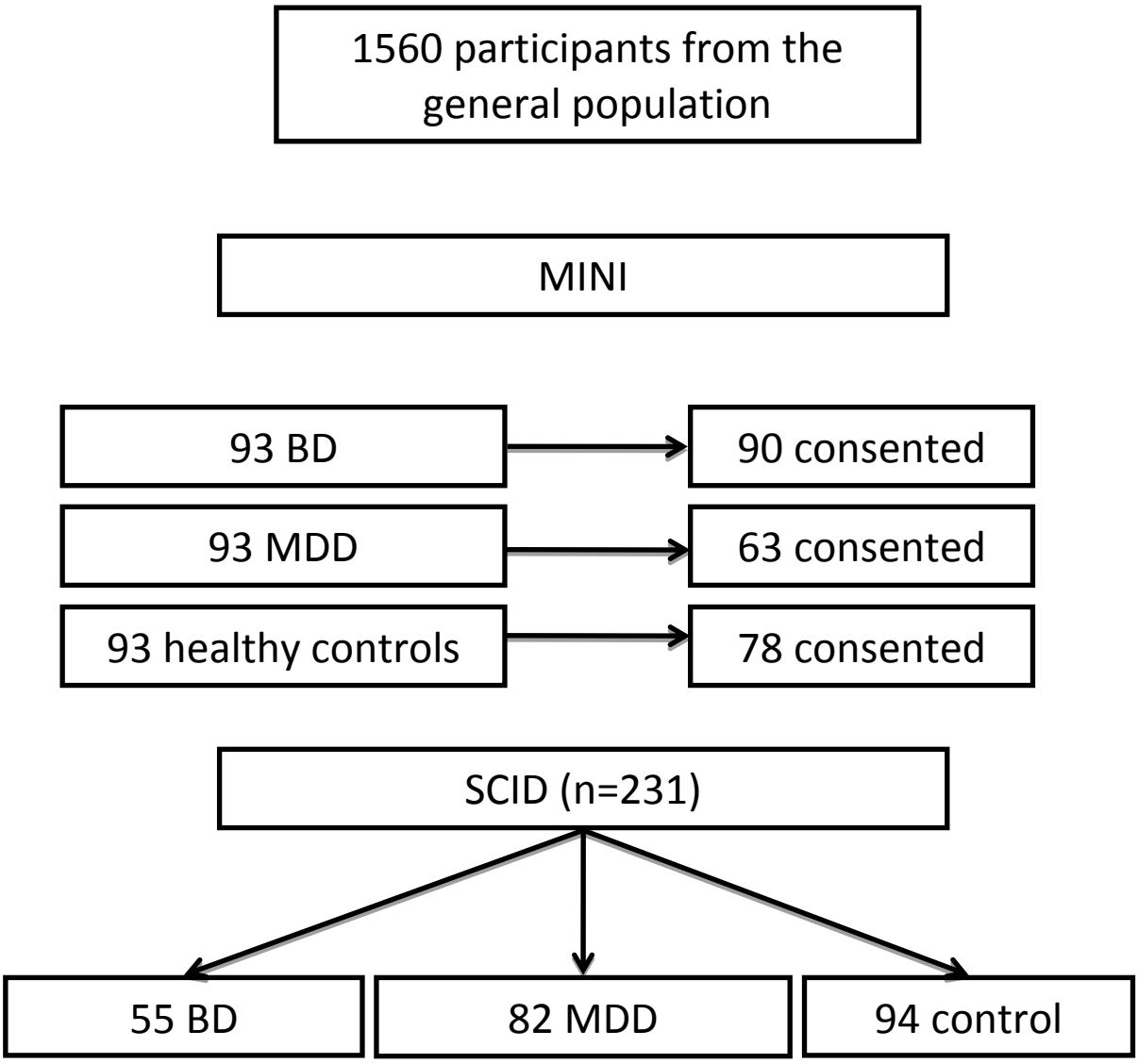
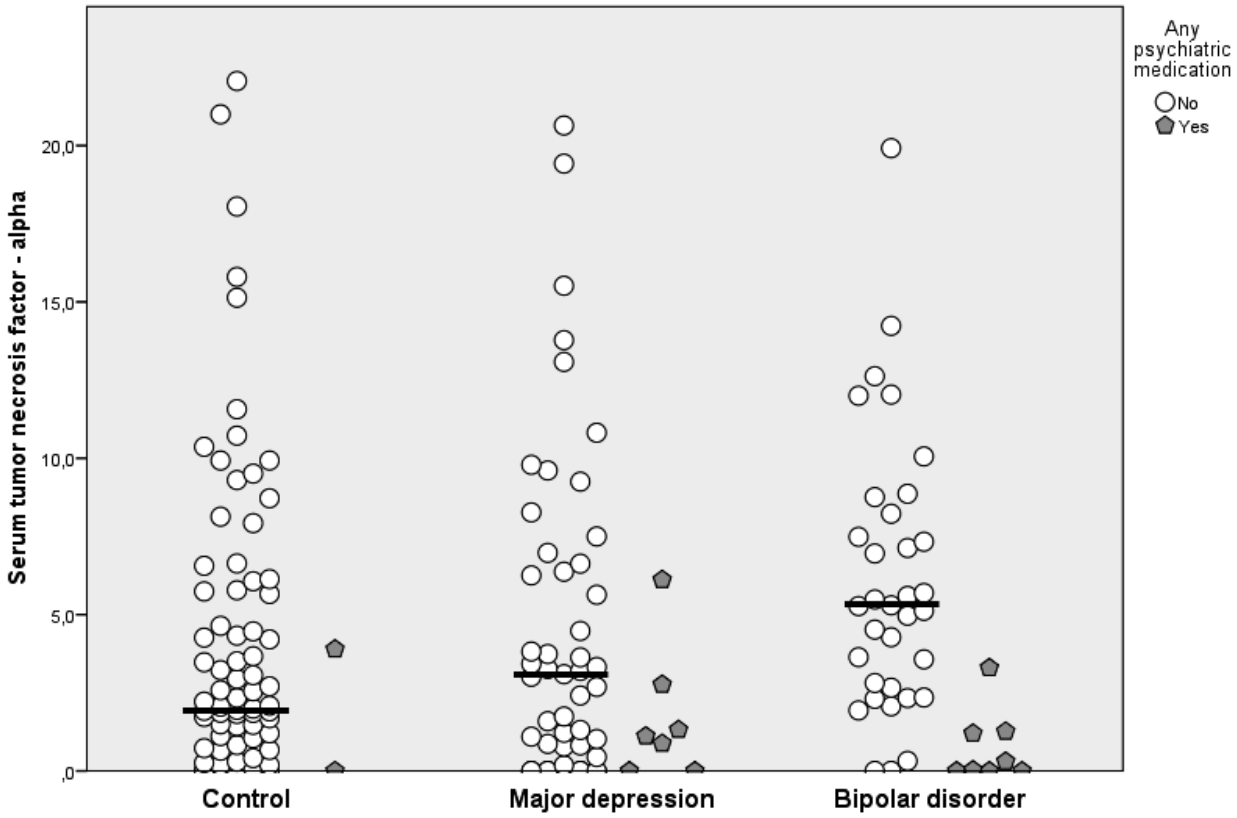


Figure 2. Scatter diagram showing differences in tumor necrosis factor alpha levels according to diagnostic group and medication status



* Horizontal bars represent group medians. Top decile and use of illicit drugs excluded (n=177)

Systemic toxicity in early-stage mood disorders[7]

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We recently reported acute episodes to be associated with significant systemic toxicity in a sample of patients with bipolar disorder (Kapczinski et al., 2011; Kapczinski et al., 2010). As peripheral markers clustered together, we extracted an "index" from the variance shared by inflammation and oxidative stress markers and neurotrophins. This systemic summary variable sharply set apart patients with mania or depression from healthy control subjects. As bipolar disorder is increasingly understood as a multisystem disease, demonstrating peripheral pathophysiology is of clear relevance since it may indicate shared pathways between chronic medical conditions and mood disorders (Berk et al., 2011; Pasco et al., 2010; Soreca, Frank, & Kupfer, 2009).

Changes in specific peripheral mediators have been repeatedly replicated (Berk et al., 2011). However, most samples were weighed towards patients with chronic disease, with a long-term exposure both to the illness and multiple pharmacological treatments. Not much is known on whether a similar toxicity is present in early illness stages. Given the notion that bipolar disorder can be a progressive disease, this is clearly meaningful, as the pathophysiology would be expected to be particular, the effect sizes different, for each stage (Berk et al., 2011).

Here, we attempted to replicate the finding of a general systemic toxicity in a sample with early-stage bipolar disorder. To that end, we nested a case-control study in a population-based sample, of people 18 to 24 years old. The original sample was systematically selected by clusters, where 89 census-based sectors of the city were drawn. It consisted of 1560 participants (Jansen et al., 2011). They underwent the Mini-International Neuropsychiatric Interview as an initial screen; every person with a past or current history of a manic episode was recruited. Two groups of controls were randomly selected from the same population, one with only depressive episodes and

the other without any mood episodes. Consent was obtained from 231 individuals (83% of the intended sample). They further underwent the Structured Clinical Interview for DSM-IV (SCID) to confirm diagnoses, the group-defining criterion for this study. The sample for the case-control study consisted of 94 control subjects, 82 participants with major depression and 55 with bipolar disorder (33 type I and 22 type II). While constrained by the sample size of the population sample, we aimed at recruiting a number of participants comparable to previous clinical studies (Kapczinski et al., 2011; Kauer-Sant'Anna et al., 2009). Individuals provided written informed consent. The study was approved by the ethics committee of the Catholic University of Pelotas.

Serum samples were assayed by technicians blinded to any participant characteristics, using the exact same methodology as in a previous report (Kapczinski et al., 2011). For this analysis, we use thiobarbituric acid reactive substances (TBARS) as a measure of oxidative damage, interleukin-6 (IL-6), IL-10 and tumor necrosis factor-alpha (TNF-α) as inflammation markers and brain-derived neurotrophic factor (BDNF). We chose not to include protein carbonyl content because there were more missing values in this variable, which would necessarily reflect in the summary variable. Clinical illness was self-reported. Drug misuse was evaluated with the Alcohol, Smoking and Substance Involvement Screening Test (Henrique, De Micheli, Lacerda, Lacerda, & Formigoni, 2004).

For the primary component analysis (PCA), BDNF and IL-6 levels were log-transformed and TBARS levels were square-root transformed. IL-10 and TNF were transformed in eight even ordinal categories, as previously reported (Kapczinski et al., 2010). We use simple regression to report bivariate differences in the variable extracted from the PCA. Linear regression with bias-corrected accelerated

bootstrapping with 2000 resamples was used with sex, social class, current mood state, mood diagnoses, substance abuse and self-reported clinical illness as predictors. Bootstrapping is robust when handling data for which the population distribution in unclear (Henderson, 2005).

Due to different missing patterns in biomarkers, the sample size available with all parameters was 204 (49 with bipolar disorder, 72 with major depression and 83 participants without any mood disorders). Women comprised 66% of the sample and were slightly underrepresented in control subjects (55.4%, p=0.037). Median age was 22 and age at onset for the mood disorder groups, 16. Of the people with major depression, 76% were in a current depressive episode. Of those with bipolar disorder, 19% were euthymic, 67% were in a depressive episode and 14% were in a mixed or manic episode. Only 17 participants were using any psychotropic medication (seven with bipolar disorder, 8 with major depression, two controls). Of these, five were on antidepressants, two on mood stabilizers, three on benzodiazepines, one on an antipsychotic and six were not sure on the name or class of the medication.

The first component of the analysis explained 31% of the shared variance. Individual weights were 0.80 for TNF, 0.76 for IL-6, 0.40 for IL-10, 0.32 for TBARS and -0.21 for BDNF. Bivariate analysis did not reveal mood state or diagnostic group effects in this systemic toxicity variable; use of medications, however, was associated with significantly lower toxicity (β=-0.15, p=0.03). Bipolar disorder emerged as a second predictor of toxicity in the multivariable model (Table; β=0.19, p=0.04).

The early-stage and population-based sample of this study offered the possibility of controlling for medication status. This proved relevant, since those on medication had a lower systemic toxicity. A previous report had already shown

alterations in peripheral markers, particularly pro-inflammatory cytokines and nitration-induced damage after only one mood episode (Andreazza et al., 2009; Kauer-Sant'Anna et al., 2009). One caveat in trying to reconcile these results is that this is originally a population-based, not a clinical sample. The latter may have special characteristics such as greater severity, chronicity and more frequent comorbidity. We are also not implying that these are the only relevant mediators of toxicity in bipolar disorder. They have, however, been previously shown to be altered in this condition. It is also possible that they are related not only to neuroprogression, but also to “somatoprogression” (i.e., an increase in the likelihood of medical comorbidity with illness progression) (Goldstein, Kemp, Soczynska, & McIntyre, 2009).

That acute mood states were not particularly associated with toxicity here may be an indication that a greater illness severity may be necessary to cause the full-fledged systemic dysregulation previously reported. A longitudinal design is needed to effectively demonstrate which individuals, via which pathophysiological pathways, are at greater risk for illness progression.

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Table. Bivariate and multivariate associations between systemic toxicity and clinical and demographic predictors (n=204)

Variable	**Systemic toxicity**			
	Bivariate		Multivariate	
	β	P value	β	P value
Sex	-0.02	0.77	-0.04	0.61
Lower social class	0.14	0.05	0.12	0.09
Smoking	0.12	0.10	0.10	0.18
Alcohol abuse	-0.02	0.78	-0.10	0.18
Abuse of Illicit drugs	0.08	0.25	0.10	0.15
Clinical illness	-0.03	0.65	-0.08	0.25
Current depression	-0.01	0.83	-0.01	0.98
Current mania	-0.04	0.60	-0.05	0.38
Major depression	0.04	0.66	0.02	0.85
Bipolar disorder	0.15	0.09	0.19	0.04
Any medication	-0.15	0.03	-0.17	<0.01

* Standardized coefficients and p values from simple linear regressions for bivariate analysis and linear regression with bias-corrected accelerated bootstrapping for the multivariate model

CONCLUSÕES E CONSIDERAÇÕES FINAIS

Duas conclusões gerais podem ser retiradas dos resultados aqui apresentados. A primeira é que o transtorno bipolar está associado a alterações sistêmicas desde seus estágios iniciais. Especificamente, foram encontrados indícios de estados pró-oxidante e pró-inflamatório. Os dados desta tese mostraram que esta associação foi independente de alguns fatores clínicos e demográficos que poderiam ser confundidores. Uma minoria dos participantes utilizava medicações. Isso foi bastante interessante, já que foi possível separar o efeito das últimas, e também demonstrar uma associação delas com menores níveis de fator de necrose tumoral e menos toxicidade em geral.

Os principais objetivos do estudo eram avaliar a relação do diagnóstico de transtorno bipolar em jovens indivíduos provenientes da comunidade com alguns marcadores periféricos. Especificamente, observou-se um aumento no dano oxidativo a proteínas e nos níveis circulantes do fator de necrose tumoral no transtorno bipolar. Esses foram achados consistentes, confirmados pelo modelo multivariado proposto *a priori*. Os outros fatores em estudo não separaram os grupos diagnósticos. Isso leva à segunda conclusão maior da tese. Mesmo que presentes, esses achados são bastante mais sutis que os observados em amostras clínicas. Isso apóia uma idéia de progressão do transtorno bipolar, possivelmente diferentes estágios estando associados a fisiopatologias diversas. Como os marcadores não se associaram tão fortemente, uma especulação possível seria que, em estágios iniciais, ainda seja possível observar uma heterogeneidade fisiopatológica da doença. Isso se perderia com o tempo, pela influência de múltiplos fatores, como cronicidade e mesmo o tratamento.

Um cuidado importante na interpretação desses resultados é que a comparação de diferentes amostras transversais pode ser problemática. Os estudos anteriores utilizaram basicamente amostras clínicas, com todas as repercussões e implicações de doenças crônicas. Dessa forma, não apenas a progressão da doença, mas outros fatores diferenciais entre as amostras poderiam ser responsáveis pelos achados. Os estudos de base populacional em psiquiatria também não deixam de ter problemas particulares. O estabelecimento de um diagnóstico fora da clínica freqüentemente é complicado pela baixa acurácia dos instrumentos disponíveis. O uso de entrevistas semi-estruturadas, que são consideradas o “padrão-ouro” atualmente, implica em um profissional treinado, o que praticamente impossibilita a inclusão de centenas a milhares de participantes, o escopo usual de estudos epidemiológicos. A solução parcial aqui foi realizar o estudo em duas etapas, com uma confirmação diagnóstica. Isso também não evita possíveis questões sobre distorções do desenho original, mas pareceu aos autores a melhor opção disponível.

A ausência de estudos prospectivos dificulta uma melhor compreensão de como e em que pessoas o transtorno bipolar tende a progredir. Embora estudos populacionais em jovens potencialmente forneçam dados menos enviesados, relações causais não são possíveis de serem estabelecidas. Realmente, um desenvolvimento lógico para o campo é a incorporação de desenhos longitudinais. Com eles, é possível avaliar mudança, uma dimensão de extrema relevância em psicopatologia. Eles vão permitir avaliar não apenas como a fisiopatologia afeta o prognóstico, mas possíveis cadeias causais entre as várias vias biológicas de importância para progressão e resiliência aos efeitos da doença.

Mesmo assim, estudos transversais em amostras representativas são relevantes ao demonstrar a existência de dano nos transtornos psiquiátricos e gerar hipóteses. Isso porque mesmo que as associações sejam certamente complexas e intricadas, a demonstração de áreas de disfunção e patologia é fundamental. Tanto o uso de antioxidantes quanto de antiinflamatórios já foi investigado em estudos clínicos. Para alguns compostos, os resultados são encorajadores, mesmo que não definitivos.

É indubitável a confluência de dados recentes em apontar envolvimento multissistêmico no transtorno bipolar. Isso tem organizado a busca por marcadores periféricos relevantes, e há diversas indicações de relações entre sistemas metabólicos, pró e antiinflamatórios, pró-oxidantes e antioxidantes, entre outros. Que toxicidade generalizada seja encontrada desde os estágios iniciais é um alerta importante, e uma lembrança sobre a necessidade de investir na prevenção secundária desde os estágios iniciais da doença. Uma abordagem translacional ao problema sugere que dados provenientes de estudos clínicos, pré-clínicos e comunitários sejam utilizados em conjunto para o entendimento da fisiopatologia das doenças afetivas. Assim, minimizam-se as limitações inerentes a cada um dos métodos.

ANEXOS

ANEXO A – Aprovação do Comitê de Ética da Universidade Católica de Pelotas e Termo de consentimento livre e esclarecido

UNIVERSIDADE CATÓLICA DE PELOTAS
PRÓ-REITORIA ACADÊMICA
COMITÊ DE ÉTICA EM PESQUISA – CEP/UCPel

RESULTADO

O Comitê de Ética em Pesquisa da Universidade Católica de Pelotas analisou o projeto:

Número: 2008/118

Título do projeto: *"Fisiopatologia do transtorno do humor bipolar em uma amostra de base populacional"*

Investigador(a) principal: Ricardo Azevedo da Silva

O projeto foi aprovado pelo Comitê de Ética em Pesquisa – CEP da UCPel, em reunião datada de 19 de março de 2009, ata nº 02.

A avaliação foi realizada pelos membros do comitê, baseada na análise minuciosa do projeto, apresentada por um dos membros.

Outrossim, informamos que é obrigatório a entrega do relatório de conclusão pela coordenação do referido projeto ao Comitê de Ética – CEP/UCPel, na Secretaria da Pró-Reitoria Acadêmica da Universidade Católica de Pelotas.

Pelotas, 03 de abril de 2009

Prof. Dr. Ricardo Tavares Pinheiro
Coordenador CEP/UCPel

UNIVERSIDADE CATÓLICA DE PELOTAS
PROGRAMA DE PÓS-GRADUAÇÃO EM SAÚDE E COMPORTAMENTO

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Projeto: *Fisiopatologia do transtorno de humor bipolar em uma amostra de base populacional*

Informações sobre o estudo ao participante

Esta folha informativa tem o objetivo de fornecer a informação suficiente para quem considerar participar neste estudo. Ela não elimina a necessidade do pesquisador de explicar, e se necessário, ampliar as informações nela contidas.

Antes de participar deste estudo, gostaríamos que você tomasse conhecimento do que ele envolve. Damos abaixo alguns esclarecimentos sobre dúvidas que você possa ter.

Qual é o objetivo da pesquisa?
Com este estudo buscamos identificar algumas substâncias no sangue que podem estar relacionadas aos transtornos de humor. Para isso, será coletado sangue de pessoas com indicativo de transtornos de humor e de pessoas sem esses indícios para comparação. Assim, será possível entender melhor de que maneira essas substâncias se associam aos transtornos.

Como o estudo será realizado?
Será realizada uma coleta de sangue do seu braço, na qual serão retirados 15 ml de sangue, o que não compromete a sua saúde. Esta coleta será realizada por pesquisadores da área da saúde devidamente treinados para tal função.

Quais são os riscos em participar?
Os riscos da coleta são mal-estar passageiro ou mancha roxa no local. O procedimento será feito com material esterilizado e descartável por profissionais da área de saúde. A coleta será feita para que sejam analisadas algumas substâncias que poderão estar alteradas em função dos transtornos de humor.

Itens importantes:
Você tem a liberdade de desistir do estudo a qualquer momento, sem fornecer um motivo, assim como pedir maiores informações sobre o estudo e o procedimento a ser feito. Isto de maneira alguma irá influenciar na qualidade de seu atendimento neste hospital.

O que eu ganho com este estudo?
Sua colaboração neste estudo pode ajudar a aumentar o conhecimento científico sobre fatores relacionados aos transtornos de humor, que poderão eventualmente beneficiar você ou outras pessoas. Ao saber melhor quais substâncias estão relacionadas aos transtornos, um tratamento médico mais direcionado pode ser esperado no futuro.

Quais são os meus direitos?
Os resultados deste estudo poderão ser publicados em jornais científicos ou submetidos à autoridade de saúde competente, mas você não será identificado por nome. Sua participação neste estudo é voluntária.

DECLARAÇÃO:

Eu, ...declaro que:

1. Concordo total e voluntariamente em fazer parte deste estudo.
2. Recebi uma explicação completa do objetivo do estudo, dos procedimentos envolvidos e o que se espera de mim. O pesquisador me explicou os possíveis problemas que podem surgir em conseqüência da minha participação neste estudo.
3. Informei o pesquisador sobre medicamentos que estou tomando.
4. Concordo em cooperar inteiramente com o pesquisador supervisor.
5. Estou ciente de que tenho total liberdade de desistir do estudo a qualquer momento e que esta desistência não irá, de forma alguma, afetar meu tratamento ou administração médica futura.
6. Estou ciente de que a informação nos meus registros médicos é essencial para a avaliação dos resultados do estudo. Concordo em liberar esta informação sob o entendimento de que ela será tratada confidencialmente.
7. Estou ciente de que não serei referido por nome em qualquer relatório relacionado a este estudo. Da minha parte, não devo restringir, de forma alguma, os resultados que possam surgir neste estudo.

Nome completo do paciente: _____

Assinatura do Paciente: _____

Data: __ / __ / _____

Assinatura do Pesquisador: _____

Para maiores informações entre em contato com Karen Jansen pelo telefone: 81254906 - 21288404

Coordenador do projeto: Prof. Dr..Ricardo Azevedo da Silva
Programa de Pós-Graduação em Saúde e Comportamento
Universidade Católica de Pelotas
Fone: 21288404 - 81228378

ANEXO B - N-acetyl cysteine add-on treatment for bipolar II disorder: a subgroup analysis of a randomized placebo-controlled trial[8]

Magalhães PV, Dean OM, Bush AI, Copolov DL, Malhi GS, Kohlmann K, Jeavons S, Schapkaitz I, Anderson-Hunt M, Berk M.

[8] Publicado no *Journal of Affective Disorders*, v.129, p.317 - 320, 2011.

ABSTRACT

Background The evidence base for the pharmacological treatment of bipolar II disorder is limited. In bipolar disorder, there is evidence for glutathione depletion and increased oxidative stress, as well as dysregulation of glutamate; N-acetyl cysteine (NAC) has effects on both of these systems. Add-on NAC has been shown to have a significant benefit on depressive symptoms in a randomized placebo controlled-trial. In this report, we explore the effects of this compound in a subset of patients with bipolar II disorder from that trial.

Methods Individuals were randomized to NAC or placebo in addition to treatment as usual, in a double-blind fashion. Mood and functional outcomes were assessed up to 24 weeks of treatment.

Results Fourteen individuals were available for this report, seven in each group. Six people achieved full remission of both depressive and manic symptoms in the NAC group; this was true for only 2 people in the placebo group (Chi^2= 4.67, p=0.031).

Limitations Subgroup analyses in a small subsample of patients. Not all participants had elevated depression scores at baseline.

Conclusion Notwithstanding all the limitations that subgroup analysis of trials carry, this data could serve as a hypothesis-generating stimulus for further clinical trials of pharmacologic treatment for bipolar II depression.

Keywords: bipolar disorder, n-acetyl cysteine, oxidative stress, treatment, remission, depression, mania.

INTRODUCTION

The evidence base for the pharmacological treatment of bipolar II disorder, a condition where depressive symptoms are the hallmark, is limited (Yatham et al., 2009). With high rates of misdiagnosis and lack of clarity about appropriate management, there is an important void in treatment research in bipolar II depression, which is all the more salient given the high prevalence of the disorder (Berk and Dodd, 2005). Based on the current dearth of studies, a recent consensus specifically encouraged the conduct of trials testing pharmacologic strategies in bipolar II depression (Kasper et al., 2008).

One promising avenue has been the study of the glutathione system (Dean et al., 2009; Dodd et al., 2008; Ng et al., 2008), since redox imbalance has been repeatedly demonstrated in bipolar disorder (Andreazza et al., 2008; Kapczinski et al., 2010). Glutathione is a generic non-enzymatic cellular free radical scavenger (Berk et al., 2008b). In this respect, N-acetyl cysteine (NAC), a glutathione precursor, may provide symptomatic relief by mitigating redox imbalance (Berk, 2008b; Berk et al., 2009). There is similarly evidence of glutamatergic involvement in bipolar disorder (Berk et al., 2000; Singh et al., 2009), and NAC has potent effects on glutamate via cystine glutamate exchange (Moussawi et al., 2009).

As previously reported, add-on NAC had a significant effect on depressive symptoms in a randomized placebo controlled-trial (Berk et al., 2008a). In this report, we explore the effects of this compound in a small subset of patients with bipolar II disorder from that trial.

METHODS

A detailed description on the study recruitment and evaluation procedures have been published elsewhere (Berk et al., 2008a). Briefly, consenting individuals were randomized to NAC or placebo in addition to treatment as usual, in a double-blind fashion. They needed to fulfill DSM-IV criteria for bipolar I or II disorder, and be on stable therapy for at least one month prior to randomization. In this report, only data pertaining to the individuals with bipolar II disorder are described. The trial was conducted in public and private outpatient settings. Exclusion criteria were kept to a minimum, as the trial was intended to be naturalistic as possible, and included systemic medical disorders, pregnant or lactating women and previous known intolerance or contraindication to NAC. All participants provided written informed consent. The participating institutions' research and ethics committees approved the trial. The study was registered with the Australian Clinical Trials Registry (Registration number: 12605000362695).

The participants received two NAC (500mg) capsules twice daily or matching placebo. Withdrawal occurred if participants stopped effective contraception, taking their medication for 7 consecutive days or became pregnant, and also if they withdrew the consent or developed a serious adverse event necessitating withdrawal. They were assessed at baseline with the Mini-International Neuropsychiatric Interview (Sheehan et al., 1998). Interviewers assessed mood and functional outcomes at baseline and at weeks 2, 4, 8, 12, 16, 20 and 24. The analysis included all randomized patients with at least one post-baseline assessment.

Interviewers assessed mood using the Bipolar Depression Rating Scale (Berk et al., 2004; Berk et al., 2007) (BDRS), the Montgomery-Asberg Rating Scale (Montgomery and Asberg, 1979) (MADRS), and the Young Mania Rating Scale

(Young et al., 1978) (YMRS). Remission rates at endpoint were obtained using validated criteria (Berk et al., 2008c), that is, a MADRS and YMRS score of less than 8. The Clinical Global Impression (Spearing et al., 1997) (21) (CGI) was obtained as a measure of overall illness severity. Functioning and quality of life were assessed with the Global Assessment of Functioning (Jones et al., 1995) (GAF), the Social and Occupational Functioning Assessment Scale (Morosini et al., 2000) (SOFAS), the Streamlined Longitudinal Interview Clinical Evaluation for the Longitudinal Interval Follow-up Evaluation (SLICE-LIFE), the Longitudinal Interval Follow-up Evaluation – Range of Impairment Functioning Tool (Keller et al., 1987) (LIFE-RIFT) and the Quality of life Enjoyment and Satisfaction Questionnaire (Endicott et al., 1993) (Q-LES-Q).

All analyses are based on the intention-to-treat population. Sample size precluded parametric analyses, so the investigation of change trajectory in this report was not possible (for results on the whole sample as well as original sample size estimation see (Berk et al., 2008a). We present distributions on quantitative measures as medians and ranges to give an idea of extremes. Changes from baseline to endpoint were compared using Mann-Whitney's U. We employed Chi^2 tests to compare differences in remission rates.

RESULTS

Fourteen participants were available for this report, seven in each group (see Table 1), where baseline data and change in outcomes are illustrated. Participants typically had residual depressive symptoms, with a median MADRS of 11, and a CGI of 3.

For every outcome, change was more pronounced in the NAC group (Table 2), although it only achieved significance for the YMRS (Z=2.40, p=0.016) and there was a non-significant trend for the MADRS (Z=1.80, p=0.072). Of note, MADRS and BDRS change scores were closely associated (rho=0.71, p=0.005), as were GAF and BDRS changes (rho=-0.73, p=0.003).

Using a composite outcome including remission of both symptoms of mania and depression, 6 participants achieved remission from both mania and depression in the NAC group, while only 2 participants had the same outcome in the placebo group; this was statistically significant (Figure 1, Chi^2= 4.67, p=0.031). Regarding depressive symptoms, 6 out of the 7 participants in the NAC group and 3 out of 7 achieved remission at endpoint (Chi^2=2.80, p=0.094). All participants in the NAC group and 6 in the placebo group remitted from manic symptoms (Chi^2=1.08, p=0.299).

One participant in the NAC group (withdrew consent) and three in the placebo group (two withdrew consent, one was non-adherent) failed to complete all assessments. None of the patients in the trial reported a serious adverse event for the trial duration. Three participants in the NAC (sweating, thirst and headache) and three in the placebo group (palpitations, nausea, diarrhea) reported side effects during the trial.

DISCUSSION

In this small subset of subjects with bipolar II disorder, N-acetyl cysteine demonstrated an interesting pattern of efficacy after six months of treatment. Six out of seven participants achieved full remission of both depressive and manic symptoms; this was true for only 2 participants in the placebo group.

NAC has been demonstrated to boost glutathione levels in animal models (Dean et al., 2009). It is as a precursor of cysteine, the rate-limiting step in glutathione synthesis (Dodd et al., 2008). Its main mechanisms of action in the treatment of bipolar disorder likely involve glutathione replenishment and changes in glutamate through cystine glutamate exchange (Moussawi et al., 2009). Increased plasma glutathione levels have been shown following this dose regimen; NAC equally appears to reverse the deficit in mismatch negativity, and index of glutamate dysregulation (Lavoie et al., 2008). Indeed, previous literature suggests both a deficit in oxidative defenses and changes in glutamatergic function in bipolar disorder. Buffering glutathione deficits may ultimately counteract neurotransmitter defects or excessive neurotoxicity. Whether NAC will have a role in preventing neuroprogression is still a matter that needs clinical testing (Berk, 2009; Berk et al., 2009). Additionally, NAC has a role in decreasing inflammation, as well as increasing neurogenesis and neurite growth and reversing mitochondrial dysfunction, which is relevant given the role of those factors in the pathophysiology of mood disorders (Andreazza et al., 2010; Laux and Nel, 2001; Qian and Yang, 2009; Welin et al., 2009).

Subgroup analyses may pose significant interpretation problems. Clearly, the smaller the trial, the higher the likelihood of chance findings. The sample size also prevented controlling for any possible confounders, as well as for checking for possibly relevant interactions. However, the results of this sub-analysis are congruent with the larger study (n=76), providing support to the current findings (Berk et al., 2008a). It is also relevant that not all participants had elevated depression scores at baseline, and symptoms were typically mild to moderate. To measure the effect of NAC on moderate to severe depressive episodes a clinical trial with a representative

sample is necessary. With that said, we did not detect a large group difference biasing the results toward better outcomes for those on NAC. For example, there were more participants randomized to placebo on antidepressants at baseline. Subjects were also very similar in respect of illness severity and functioning at baseline.

Clinical trials for bipolar II disorder are sparse (Fountoulakis and Vieta, 2008). It has been suggested that most individuals need combination treatment (Fountoulakis, 2010). Given the poor performance of many agents in bipolar depression, especially antidepressants (Yatham et al., 2009), research into novel treatments with novel mechanisms of action has been called for, especially in the context of bipolar II depression (Kasper et al., 2008). While this report is clearly hypothesis generating rather than hypothesis confirming, add-on n-acetyl cysteine may have clinically meaningful effects on the characteristic symptoms of bipolar II disorder. This could serve as stimulus for the design of further clinical trials for bipolar II depression.

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Table 1. Demographical, clinical and treatment characteristics of the study sample at baseline

Characteristic	**Euthymia (n=19)**	**Depression (n=33)**	**Mixed (n=10)**
Age [a]	43 (35 – 54)	45 (37 – 55)	40 (33 – 55)
Female sex	58%	58%	70%
On NAC	42%	58%	50%
Age at diagnosis [a]			
MADRS**	5 (2 – 9)	20 (11 – 25)	19 (12 – 25)
YMRS*	2 (0 -3)	2 (0 – 4)	4 (2 – 6)
CGI-BP	2 (2 – 2)	3 (3 – 5)	3 (3 – 3)
GAF	75 (61 – 81)	60 (51 – 65)	63 (54 – 70)
Medication			
Lithium	37%	39%	30%
Other mood stabilizers	68%	51%	70%
Atypical antipsychotics	32%	27%	60%
Antidepressants	63%	55%	50%
Benzodiazepines	5%	12%	30%
Monotherapy			

[a] Results are shown as median (IQR)

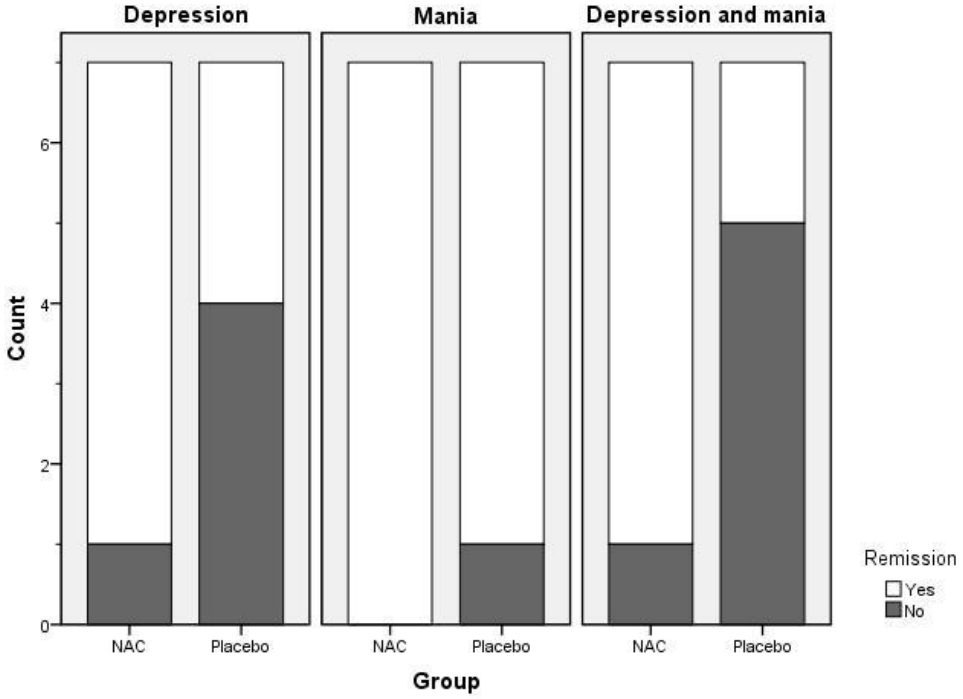
Table 2. Change from baseline to endpoint in rating scales

Characteristic	**NAC group (n=7)**		**Placebo group (n=7)**	
	Baseline	*Change*	*Baseline*	*Change*
MADRS	9 (21)	-7 (43)	12 (21)	-1 (8)
BDRS	10 (18)	-10 (26)	13 (21)	-1 (13)
YMRS*	4 (11)	-2 (13)	2 (3)	0 (9)
CGI	3 (3)	-1 (7)	3 (2)	0 (2)
GAF	66 (24)	4 (45)	60 (46)	0 (11)
LIFE-RIFT	9 (10)	-3 (11)	11 (10)	-1 (4)
SLICE-LIFE	18 (19)	-3 (16)	18 (15)	-2 (9)
Q-LES-Q	58 (28)	1 (51)	54 (25)	-1 (11)
SOFAS	66 (20)	4 (46)	60 (46)	0 (11)

Results are shown as median (range). MADRS - Montgomery-Asberg Rating Scale; YMRS - Young Mania Rating Scale; BDRS - Bipolar Depression Rating Scale; CGI - Clinical Global Impression ; GAF - Global Assessment of Functioning; SOFAS - Social and Occupational Functioning Assessment Scale ; SLICE-LIFE - Streamlined Longitudinal Interview Clinical Evaluation for the Longitudinal Interval Follow-up Evaluation; LIFE-RIFT - Longitudinal Interval Follow-up Evaluation – Range of Impairment Functioning Tool; Q-LES-Q - Quality of life Enjoyment and Satisfaction Questionnaire.

* $p<0.05$ (Mann-Whitney's U) for difference in change scores between NAC and placebo groups

Figure. Remission rates for n-acetyl cysteine and placebo groups at endpoint



ANEXO C – Dimensions of improvement in a clinical trial of n-acetyl cysteine for bipolar disorder[9]

Magalhães PV, Dean OM, Bush AI, Copolov DL, Malhi GS, Kohlmann K, Jeavons S, Schapkaitz I, Anderson-Hunt M, Berk M.

[9] Publicado na *Acta Neuropsychiatrica* v.23, p.87 - 88, 2011.

In addition to mood symptoms, functioning and quality of life are dimensions of outcome now routinely assessed in bipolar disorder [1]. In cross-section, the general finding is that symptoms, quality of life and functioning will correlate to some degree [2, 3]. A common view, thus, is that these domains are related and similar. Nonetheless, more complex associations may underlie these simple findings. Dimensions may correlate and still be discernible. This, in fact, is often the case with psychiatric instruments [4].

While disability during euthymia has been identified, the focus has habitually been on its relation with residual symptoms. Although symptoms and functioning clearly fluctuate together [5], the extent to which these dimensions are linked has not been thoroughly explored. In this report, we use data from a placebo-controlled trial investigating N-acetyl cysteine (NAC) as an adjunctive treatment for bipolar disorder to test how measures of depression, functioning and quality of life change and how these scores cluster. Given that NAC was highly effective in both depression and functioning, we further aimed to assess how much of the change in functioning brought on by the treatment was mediated via change in depression.

A thorough description of the study has been published [6]. Briefly, this was a double-blind placebo-controlled trial of NAC in addition to treatment as usual. Participants fulfilled DSM-IV criteria for bipolar disorder and were on stable therapy for at least one month prior to the study. There was no minimum rating scales score at inclusion. Only people with currently uncontrolled medical conditions and pregnant or lactating women were excluded. All participants provided written informed consent. The participating institutions' ethics committees approved the trial, which was registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12605000362695).

Participants received NAC (1000mg) twice daily or matching placebo. Outcomes were assessed at baseline and up to week 24. Participants were assessed using the Montgomery-Asberg Rating Scale (MADRS, [7]), the Young Mania Rating Scale (YMRS, [8]) and the Bipolar Depression Rating Scale (BDRS, [9, 10]). Functioning and QoL were assessed with the Global Assessment of Functioning (GAF, [11]), the Social and Occupational Functioning Assessment Scale (SOFAS, [12]), the Streamlined Longitudinal Interview Clinical Evaluation (SLICE-LIFE), the Range of Impairment Functioning Tool (LIFE-RIFT, [13]) and the Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q, [14]). Here we do not report on (hypo)manic symptoms, since both baseline and change scores were low in this sample.

Exploratory factor analysis is a method aimed at simplifying complex datasets. Unlike simple correlations, data loading in factors can be seen as a statement of the relationship between variables. It is in this sense it is used here. We used alpha factoring with Oblimin rotation to extract factors. The number of factors were selected both by inspecting the Scree plot and the most parsimonious solution to the data. The distribution of change scores was deemed acceptable for factor analysis. A simple mediation model [15] was employed to test the proportion of the effect of NAC on functioning that was mediated by change in depressive symptoms (BDRS). The functioning measure we used on the model was the LIFE-RIFT, on which NAC had the largest effect size.

Seventy-four participants were included in the trial. All bivariate associations between change scores were highly significant ($p<0.001$ for all; Table). The Kaiser-Meyer-Olkin measure was 0.82, indicating factorability. The two-factor solution, in which distinct dimensions emerged, best accounted for the data. It explained 81% of the variance. The first factor included the GAF, the SOFAS, the SLICE-LIFE and the

LIFE-RIFT, and accounted for 68% of the variance. The second factor included the depression scales and the Q-LES-Q and explained a further 13% of the variance. The factors correlated at 0.68.

In the mediation model, change in depression mediated the impact of NAC on functioning (i.e. there was a significant indirect effect; Coef=1.78, S.E.=0.71, p=0.012). The proportion of the treatment (NAC) effect on functioning mediated through depressive symptoms change was 55%. The remaining proportion of the effect was direct (i.e. unaccounted for by change in depressive symptoms).

Change in interviewer-rated functioning scores and depressive symptoms (along with quality of life) clustered into two discernible factors. This replicates cross-sectional data, where functioning and quality of life clustered in separate dimensions [16]. Furthermore, in a simple mediation model, a considerable proportion of the impact of NAC on functioning was not associated with change in depression.

Interventions may affect functional status via pathways not exclusively related to symptom change. Other mechanisms, such as cognition and biological rhythms have been associated with functioning independently of symptoms [17]. These are interesting targets for novel interventions. Perhaps because the data in clinical trials tend to be highly correlated, change too often is seen as unidimensional. Functional data should be viewed as outcomes in their own right, and not only serve to accentuate the relevance of residual symptoms.

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Table. Correlation matrix showing coefficients for associations between change scores in rating scales in participants in a clinical trial (n=74)

Score	**Mean ± SD**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1. MADRS	-2.77± 12.93	x	0.83	-0.68	-0.64	-0.64	0.70	0.40
2. BDRS	-2.59± 10.74	0.83	x	-0.66	-0.54	-0.55	0.67	0.41
3. Q-LES-Q	0.69± 12.53	-0.68	-0.66	x	0.54	0.62	-0.68	-0.43
4. GAF	4.09 ±12.17	-0.64	-0.54	0.54	x	0.91	-0.65	-0.57
5. SOFAS	3.86 ±12.17	-0.64	-0.55	0.62	0.91	x	-0.72	-0.65
6. RIFT	-1.18 ±4.61	0.70	0.67	-0.68	-0.65	-0.72	x	0.77
7. SLICE	-2.89 ±7.39	0.40	0.41	-0.43	-0.57	-0.65	0.77	x

MADRS - Montgomery-Asberg Rating Scale; BDRS - Bipolar Depression Rating Scale; GAF - Global Assessment of Functioning; SOFAS - Social and Occupational Functioning Assessment Scale ; SLICE-LIFE - Streamlined Longitudinal Interview Clinical Evaluation for the Longitudinal Interval Follow-up Evaluation; LIFE-RIFT - Longitudinal Interval Follow-up Evaluation – Range of Impairment Functioning Tool; Q-LES-Q - Quality of life Enjoyment and Satisfaction Questionnaire.

ANEXO D – Protocolos de intervenção de revisões sistemáticas sobre o uso de antioxidantes no transtorno bipolar e na esquizofrenia[10]

Pedro V S Magalhães, Olivia Dean, Ana Cristina Andreazza, Michael Berk, Flávio Kapczinski

[10] Em desenvolvimento junto à Colaboração Cochrane

Adjunctive antioxidants for bipolar disorder

Background

Description of the condition

Bipolar disorder is a chronic and recurrent psychiatric disorder. Its course is frequently complicated by misdiagnosis and a delay and low rate of effective treatment (Kessler 2007). Among the mood disorders, it is associated with more work-related impairment per case than major depressive disorder (Kessler 2006). Its main subtypes are bipolar I disorder, where one or more mixed or manic episodes are usually accompanied by depressive episodes, and bipolar II disorder, where symptoms are similar to that of a manic episode, but milder and not causing pronounced impairment (Muller-Oerlinghausen 2002). A residual diagnosis, bipolar disorder not otherwise specified (NOS), is also possible for when the disorder does not completely fulfill criteria for bipolar I or II disorder. Their relative prevalences vary in different epidemiological samples, but the replication of the National Comorbidity Survey puts them at approximately 1% for bipolar I and II disorders and 2.5% for bipolar disorder NOS (Merikangas 2007).

Bipolar disorder is increasingly recognized as being associated with dysfunction and disability (Huxley 2007). Initially conceived as relatively benign, with mood episodes alternating with euthymia (define), the picture emerging from the recent literature is one of poor inter-episode functioning and increasing treatment resistance associated with illness progression (Kapczinski 2008; Berk 2009). This condition has been increasingly seen as an illness not only characterized by central nervous system dysfunction, but also systemic toxicity (Soreca 2009). Peripheral oxidative damage has been consistently demonstrated (Andreazza 2008), and

evidence also points to alterations in antioxidant pathways (Selek 2008; Andreazza 2009). Also of note, these changes have often been associated with other peripheral biomarker changes involving neurotrophic and inflammatory pathways (Kapczinski 2010), making for an overall picture of progressive vulnerability to mood episodes and possibly further toxicity (Kapczinski 2008).

How this vicious cycle is brought on and how oxidative stress is causally related to illness activity is not fully understood at present. Recent post-mortem evidence indicate abnormalities in mitochondrial structure and increased oxidative stress and damage to the mitochondrial electron transport chain in the brain, as well as low glutathione - the major antioxidant in the brain - levels (Wang 2009; Cataldo 2010; Andreazza 2010; Gawryluk 2010). These new findings support the involvement of oxidative stress in the pathophysiology of bipolar disorder and have rendered it one of several emerging targets for improving outcomes in this condition (Machado-Vieira 2010).

Description of the intervention

Antioxidants are exogenous or endogenous molecules that mitigate any form of oxidative stress or its consequences. Exogenous antioxidants may act from directly scavenging free radicals to stimulating anti-oxidant defences (Uttara 2009). There is evidence that current treatments such as lithium, valproate and atypical antipsychotics impact oxidative pathways and may to some extent reverse pro-oxidative states in bipolar disorder (Andreazza 2008). These agents are the cornerstone of treatment in published guidelines (Yatham 2009, Goodwin 2009, NICE 2006). Nevertheless, there is emerging interest in adjunctive treatments that target specific pathways (Ng 2008). Among these, oxidative stress - along with other

promising pharmacological approaches - has been identified as a target of interest (Machado-Vieira 2010).

Thus far, antioxidants have been promoted as treatments for several diseases, not always fulfilling their promise. Oral adjunctive antioxidants have demonstrated benefits in specific cases, such as vitamin E for nonalcoholic steatohepatitis (Sanyal 2010) and protection of cisplatin neuropathy (Pace 2010), n-acetyl cysteine for trichotillomania (Grant 2009), ginko biloba for symptoms of schizophrenia (Singh 2010), selenium for improving semen parameters in infertile men (Safarinejad 2009) and a package of antioxidants including selenium, ascorbic acid, beta-carotene, tocopherol and methionine for relieving the pain associated with pancreatitis (Bhardwaj 2009). Healthy skepticism remains as to the general utility of antioxidants, however, and a Cochrane review did not find a general effect of antioxidants on mortality (Bjelakovic 2008). This may be in part related to their being seen as an indistinct class in spite of very different pharmacokinetic and pharmacodynamic profile (Berk 2008).

How the intervention might work

The underlying mechanisms underpinning the process of disease neuroprogression and subsequent brain change are incompletely understood. There is, however, evidence pointing to central and peripheral pro-oxidative changes, including lowered oxidative defences particularly the glutathione system and oxidative damage to proteins, lipids and DNA (Berk 2009a).

Ascorbic acid, α-tocopherol, carotenoids and flavonoids are non-enzymatic antioxidants (Valko 2007, Rahman 2007). Selenium acts by stimulating selenoproteins, such as glutathione peroxidase (Steinbrenner 2009). Some

antioxidants exert their effects both by scavenging free radicals and stimulating enzymatic defences; this appears to be the case with melatonin, pramipexole, selegiline and the standardized extract of G. biloba (EGb 761) (Kaur 2008, Le 2000, Smith 2004). The main mechanism of action of allopurinol is the inhibition of xantine oxidase - thus lowering the formation of reactive oxigen species - but it also directly scavenges free-radicals (George 2009). In the case of N acetyl cysteine, the main pathway for its antioxidant properties is the replenishment of glutathione (Dean 2009). In this fashion, antioxidants with different mechanisms have been studied in different progressive illnesses.

Why it is important to do this review

The available data on oxidative stress in bipolar disorder has increased exponentially in the past decade (Ng 2008). If antioxidants are to have a place in the treatment of this serious condition, the relevant and up-to-date information should be available to clinicians and investigators.

Objectives

Mood stabilizers are the cornerstone of the treatment of bipolar disorder, with recent guidelines recommending their use in every phase of this illness (Yatham 2009, NICE 2006, Goodwin 2009). As such, the overall objective of the review is to evaluate the impact of adding of antioxidant pharmacotherapy in comparison with placebo as add-on treatments to standard mood-stabilizing treatment for improving acute mood episodes and preventing relapse in people with bipolar disorder.

Methods

Criteria for considering studies for this review

Types of studies

Randomised controlled trials with at least a 4-week follow-up are to be included in this review. We will exclude quasi-randomised studies, such as those allocating by using alternate days of the week. Randomised cross-over studies will be eligible but only data up to the point of first cross-over.

As the level of blindness has not been definitely linked to bias (Petiti 2000), studies with any level of blinding will be eligible. Non-English language will not be an obstacle to inclusion.

Types of participants

Adults (18-74 years old) with a diagnosis of bipolar disorder (I, II or NOS), with a diagnosis approximating ICD-10 (or ICD-9) or DSM-IV (or DSM-III/IIIR) criteria. Patients can either be recruited during an acute episode (depressive, manic or mixed) or during euthymia. Acute and maintenance investigations are expected to form different study subsets, looking into improvement and relapse prevention, respectively. Patients may be provenient from inpatient, outpatient or primary care settings (or a combination of these). Studies specifically including patients with any psychiatric or medical comorbidities will be eligible.

Types of interventions

Antioxidants: any pharmacologically active substance explicitly administered with the purpose of redox modulation. This includes, for instance, n-acetyl cysteine, vitamin E, ginko biloba, vitamin C, allopurinol and other substances that primarily influence oxidative stress or antioxidant pathways. Typical doses and formulations can be seen in the supplementary table (Table 1).

As mood-stabilizers are interventions with a large evidence-base of efficacy, we will to include only add-on studies. In these, participants already using treatment are randomised to an antioxidant or placebo in addition to their previous treatment. Alternatively, for maintenance studies they could be randomised to stay on the antioxidant or have it substituted for a placebo (i.e. an “enriched” design).

Types of outcome measures

Outcomes will be grouped into short term (up to 12 weeks), medium term (13-26 weeks) and long term (over 26 weeks).

Primary outcomes

For acute studies

Global state: clinically important response or remission as defined by the individual studies - for example a 50% reduction on a rating scale (for example the Montgomery-Asberg Depression Rating Scale (Montgomery 1979) or the Young Mania Rating Scale (Young 1978)). Cut-offs using empirically validated criteria will be given preference (Berk 2008a).

Change in a general functioning instrument (for instance, the Global Assessment of Functioning (Patterson 1995), the Functioning Assessment Short Test (Cacilhas 2009) or the Range of Impaired Functioning Tool (Leon 1999)).

Leaving the studies early (any reason, adverse events, inefficacy of treatment, manic switch).

For maintenance studies

Relapse rates or time to relapse.

Leaving the studies early (any reason, adverse events, inefficacy of treatment, manic switch).

Secondary outcomes

1. Mental state (with particular reference to depressive and manic symptoms).

2. Remission rates from acute episode. Recent guidelines have been published defining those terms for those with bipolar disorder (Tohen 2009). Response can be defined as a 50% or more decrease in commonly used rating scales. For remission, narrower cut-off scores are employed, such as a MADRS and YMRS scores of less than 8.

2.1. Average endpoint general mental state score.

2.2. Average change in general mental state score.

3. Change in general quality of life (using, for instance, the World Health Organization Quality of Life Assessment (New Reference) or the Quality of Life Enjoyment and Satisfaction Questionnaire (Endicott 1993)).

4. Change in overall cognitive functioning.

5. Number of participants hospitalised (in maintenance studies).

6. Adverse effects:

6.1. Number of participants with at least one adverse effect.

6.2. Number of participants with at least one serious adverse event.

6.3 Death, including suicide.

6.4 Clinically important specific adverse effects (cardiac effects, movement disorders, sedation, seizures, weight gain, effects on white blood cell count).

7. Clinical effects on comorbid medical conditions (e.g. change in blood pressure, weight, insulin resistance, waist circumference).

8. Laboratory data:

8.1 Change in tests of oxidative stress or antioxidant defences / potential.

Search methods for identification of studies

Trial Registers (CCDANCTR)

The Cochrane Depression, Anxiety and Neurosis Group (CCDAN) maintain two clinical trials registers at their editorial base in Bristol, UK: a references register and a studies-based register.

The CCDANCTR-References Register contains over 24,500 reports of trials in depression, anxiety and neurosis. Approximately 70% of these references have been tagged to individual, coded trials. The coded trials are held in the CCDANCTR-Studies Register and records are linked between the two registers through the use of unique Study ID tags. Coding of trials is based on the EU-Psi coding manual. Please contact the CCDAN Trials Search Coordinator for further details.

Reports of trials for inclusion in the Group's registers are collated from routine (weekly), generic searches of MEDLINE, EMBASE and PsycINFO; quarterly searches of the Cochrane Central Register of Controlled Trials (CENTRAL) and review specific searches of additional databases. Reports of trials are also sourced from international trials registers c/o the World Health Organisation's trials portal (ICTRP) (http://apps.who.int/trialsearch/), drug companies, the hand-searching of key journals, conference proceedings and other (non-Cochrane) systematic reviews and meta-analyses.

Details of CCDAN's generic search strategies can be found in the 'Specialized Register' section of the Cochrane Depression, Anxiety and Neurosis Group's module text.

Electronic searches

The CCDAN trial registers will be searched using terms for bipolar disorder and the following terms for antioxidants: 'vitamin C', 'ascorbic acid', 'vitamin E', 'alpha-tocopherol', 'selegiline', 'pramipexole', 'beta-carotene', 'tocopherol', 'deprenyl', 'n-acetyl cysteine', 'n-acetyl-l-cysteine', 'n-acetylcysteine', 'acetylcysteine', 'thioredoxin', 'glutathione', 'conzyme Q10', 'glutathione peroxidase', 'GPx', 'glutathione reductase', glutathione S transferase', 'GST, 'catalase', 'superoxide dismutase', 'SOD', 'dehydroepiandrosterone', 'ginko biloba', 'allopurinol', 'selenium', 'melatonin', and the generic terms 'oxidative', 'redox' and 'antioxidant*'.

Searching other resources

Reference searching

The reference lists of all retrieved articles, previous reviews and major text books of bipolar disorder will be examined for additional trials.

Personal contact

The authors of significant papers, as well as other experts in the field, will be contacted and asked for their knowledge of further studies, published or unpublished, relevant to the review.

Data collection and analysis

Selection of studies

Two of the review authors (PVM and MB) will independently inspect the identified citations. Potentially relevant reports will be ordered as full papers for assessment. Retrieved articles will again be assessed by the two review authors for inclusion

according to the previously defined inclusion criteria. Disagreements will be resolved by consensus with the other authors.

Data extraction and management

Extraction

Two of the authors (PVM and ACA) will independently extract data from the included studies.

Management

Data will be extracted onto standard, simple forms.

Scale-derived data

We will include continuous data from rating scales only when the psychometric properties of the measuring instrument had been described in a peer-reviewed journal.

Assessment of risk of bias in included studies

Two review authors (PM and OD) will independently assess risk of bias in accordance with the Cochrane Collaboration's tools for assessing quality and risk of bias (Higgins 2008a). This tool encourages consideration of how the sequence was generated, how allocation was concealed, the integrity of blinding, the completeness of outcome data, selective reporting and other biases.

The risk of bias in each domain and overall will be categorised into:

A. Low risk of bias: plausible bias unlikely to seriously alter the results (categorised as 'Yes' in Risk of Bias table)

B. High risk of bias: plausible bias that seriously weakens confidence in the results (categorised as 'No' in Risk of Bias table)

C. Unclear risk of bias: plausible bias that raises some doubt about the results (categorised as 'Unclear' in Risk of Bias table)

We will not include trials with high risk of bias (defined as at least 3 out of 5 domains were categorised as 'No') in the meta-analysis.

Where inadequate details of randomisation or other trial characteristics are provided, authors of the studies will be contacted.

Measures of treatment effect

1. Dichotomous data

When possible, dichotomous data will be used. We will use relative risks to summarize binary data, since they may be easier to interpret (Freemantle 1999).This could be done by identifying cutoff points on rating scales and dividing participants accordingly into 'clinically improved' or 'not clinically improved'. If data based on these thresholds are not available, we will use the primary cut-off presented by the original authors.

We will calculate the relative risk (RR) and its 95% confidence interval (CI) based on the random-effects model, as this takes into account any differences between studies even if there is no statistically significant heterogeneity. When results are significant we will provide number needed to treat or the number needed to harm (NNT or NNH, respectively).

Although the choice of model for meta-analysis remains controversial (Freemantle 1999), a random effects model (DerSimonian 1986), which assumes that studies analysed actually comes from pool of hypothetical studies, will be used in this meta-analysis.

2. Continuous data

2.1 Summary statistic

For continuous outcomes we will estimate a Mean Difference (MD) between groups when the same measurement scale is reported from original studies (or where necessary, a Standardised Mean Difference (SMD)). Summary effects are to be based on the random-effects model.

2.2 Endpoint versus change data

Where available, endpoint data will be used. If endpoint data are not available, we will use change scores.

2.3 Skewed data

Continuous data on clinical and social outcomes are often not normally distributed. To avoid the pitfall of applying parametric tests to non-parametric data, we will apply the following standards to all data before inclusion:

(a) standard deviations and means are reported in the paper or obtainable from the authors; (b) when a scale starts from the finite number zero, the standard deviation, when multiplied by two, is less than the mean (as otherwise the mean is unlikely to be an appropriate measure of the centre of the distribution; (c) if a scale starts from a positive value (such as PANSS which can have values from 30 to 210) the calculation described above will be modified to take the scale starting point into

account. In these cases skew is present if 2SD>(S-S min), where S is the mean score and S min is the minimum score. Endpoint scores on scales often have a finite start and end point and these rules can be applied. When continuous data are presented on a scale which includes a possibility of negative values (such as change data), it is difficult to tell whether data are skewed or not. Sensitivity analyses will be performed to verify the effect of inclusion of skewed data.

2.4 Data synthesis

If standard errors instead of standard deviations are presented, the former will be converted to standard deviations. If standard deviations are not reported and can not be calculated from available data, authors will be asked to supply the data.

2.5 Multiple doses

If a study investigating a number of fixed doses of an antioxidant is to be included, we will use the method described in section 16.5 of the Cochrane Handbook (Higgins 2008) to combine data from multiple groups.

Unit of analysis issues

Where a study involves more than two treatment groups, if relevant, the additional treatment groups will be presented in additional relevant comparisons using methods described in section 16.5 of the Cochrane Handbook (Higgins 2008) to avoid unit-of-analysis errors. Should any relevant cross-over studies be identified, we will only use data of the first randomised phase.

Dealing with missing data

1. Overall loss of credibility

Should more than 50% of data be unaccounted for we will not reproduce these data or use them within analyses. Outcomes can be influenced by excessive or differential drop-out, and since there is no ideal way of dealing with this problem, caution is called for in these situations.

2. Binary

In the case where attrition for a binary outcome is between 0 and 50% and outcomes of these people are described, we will include these data as reported. if the data are not clearly described, data will be presented on a 'once-randomised always-analyse' basis, assuming an intention to treat analysis. Those lost to follow-up will be assumed to have a negative outcome, with the exception of the outcome of death.

3. Continuous

Intention-to-treat (ITT) data will be used when available. In the case where attrition for a continuous outcome is between 0 and 50% and completer-only data are reported, we will reproduce these. However, a sensitivity analysis will be conducted where completer data are excluded.

Assessment of heterogeneity

1. Clinical heterogeneity

We will consider all included studies to judge clinical heterogeneity.

2. Statistical heterogeneity

2.1 Visual inspection.

We will inspect the graphs to investigate the possibility of statistical heterogeneity.

2.2 Employing the I-squared statistic

This will be employed to estimate the percentage of inconsistency thought to be due to chance. When heterogeneity exists without a plausible explanation, the quality of evidence decreases, and the conclusions have to be adjusted (Higgins 2008).

Assessment of reporting biases

Reporting biases arise when the dissemination of research findings is influenced by the nature and direction of results. These are described in section 10.1 of the Cochrane Handbook (Higgins 2008). We are aware that funnel plots may be useful in investigating small-study effects but are of limited power to detect such effects when there are few studies. Therefore, they will be employed when at least 10 studies are available for a particular outcome.

Data synthesis

Where possible we will employ a random-effects model for analyses. We understand that there is no closed argument for preference for use of fixed or random-effects models. The random-effects method incorporates an assumption that the different studies are estimating different, yet related, intervention effects.

Subgroup analysis and investigation of heterogeneity

In the case of clearly heterogeneous data, we will check that data are correctly extracted and entered and that we there are no unit-of-analysis errors. We will not

undertake meta-analysis If the high levels of heterogeneity remain (i.e., an I2 statistic over 50% (Higgins 2008)).

Planned subgroup analyses include:

Specific antioxidant used

Bipolar disorder subtype (I or II)

Recruitment setting (inpatients, outpatients, primary care)

Sensitivity analysis

Sensitivity analysis, as mentioned above, will be conducted regarding study quality (including allocation concealment and blinding), handling of missing observations in the original study, outcomes with included studies with skewed data and statistical model for the synthesis.

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Antioxidant treatments for schizophrenia

Background

Description of the condition

Schizophrenia is a severely debilitating and progressive illness. Although not especially highly prevalent, it carries a disproportionate share of illness related disability, partially due to its early age at onset, associated impact in functioning and chronic course. This effect is even more devastating as the illness tends to be deteriorating, with increased disability and personal and societal burden (Berk 2009, Lieberman 2006). Furthermore, only a minority of this burden is currently averted with standard treatment (Rossler 2005).

There is accumulating evidence that progressive brain changes take place as the disease unfolds (DeLisi 2008). Among many possible candidates, oxidative stress may be one of the mediators of neuroprogression, grey matter loss and subsequent cognitive and functional impairment (Lieberman 2006, Wood 2009, Dean 2009). Specifically, oxidative imbalance has been evidenced by the increased levels of 8-OH deoxyguanosine, (an indicator of DNA damage and potentially of apoptotic events), protein carbonylation (leading to cellular dysfunction) and lipid peroxidation (potentially leading to alterations in membrane structure and permeability) shown in individuals with schizophrenia. Oxidative defences have also been shown to be impaired, including decreased glutathione (the primary antioxidant in the brain) levels, polymorphisms in gene pathways associated with oxidative defence and changes in other antioxidants including superoxide, dismutase, catalase and glutathione peroxidase (Wood 2009).

Description of the intervention

Antioxidants are exogenous or endogenous molecules that mitigate any form of oxidative stress or its consequences. They may act from directly scavenging free radicals to increasing anti-oxidative defences (Uttara 2009). In this fashion, antioxidants with different mechanisms have been studied in different progressive illnesses (Berk 2009).

There is evidence that current treatments impact oxidative pathways and may to some extent reverse pro-oxidative states in schizophrenia. Indeed, second generation antipsychotics have been shown to have effects in neuroprotection. The existing literature, however, indicates that these treatments do not fully restore the deficits in antioxidant levels or restore levels of oxidants in schizophrenia (Lieberman 2005, Padurariu 2010, Wang 2008).

How the intervention might work

Oxidative stress occurs when there is an overproduction of free radicals or a deficiency in antioxidant defences (Wood 2009). This has had theoretical appeal to neurodegenerative disorders, since the brain is considered particularly vulnerable to oxidative damage. This process has been implicated in many psychiatric disorders, and most robust evidence of its importance comes from studies of schizophrenia (Ng 2008). The underlying mechanisms underpinning the process of disease neuroprogression and subsequent brain changes is incompletely understood. There is, however, some evidence pointing to central and peripheral pro-oxidative changes, including lower oxidative defences and oxidative damage to proteins, lipids and DNA (Wood 2009).

As such, there has been interest in developing interventions aimed at restoring this oxidative balance beyond the benefits of antipsychotics in this direction (Dean 2009). Some work has been done investigating the modulation of antioxidants as a therapeutic target for the treatment of schizophrenia. Similarly, mechanisms of action are believed to vary between compounds. Omega-3 for example, is proposed to have some direct antioxidant properties, but works primarily by protecting against oxidative attack by reinforcing lipid membranes and lipid-associated structures (such as myelin). Alternatively, N-acetyl cysteine is believed to act predominantly on the glutathione pathway and may also modulate glutamate function (Dean 2009).

Why it is important to do this review

The investigation on oxidative stress in schizophrenia has increased exponentially in the past decade (Ng 2008). If antioxidants are to have a place in the treatment of this serious condition, the relevant and up-to-date information should be available to clinicians and investigators.

Objectives

The overall objective of the review is to evaluate the effect of antioxidants as add-on treatments to standard antipsychotic medication for improving acute psychotic episodes and core symptoms and preventing relapse in people with schizophrenia.

Methods

Criteria for considering studies for this review

Types of studies

All relevant randomised trials. We will exclude quasi-randomised studies, such as those allocating by using alternate days of the week. As the level of blindness has not been definitely linked to bias (Petiti 2000), studies with any level of blinding will be eligible. Non-English language will not be an obstacle to inclusion.

Types of participants

Adults (18+ years) with schizophrenia or other types of schizophrenia-like psychosis (e.g. schizophreniform and schizoaffective disorders), irrespective of diagnostic criteria used. There is no clear evidence that the schizophrenia-like psychoses are caused by fundamentally different disease processes or require different treatment approaches (Carpenter 1994).

We are interested in making sure that information is as relevant to the current care of people with schizophrenia as possible so propose to clearly highlight the current clinical state (acute, early post-acute, partial remission, remission) as well as the stage (prodromal, first episode, early illness, persistent) and as to whether the studies primarily focused on people with particular problems (for example, negative symptoms, treatment-resistant illnesses).

Types of interventions

1. Antioxidants

Any pharmacologically active substance explicitly administered with the purpose of antioxidation.

2. Placebo

As antipsychotics are interventions with a large evidence-base of efficacy, we expect to include only add-on studies. In these, participants already using a first line agent are randomised to an antioxidant or placebo in addition to their previous treatment. Although we will require that participants are on antipsychotics, more 'naturalistic'

studies including those on 'poly-therapy' will be included, provided participants are randomised to placebo or an antioxidant. Alternatively, for maintenance studies they could be randomised to stay on the antioxidant or have it substituted for a placebo.

Types of outcome measures

Outcomes will be grouped into immediate (4 weeks or less), short term (4-12 weeks), medium term (13-26 weeks) and long term (over 26 weeks).

Primary outcomes

1. Global state

1.1 No clinically important response as defined by the individual studies

For example, global impression less than much improved or less than 50% reduction on a rating scale.

Secondary outcomes

1. Leaving the studies early

1.1 Any reason, adverse events, or inefficacy of treatment

2. Global state

2.1 No clinically important change in global state (as defined by individual studies)

2.2 Relapse (as defined by the individual studies)

3. Mental state

3.1 No clinically important change in general mental state score

3.2 Average endpoint general mental state score

3.3 Average change in general mental state scores

3.4 No clinically important change in specific symptoms (positive symptoms of schizophrenia, negative symptoms of schizophrenia)

3.5 Average endpoint specific symptom score

3.6 Average change in specific symptom scores

4. General functioning

4.1 No clinically important change in general functioning

4.2 Average endpoint general functioning score

4.3 Average change in general functioning scores

5. Quality of life/satisfaction with treatment

5.1 No clinically important change in general quality of life

5.2 Average endpoint general quality of life score

5.3 Average change in general quality of life scores

6. Cognitive functioning

6.1 No clinically important change in overall cognitive functioning

6.2 Average endpoint of overall cognitive functioning score

6.3 Average change of overall cognitive functioning scores

7. Service use

7.1 Number of participants hospitalised

7.2 Duration of hospitalisation

8. Adverse effects

8.1 Number of participants with at least one adverse effect

8.2 Clinically important specific adverse effects (cardiac effects, death, movement disorders, probating increase and associated effects, sedation, seizures, weight gain, effects on white blood cell count)

8.3 Average endpoint in specific adverse effects

8.4 Average change in specific adverse effects

9. Laboratory data

9.1 Change in tests of oxidative stress

9.2 Change in tests of antioxidant defences / potential

Search methods for identification of studies

No language restriction is to be applied.

Electronic searches

1. Cochrane Schizophrenia Group Trials Register (November 2010)

We will search the register using the phrase:

[(*vitamin C* OR *ascorbic acid* OR *vitamin E* OR *alpha-tocopherol* OR *selegiline* OR *deprenyl* OR *n-acetyl cysteine* OR *n-acetyl-l-cysteine* OR *n-acetylcysteine* OR *acetylcysteine* OR *superoxide dismutase* OR *SOD * OR *dehydroepiandrosterone* OR *antioxidant*) in title, abstract and index terms of REFERENCE and Intervention of STUDY]

This register is compiled by systematic searches of major databases, hand searches and conference proceedings (see Group Module).

Searching other resources

1. Reference searching

The reference lists of all retrieved articles, previous reviews and major text books of schizophrenia will be examined for additional trials.

2. Personal contact

The authors of significant papers, as well as other experts in the field, will be contacted and asked for their knowledge of further studies, published or unpublished, relevant to the review.

Data collection and analysis

Selection of studies

PVM and ACA will independently inspect citations from the searches and identify relevant abstracts. A random 20% sample will be independently re-inspected by MB and FK to ensure reliability. Where disputes arise, the full report will be acquired for more detailed scrutiny. Full reports of the abstracts meeting the review criteria will be obtained and inspected by PVM and ACA. Again, a random 20% of reports will be re-inspected by MB and FK in order to ensure reliable selection. Where it is not possible to resolve disagreement by discussion, we will attempt to contact the authors of the study for clarification.

Data extraction and management

1. Extraction

Reviewer PVM will extract data from all included studies. In addition, to ensure reliability, ACA will independently extract data from a random sample of these studies, comprising 10% of the total. Again, any disagreement will be discussed, decisions documented and, if necessary, authors of studies will be contacted for clarification. With remaining problems MB will help clarify issues and these final decisions will be documented. Data presented only in graphs and figures will be extracted whenever possible, but included only if two reviewers independently have the same result. Attempts will be made to contact authors through an open-ended request in order to obtain missing information or for clarification whenever necessary. If studies are multi-centre, where possible, we will extract data relevant to each component centre separately.

2. Management

2.1 Forms

Data will be extracted onto standard, simple forms.

2.2 Scale-derived data

We will include continuous data from rating scales only if:a. the psychometric properties of the measuring instrument have been described in a peer-reviewed journal (Marshall 2000); andb. the measuring instrument has not been written or modified by one of the trialists for that particular trial.

Ideally the measuring instrument should either be i. a self-report or ii. completed by an independent rater or relative (not the therapist). We realise that this is not often reported clearly, in Description of studies we will note if this is the case or not.

2.3 Endpoint versus change data

There are advantages of both endpoint and change data. Change data can remove a component of between-person variability from the analysis. On the other hand calculation of change needs two assessments (baseline and endpoint) which can be difficult in unstable and difficult to measure conditions such as schizophrenia. We have decided to primarily use endpoint data, and only use change data if the former are not available. Endpoint and change data will be combined in the analysis as we will use weighted mean differences (MD) rather than standardised mean differences throughout (Higgins 2009, Chapter 9.4.5.2).

2.4 Skewed data

Continuous data on clinical and social outcomes are often not normally distributed. To avoid the pitfall of applying parametric tests to non-parametric data, we aim to apply the following standards to all data before inclusion: a) standard deviations and means are reported in the paper or obtainable from the authors; b) when a scale starts from the finite number zero, the standard deviation, when multiplied by two, is less than the mean (as otherwise the mean is unlikely to be an appropriate measure

of the centre of the distribution, (Altman 1996); c) if a scale started from a positive value (such as PANSS which can have values from 30 to 210) the calculation described above was modified to take the scale starting point into account. In these cases skew is present if 2SD>(S-S min), where S is the mean score and S min is the minimum score. Endpoint scores on scales often have a finite start and end point and these rules can be applied. When continuous data are presented on a scale that includes a possibility of negative values (such as change data), it is difficult to tell whether data are skewed or not. Skewed data from studies of less than 200 participants will be entered in additional tables rather than into an analysis. Skewed data pose less of a problem when looking at means if the sample size is large and will be entered into syntheses.

2.5 Common measure

To facilitate comparison between trials, we intend to convert variables that can be reported in different metrics, such as days in hospital (mean days per year, per week or per month) to a common metric (e.g. mean days per month).

2.6 Conversion of continuous to binary

Where possible, efforts will be made to convert outcome measures to dichotomous data. This can be done by identifying cut-off points on rating scales and dividing participants accordingly into 'clinically improved' or 'not clinically improved'. It is generally assumed that if there is a 50% reduction in a scale-derived score such as the Brief Psychiatric Rating Scale (BPRS, Overall 1962) or the Positive and Negative Syndrome Scale (PANSS, Kay 1986), this could be considered as a clinically significant response (Leucht 2005, Leucht 2005a). If data based on these thresholds are not available, we will use the primary cut-off presented by the original authors.

2.7 Direction of graphs

Where possible, we will enter data in such a way that the area to the left of the line of no effect indicates a favourable outcome for social skills training. Where keeping to this makes it impossible to avoid outcome titles with clumsy double-negatives (e.g. 'Not improved') we will report data where the left of the line indicates an unfavourable outcome. This will be noted in the relevant graphs.

2.8 Multiple doses

If a study investigating a number of fixed doses of an antioxidant is to be included, we will use the method described in section 16.5 of the Cochrane Handbook (Higgins 2009) to combine data from multiple groups.

2.9 Summary of findings table

We anticipate including the following short or medium term outcomes in a summary of findings table.

1. Global state

Clinically significant response - as defined by each of the studiesRelapse

2. Mental state

Clinically significant response in mental state - as defined by each of the studies

3. Service utilisation outcome

Hospital admissionDays in hospital

4. Adverse effect

Any important adverse event

5. Quality of life

Improved to an important extent

Assessment of risk of bias in included studies

Again PM and ACA will work independently to assess risk of bias by using criteria described in the Cochrane Collaboration Handbook (Higgins 2009) to assess trial quality. This set of criteria is based on evidence of associations between overestimate of effect and high risk of bias of the article such as sequence generation, allocation concealment, blinding, incomplete outcome data and selective reporting.

If the raters disagree, the final rating will be made by consensus, with the involvement of another member of the review group. Where inadequate details of randomisation and other characteristics of trials are provided, authors of the studies will be contacted in order to obtain further information. Non-concurrence in quality assessment will be reported, but if disputes arise as to which category a trial is to be allocated, again, resolution will be made by discussion.

The level of risk of bias will be noted in both the text of the review and in the Summary of findings table 1.

Measures of treatment effect

1. Binary data

For binary outcomes we will calculate a standard estimation of the risk ratio (RR) and its 95% confidence interval (CI). It has been shown that RR is more intuitive (Freemantle 1999) than odds ratios and that odds ratios tend to be interpreted as RR by clinicians (Deeks 2000). For statistically significant results we had planned to calculate the number needed to treat to provide benefit /to induce harm statistic (NNTB/H), and its 95% confidence interval (CI) using Visual Rx (http://www.nntonline.net/) taking account of the event rate in the control group. This,

however, has been superseded by Summary of findings table 1 and calculations therein.

2. Continuous data

For continuous outcomes will estimate mean difference (MD) between groups. We prefer not to calculate effect size measures (standardised mean difference SMD). However, if scales of very considerable similarity are used, we would have presumed there was a small difference in measurement, and we would have calculated effect size and transformed the effect back to the units of one or more of the specific instruments.

Unit of analysis issues

1. Cluster trials

Studies increasingly employ 'cluster randomisation' (such as randomisation by clinician or practice) but analysis and pooling of clustered data poses problems. Firstly, authors often fail to account for intra-class correlation in clustered studies, leading to a 'unit of analysis' error (Divine 1992) whereby p values are spuriously low, confidence intervals unduly narrow and statistical significance overestimated. This causes type I errors (Bland 1997, Gulliford 1999).

Where clustering is not accounted for in primary studies, we will present data in a table, with a (*) symbol to indicate the presence of a probable unit of analysis error. In subsequent versions of this review we will seek to contact first authors of studies to obtain intra-class correlation coefficients for their clustered data and to adjust for this by using accepted methods (Gulliford 1999). Where clustering has been incorporated into the analysis of primary studies, we will present these data as if from a non-cluster randomised study, but adjust for the clustering effect.

We have sought statistical advice and have been advised that the binary data as presented in a report should be divided by a 'design effect'. This is calculated using the mean number of participants per cluster (m) and the intra-class correlation coefficient (ICC) [Design effect = 1+(m-1)*ICC] (Donner 2002). If the ICC is not reported it will be assumed to be 0.1 (Ukoumunne 1999).

If cluster studies have been appropriately analysed taking into account intra-class correlation coefficients and relevant data documented in the report, synthesis with other studies would have been possible using the generic inverse variance technique.

2. Cross-over trials

Randomised cross-over studies will be eligible but only data up to the point of first cross-over. A major concern of cross-over trials is the carry-over effect. It occurs if an effect (e.g. pharmacological, physiological or psychological) of the treatment in the first phase is carried over to the second phase. As a consequence on entry to the second phase the participants can differ systematically from their initial state despite a wash-out phase. For the same reason cross-over trials are not appropriate if the condition of interest is unstable (Elbourne 2002). As both effects are very likely in severe mental illness, we will only use data of the first phase of cross-over studies.

3. Studies with multiple treatment groups

Where a study involves more than two treatment arms, if relevant, the additional treatment arms will be presented in comparisons. If data are binary these will be simply added and combined within the two-by-two table. If data are continuous we will combine data following the formula in section 7.7.3.8 (Combining groups) of the Cochrane Handbook. Where the additional treatment arms are not relevant, these data will not be reproduced.

Dealing with missing data

1. Overall loss of credibility

At some degree of loss of follow-up data must lose credibility (Xia 2009). We choose that, for any particular outcome, should more than 40% of data be unaccounted for, we will not reproduce these data or use them within analyses. If, however, more than 40% of those in one arm of a study are lost, but the total loss was less than 40%, we will mark such data with (*) to indicate that such a result may well be prone to bias.

2. Binary

In the case where attrition for a binary outcome is between 0 and 40% and where these data are not clearly described, data will be presented on a 'once-randomised-always-analyse' basis (an intention to treat analysis). Those leaving the study early are all assumed to have the same rates of negative outcome as those who completed, with the exception of the outcome of death and adverse effects. For these outcomes the rate of those who stayed in the study - in that particular arm of the trial - will be used for those who did not. A sensitivity analysis will be undertaken testing how prone the primary outcomes are to change when 'completer' data only are compared to the intention to treat analysis using the above assumptions.

3. Continuous

3.1 Attrition

In the case where attrition for a continuous outcome is between 0 and 40% and completer-only data will be reported, we will reproduce these.

3.2 Standard deviations

If standard deviations are not reported, we will first try to obtain the missing values from the authors. If not available, where there are missing measures of variance for continuous data, but an exact standard error and confidence intervals available for

group means, and either 'p' value or 't' value available for differences in mean, we can calculate them according to the rules described in the Cochrane handbook (Higgins 2009): When only the standard error (SE) is reported, standard deviations (SDs) are calculated by the formula SD = SE * square root (n). Chapters 7.7.3 and 16.1.3 of the Cochrane handbook (Higgins 2009) present detailed formula for estimating SDs from p-values, t or F values, confidence intervals, ranges or other statistics. If these formula do not apply, we will calculate the SDs according to a validated imputation method which is based on the SDs of the other included studies (Furukawa 2006). Although some of these imputation strategies can introduce error, the alternative would be to exclude a given study's outcome and thus to lose information. We nevertheless will examine the validity of the imputations in a sensitivity analysis excluding imputed values.

3.3 Last observation carried forward

We anticipate that in some studies the method of last observation carried forward (LOCF) will be employed within study reports. As with all methods of imputation to deal with missing data, LOCF introduces uncertainty about the reliability of the results (Leucht 2007). Therefore, where LOCF data have been used in the trial, if less than 50% of the data have been assumed, we will reproduce these data and indicate that they are the product of LOCF assumptions.

Assessment of heterogeneity

1. Clinical heterogeneity

We will consider all included studies initially, without seeing comparison data, to judge clinical heterogeneity. We will simply inspect all studies for clearly outlying people or situations which we had not predicted would arise. When such situations or participant groups arise, these will be fully discussed.

2. Methodological heterogeneity

We will consider all included studies initially, without seeing comparison data, to judge methodological heterogeneity. We will simply inspect all studies for clearly outlying methods which we had not predicted would arise. When such methodological outliers arise these will be fully discussed.

3. Statistical heterogeneity

3.1 Visual inspection

We will visually inspect graphs to investigate the possibility of statistical heterogeneity.

3.2 Employing the I2 statistic

Heterogeneity between studies will be investigated by considering the I2 method alongside the Chi2 'p' value. The I2 provides an estimate of the percentage of inconsistency thought to be due to chance (Higgins 2003). The importance of the observed value of I2 depends on i. magnitude and direction of effects and ii. strength of evidence for heterogeneity (e.g. 'p' value from Chi2 test, or a confidence interval for I2). I2 estimate greater than or equal to around 50% accompanied by a statistically significant Chi2 statistic, will be interpreted as evidence of substantial levels of heterogeneity (Section 9.5.2 - Higgins 2009). When substantial levels of heterogeneity are found in the primary outcome, we will explore reasons for heterogeneity (Subgroup analysis and investigation of heterogeneity).

Assessment of reporting biases

Reporting biases arise when the dissemination of research findings is influenced by the nature and direction of results (Egger 1997). These are described in Section 10 of the Cochrane Handbook for Systematic Reviews of Interventions (Higgins 2009).

We are aware that funnel plots may be useful in investigating reporting biases but are of limited power to detect small-study effects. We will not use funnel plots for outcomes where there are ten or fewer studies, or where all studies are of similar sizes. In other cases, where funnel plots are possible, we will seek statistical advice in their interpretation.

Data synthesis

Although the choice of model for meta-analysis remains controversial (Freemantle 1999), a random effects model (DerSimonian 1986), which assumes that studies analysed actually comes from pool of hypothetical studies, will be used in this meta-analysis. We understand that there is no closed argument for preference for use of fixed or random-effects models. The random-effects method incorporates an assumption that different studies are estimating different, yet related, intervention effects. The random-effects methods does put added weight onto the smaller of the studies - those that may be most prone to bias. We nevertheless favour using a random-effects model.

Subgroup analysis and investigation of heterogeneity

1. Subgroup

Subgroup analyses will be conducted regarding the specific antioxidant used - for primary outcomes.

We are interested in making sure that information is as relevant to the current care of people with schizophrenia and broad clinical groups (see Types of participants). We want to be able to present these data clearly but current clinical state, stage and for people with particular problems.

2. Investigation of heterogeneity

2.1 Unanticipated heterogeneity

If inconsistency is high, this will be reported. First we will investigate whether data has been entered correctly. Second, if data is correct, the graph will be visually inspected and studies outside of the company of the rest will be successively removed to see if heterogeneity is restored. For this review we have decided that should this occur with data contributing to the summary finding of no more than around 10% of the total weighting, data will be presented. If not, data are not pooled and issues will be discussed. We know of no supporting research for this 10% cut off but are investigating use of prediction intervals as an alternative to this unsatisfactory state.

When unanticipated clinical or methodological heterogeneity are obvious we will simply state hypotheses regarding these for future reviews or versions of this review. We do not anticipate undertaking analyses relating to these.

2.2 Anticipated heterogeneity

We do not anticipate important levels of heterogeneity.

Sensitivity analysis

1. Quality

We aim to include trials in a sensitivity analysis if they are described in some way as to imply randomisation. For the primary outcomes we will include these studies and if there is no substantive difference when the implied randomised studies are added to those with better description of randomisation, then all data will be employed from these studies.

2. Handling of missing observations

Where assumptions have to be made regarding people lost to follow-up (see Dealing with missing data) we will compare the findings of the primary outcomes when we use our assumption compared with completer data only. If there is a substantial difference, we will report results and discuss them but continue to employ our assumption.

Where assumptions have to be made regarding missing SDs data (see Dealing with missing data), we will compare the findings on primary outcomes when we use our assumption compared with completer data only. A sensitivity analysis will be undertaken testing how prone results are to change when 'completer' data only are compared to the imputed data using the above assumption. If there is a substantial difference, we will report results and discuss them but continue to employ our assumption.

3. Statistical model for the synthesis

For the primary outcome we will check if using a fixed model substantively changes the final result.

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ANEXO E - Produção intelectual durante o doutorado

Artigos completos publicados em periódicos

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