# Desenvolupament i validació d'un model d'estadificació en el trastorn bipolar: variables clíniques, funcionals i biomarcadors perifèrics

Iria Grande i Fullana

Me arrastro con dos piernas izquierdas,

cada vez que el sol clava sus cristales.

Los versos caen al precipicio,

inundando de mar salada

páginas engullidas de tristeza.

No levanto cabeza de plumas,

que secaron su tinta con amarguras.

Mueren inválidos los poemas, o…

Ni tan sólo alumbran su existencia.

Hoy no puedo despertar ningún sueño.

Quiero que venga la guadaña de noche

a buscarme al País del Olvido,

inundando el latido con brindis de ron,

o hipnotizarme con rayas blancas.

He perdido los péndulos del tiempo

y no sé quién soy.

Mañana quizás resucite Dios de Dioses

que ayer ya invité a la Muerte.

UNIVERSITAT DE BARCELONA

IRIA GRANDE

DESENVOLUPAMENT I VALIDACIÓ D'UN MODEL D'ESTADIFICACIÓ EN EL TRASTORN BIPOLAR

# DESENVOLUPAMENT I VALIDACIÓ D'UN MODEL D'ESTADIFICACIÓ EN EL TRASTORN BIPOLAR: VARIABLES CLÍNIQUES, FUNCIONALS I BIOMARCADORS PERIFÈRICS

Tesi doctoral presentada per:

**IRIA GRANDE I FULLANA**

per a l'obtenció del grau de Doctora per la Universitat de Barcelona

*Director de tesi:* ***Prof. Eduard Vieta i Pascual***

*Codirector de tesi:* ***Prof. Flávio Kapczisnki***

*Programa de Doctorat en Medicina*

Barcelona, 2013

Levito con dos piernas derechas

cada orificio del amanecer.

El mundo está en mi pañuelo.

Soy As de corazones, musa de la luna,

viento de palabras.

Mi voz enamora al Universo

y amaestra los Ángeles.

Los ojos trasnochan todo el día,

iluminando pensamientos taumatúrgicos.

Escribo libros a los magos,

y me reclaman brebajes de vida eterna

los chamanes.

Nadie evapora mis huellas,

Sincronizan reverencias a los pasos…

Que soy el TODO PODEROSO.


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*Línia d'investigació:* Bases biològiques del trastorn psíquic i psiquiatria nuclear,

Servei de Psiquiatria de l'Hospital Clínic de Barcelona, Institut Clínic de Neurociències

Barcelona, 2013

### Introduction

Bipolar disorder (BD) is among the 10 most disabling conditions world-wide since several medical and psychiatric comorbidities as well as cognitive deficits are associated. Due to the weight of evidence suggesting the neuroprogressive evolution of the disorder, BD has been put forward as a systemic disorder and the applicability of a model of staging is under consideration. Factors such as cytokines (IL-6), oxidative stress and neurotrophic factors (BDNF) have been proposed to explain the evolution of the disorder. In this project, we aimed to study a staging model for BD based on clinical and functional variables as well as biological validators. Moreover, we studied the role of neurotrophins and their functional polymorphisms in the response to treatment in BD.

### Methods

In study 1, stable bipolar patients were matched for age and gender with first degree relatives of bipolar patients. Socio-demographic, clinical and functional data were collected. Cytokines, BDNF, biomarkers of oxidative stress were assessed. Cluster analysis was carried out to build a staging model and logistic regression was conducted to study any associations between the model and biomarkers.

In studies 2 and 3, bipolar patients who were medication-free at baseline and in an acute mood episode were recruited and matched with healthy controls. Clinical evaluation, serum BDNF and BDNF Val66Met polymorphism were assessed along the 16-week follow-up.

### Results

In the first group of patients, the staging model depicted an *early*- and a *late*-stage regarding functioning, number of episodes, age at onset of the disorder and time elapsed since the first episode. IL-6 was associated with this model.

In the second group of patients, the BDNF Val66Met polymorphism predicted changes in BDNF levels, and the response to treatment and the polarity of the episode were associated with variations in BDNF levels.

### Conclusions

Functional and clinical variables may determine a model of staging, defined with an early and late stage. IL-6 may be a biological validator.

BDNF levels vary along treatment according to response to treatment, the BDNF Val66Met polymorphism and polarity of the episode.

## Conclusions

1. The clinical and functional variables that may be helpful to determine a model of staging in bipolar disorder based on an early- and late stage are:
   a. Severity of functional impairment assessed by the FAST score
   b. Number of episodes
   c. Age at the onset of the disorder
   d. Time elapsed since the first episode

2. The inflammatory biomarker IL-6 has been determined to be related to a model of staging in bipolar disorder, displaying a progressive increase along the stages of the classification ranging from first-degree relatives of patients diagnosed with bipolar disorder without any psychiatric diagnosis, patients in an early stage to patients in a late stage.

3. The neurotrophins and the oxidative stress markers do not seem to define remarkable differences among the proposed stages in the model of staging in bipolar disorder.

4. Peripheral BDNF levels vary along the pharmacological treatment according to the response to treatment in patients diagnosed with bipolar disorder in an acute affective episode.

5. The BDNF Val66Met polymorphism can predict variations in the BDNF levels during the treatment in patients diagnosed with bipolar disorder in an acute affective episode.

6. The polarity of the episode seems to influence the slope of peripheral BDNF levels along the treatment in patients diagnosed with bipolar disorder in an acute affective episode.

Aquesta tesi doctoral ha estat desenvolupada en la Unitat de Trastorns Bipolars de l'Hospital Clínic de Barcelona que forma part de l'Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) i del Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM).

El present treball d'investigació ha estat realitzat gràcies a:

- *Contracte de formació en investigació Río-Hortega (CM12/00062) del Instituto de Salud Carlos III (Ministeri Espanyol d'Economia) i la financiació del Fondo Europeo de Desarrollo Regional (FEDER).*
- *Beca Premi Fi de Residència de l'Hospital Clínic, 2011.*
- *VI Beca de Rotación Externa para Residentes otorgades per la Fundación Española de Psiquiatría y Salud Mental i de la Fundación Astra Zeneca, 2010-2011.*

*Als meus dos Petits Prínceps*

*La veritat no és un fet que puguem descobrir, com no podem saber per endavant quines observacions són rellevants i quines no, tots els descobriments, tot allò que ens ajudi a comprendre millor, neix com a predicció del que pot ser. Aquesta imaginació predictiva és un acte creatiu de la ment, és un treball mental, una inspiració interior, no la conseqüència d'una investigació programada.*

Peter Medawar, *The Art of the Soluble*

*Es considera que l'obra d'art, literària, musical o plàstica, neix perfecte com Minerva del cervell de Júpiter, sense revelar el llarg i penós procés de la seva elaboració. Una altra diferència essencial entre el descobriment científic i l'obra d'art és que aquesta última és el resultat de l'activitat creativa d'un sol individu. És inconcebible modificar o ampliar una cantata de Bach, una pintura de Rafael o La Divina Comèdia de Dante. En canvi, el descobriment científic, tot i tenir origen en la feliç intuïció d'una persona, es converteix de seguida en una obra col·lectiva que profunditza i s'estén conforme es descobreixen i coneixen noves coses.*

Gunter Stent, *Paradoxes of progress*

## Agraïments

Els treballs que componen aquesta tesi doctoral comparteixen, com idea fonamental, aprofundir en el coneixement de les malalties psiquiàtriques i, en concret, en el trastorn bipolar per tal de poder ajudar a millorar el pronòstic d'aquells que les pateixen. Sense els pacients i les seves famílies, que han col·laborat en els estudis de forma activa, desinteressada i continuada, cap d'aquests projectes no s'hagués pogut dur a terme.

A l'Eduard per ser un gran mestre… en el divers ventall d'aspectes que ens mostra la vida.

Ao Flávio, quem me fez descobrir o maravilhoso, ideal mundo da pesquisa e a toda a turma brasileira pela sua hospitalidade, *bah!,...trilegal...*

Al Jose i l'Anabel… per tot, tot i tot que de forma desinteressada compartiu.

Al megacrack de les altures, Francesc, que no necessita del meu cognom per ser gran.

Al Goiko, el *papa* que ens ha vist créixer i ens ha ajudat en el camí.

Al Marc, el *germà gran* que ens ha aconsellat durant la sendera.

Al Juan, l'inestimable *company* de viatge durant aquests anys.

Al Toni, pel suport al llarg d'aquest temps.

A la Mar, per ses vicissituds viscudes tan fora com dins de "sa Roqueta".

A la Carla, la Mercè, "Estropajín" i la Maria, per donar, cadascuna de vosaltres, el vostre toc singular en la nostra comuna.

A l'Andrea (my Fisherman), l'Isa i l'Ale i la Dina, per donar un cop d'aire al soterrani.

A l'Imma, l'Esther i la Brisa, encara que estructuralment lluny, us tinc a prop i Núria, por nuestros momentos de lidiar con la correspondencia.

A la Donna, per les nostres confidències d'una hora.

A la Rosa, la *mamma* de tota la 12, 0.

A la colla "Lover", per tots els moments immemorables compartits que espero que siguin molts més.

A la Mire, per deixar-me l'oportunitat de tenir-te a prop.

A na Lida per iniciar-me en aquest camí.

Aquesta tesi no hagués estat possible sense la meva família. Gràcies *papas* per donar-me la millor educació possible, per fomentar el valor de l'esforç i el sentit crític, per encoratjar la iniciativa i ser inspiració del treball ben fet. A la super crack de la *buela* i sa padrina, i com no, a tota la *famiglia* de Mallorca, el *padrino, madrina* i el suport incondicional des de Figueres i Girona. Per últim, al Lluis, per tot allò que compartim i el teu sentit comú. Anhelo continuar aquest camí amb tu i, a partir d'ara, amb el nostre nou company de travessada.

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# 1. Abreviatures

## 1. Abreviatures

***BDNF (Brain-Derived Neurotrophic Factor):*** factor neurotròfic derivat del cervell

***CA:*** càrrega alostàtica

***CGI (Clinical Global Impression):*** escala d'impressió clínica global

***ELISA (Enzyme-Linked ImmunoSorbent Assay):*** assaig per immunoabsorció lligat a enzimes

***GAF (Global Assessment of Functioning):*** escala d'avaluació de funcionament global

***HDRS (Hamilton Depression Rating Scale):*** escala de valoració de Hamilton per a l'avaluació de la depressió

***IL:*** interleucina

***INF-□:*** interferó gamma

***Met:*** metionina

***PCC (Protein Carbonyl Content):*** contingut carbonil proteic

***PFC (PreFrontal Cortex):*** còrtex prefrontal

***Polimorfisme BDNF Met66Val:*** polimorfisme que consisteix en la substitució de la valina (Val) per metionina (Met) en la pro-regió del gen humà *BDNF*

***STEP-BD (Systematic Treatment Enhancement Program for Bipolar Disorder):*** Programa sistemàtic per a la millora del tractament en Trastorn Bipolar

***TB:*** trastorn bipolar

***TBARS (ThioBarbituric Acid Reactive Substances):*** substàncies reactives a l'àcid tiobarbitúric

***TNF-α (Tumor Necrosis Factor alfa ):*** factor de necrosi tumoral alfa

***Val:*** Valina

***YMRS (Young Mania Rating Scale):*** escala d'avaluació de Young per a l'avaluació de mania

# 2. Justificació de la tesi

## 2. Justificació de la tesi

Malgrat els ingents esforços realitzats en investigació psiquiàtrica per a la descoberta de diferents biomarcadors i classificacions amb finalitat diagnòstica i pronòstica, els resultats obtinguts encara disten dels aconseguits en altres especialitats mèdiques. Com exemples assentats en el món mèdic, trobaríem l'estudi de la troponina-I en el dolor toràcic en l'àmbit de la cardiologia o la classificació Child-Pugh en el camp de l'hepatologia.

Tres vies són les proposades en l'actualitat quant als mecanismes involucrats en el desencadenament i evolució de la malaltia bipolar així com possibles dianes terapèutiques[1]: a) els factors neurotròfics, com el factor neurotròfic derivat del cervell (BDNF), vinculats als processos neuroplàstics, de neurogènesi, supervivència i maduració neuronal[2] b) els marcadors d'inflamació, vinculats a processos neuroinflamatoris[3] i c) els marcadors d'estrès oxidatiu associats a possibles alteracions en les funcions mitocondrials[4].

Donat que existeix evidència sobre la possibilitat que s'afecti de forma sistèmica el nostre organisme al llarg de l'evolució progressiva del trastorn bipolar (TB) a partir dels mecanisme prèviament esmentats[5, 6], s'ha suggerit la possibilitat de classificar el TB en el marc d'un model d'estadificació. Aquest model, ja àmpliament integrat en el marc oncològic, és de referència per a classificar una entitat tumoral.

En aqueta tesi doctoral s'aprofundeix en el camp dels biomarcadors sèrics i de les classificacions en el TB a partir d'un compendi d'articles, segons la normativa de la Universitat de Barcelona. El treball inclou, per aquest ordre: una introducció;

una secció amb les hipòtesis i objectius a estudi; el segueix una explicació del mètode emprat i un resum dels resultats dels tres treballs inclosos al mateix temps que el corresponent article original; prosseguim amb un apartat de discussió; les conclusions d'aquest projecte de doctorat i; finalment, un llistat de la bibliografia citada.

Els tres articles inclosos en aquesta tesi doctoral són producte d'una línia d'investigació col·laborativa internacional amb l'equip del Prof. Flávio Kapczinski, anomenat expert en el camp dels biomarcadors i la proposta d'estadificació en el TB a nivell mundial:

- **I Grande**, F Kapczinksi, L Stertz, GD Colpo, M Kunz, KM Cereser, M Kauer-Sant'Anna, B Frey, E Vieta, PVS Magalhaes, Peripheral brain-derived neurotrophic factor changes along treatment with extended release quetiapine during acute mood episodes: An open-label trial in drug-free patients with bipolar disorder, *Journal of Psychiatric Research* 2012;46:1511-4.
- **I Grande**, PVS Magalhaes, I Chendo, L Stertz, GR Fries, KM Cereser, ABM Cunha, P Goi, M Kunz, M Udina, R Martín-Santos, BN Frey, E Vieta, F Kapczinski, Val66Met polymorphism and serum brain-derived neurotrophic factor in bipolar disorder: an open-label trial, *Acta Psychiatrica Scandinavica (on-line)*.
- **I Grande**, PVS Magalhaes, I Chendo, L Stertz, B Panizutti, GD Colpo, AR Rosa, CS Gama, F Kapczinski, E Vieta, Staging bipolar disorder: clinical, biochemical, and functional correlates (en revisió).

# 3. Introducció

# 3. Introducció

## 3.1. Trastorn bipolar: malaltia sistèmica

El TB és una malaltia crònica i recurrent, caracteritzada per l'aparició d'episodis de mania/hipomania i/o estats mixtos així com episodis de depressió que es troba entre les 10 malalties més discapacitants a nivell mundial[7]. Aquesta càrrega de deficiències global no només ve deguda a la pròpia cronicitat d'un trastorn afectiu amb oscil·lacions de l'estat d'ànim però també a un reguitzell de condicions comòrbides mèdiques[8] i psiquiàtriques[9] com també a un deteriorament cognitiu associat[10]. Donada l'extensa evidència d'afectació en aquests diferents àmbits, s'ha proposat la hipòtesi que el TB podria tenir un caràcter sistèmic, al igual que altres malalties neuropsiquiàtriques com la malaltia de Parkinson (*Figura 1*)[6].

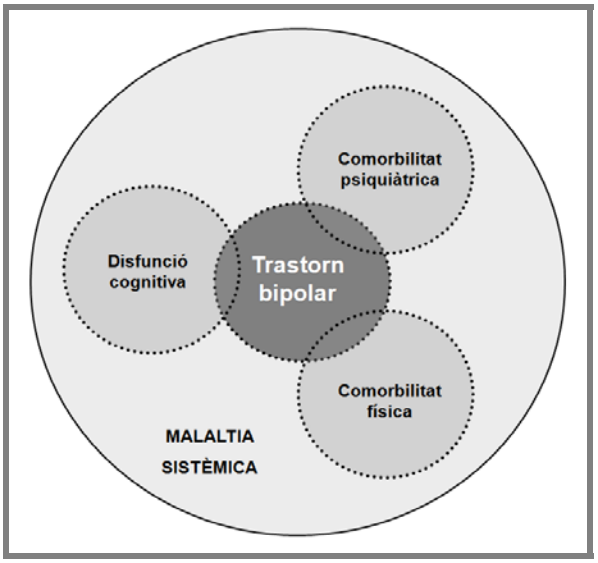


*Figura 1:* Afectació sistèmica en el trastorn bipolar[6].

### 3.1.1. Increment de les comorbilitats mèdiques, psiquiàtriques i de l'afectació cognitiva en el trastorn bipolar

Com prèviament s'ha comentat, els pacients amb TB pateixen una alta prevalença de comorbilitat, a més a més, de mortalitat mèdica. Com en la població general, els índexs de major comorbilitat mèdica són les malalties cardiovasculars[11]. Es podria argumentar que aquesta prevalença fos deguda al tractament prescrit, en major part, als antipsicòtics atípics. Tanmateix, la bibliografia recull que aquesta relació és independent i que existeix una estreta relació entre malalties cardiovasculars i el TB[12]. Aquestes malalties cardiovasculars com altres metabòliques, infeccioses, neurològiques o respiratòries, a més a més de ser més presents en els pacients amb TB també apareixen de forma més precoç[13, 14]. En canvi, recentment s'ha postulat sobre una possible comorbilitat inversa quant al càncer en els pacients amb malalties del sistema nerviós central, com és el cas del TB[15].

De manera paral·lela, la comorbilitat psiquiàtrica també és freqüent en el TB, arribant a freqüències entre el 50 i el 70%. Primen entre tots els diagnòstics psiquiàtrics, els trastorns per angoixa i de per abús de substàncies[16, 17]. La seva presència s'ha relacionat amb evolucions més tòrpides de la malaltia, menor taxa de remissió, major conducta suïcida, menor resposta al tractament i pitjor adaptació a la vida quotidiana, a més a més, de pitjor qualitat de vida[18, 19]. En definitiva, un pitjor pronòstic tant a curt com a llarg termini.

Finalment, de forma similar, però en menor grau que l'esquizofrènia, el TB també comporta dèficits neurocognitius tant en els episodis afectius com en l'estat d'eutímia[20–22]. Aquestes disfuncions estan relacionades amb la severitat de la

malaltia, la presència de símptomes psicòtics, la major duració de la malaltia i el major número d'episodis maníacs[23]. Els dèficits cognitius persistents més descrits en eutímia inclouen atenció, funcions executives i memòria verbal determinades amb tests neuropsicològics com el *California Verbal Learning Test*, el *Rey Verbal Learning Task*, el *Trail Making Test*, el *Digit Spam* i el *Wisconsin Cart Sorting Task*[24]. Aquestes funcions ens guien en la descripció dels patrons d'activitat cerebral alterats en el TB implicant el còrtex prefrontal i les vies cortico-subcortico-límbiques.

### *3.1.2. Càrrega alostàtica: manegant els esdeveniments vitals*

Els efectes sistèmics en el TB mencionats prèviament es poden entendre des del punt de vista del concepte de la *càrrega alostàtica*[25–27]. Sterling i Eyer[28] van utilitzar per primer cop el terme *d'alostasi* i en els següents anys, McEwen i Stellar[29] l'empraren per a descriure un concepte lligat a l'estrès i les seves conseqüències definint alostasi com la capacitat d'obtenir una estabilitat a través del canvi a partir de mecanismes adaptatius que ajuden a manegar les situacions de la vida quotidiana. Les conseqüències sistèmiques d'aquests mecanismes compensatoris es trobarien englobades en el terme de càrrega alostàtica (CA). Aquest darrer terme ha estat crucial en l'estudi de malalties neuropsiquiàtriques com l'Alzheimer[30], el trastorn depressiu major[31] i el TB[26] ja que proporciona un enllaç entre dimensions aparentment separades com la disfunció cognitiva i el desgast corporal evidenciat en els pacients amb malalties mentals cròniques. Aquestes suposadament distants àrees estarien vinculades a través del cervell que és el director d'orquestra de la resposta a l'estrès i determina allò que pot ser amenaçador, i per tant, generador

d'estrès (*Figura 2*). Així mateix, el cervell també controla la conducta i la resposta fisiològica a nivell global de l'organisme en funció de les singularitats i característiques de cada individu i experiències viscudes al llarg de la vida. A partir del circuit hipocampo-amígdalo-prefrontal, s'activen els processos lligats a les emocions generant cascades regulatòries neuroendocrines, autonòmiques i del sistema immunitari que repercuteixen en tot l'organisme tant a nivell cognitiu com induint malalties mèdiques i psiquiàtriques (*Figura 2*). Els diferents biomarcadors estudiats en el TB, estan relacionats amb aquestes reaccions en cascada.

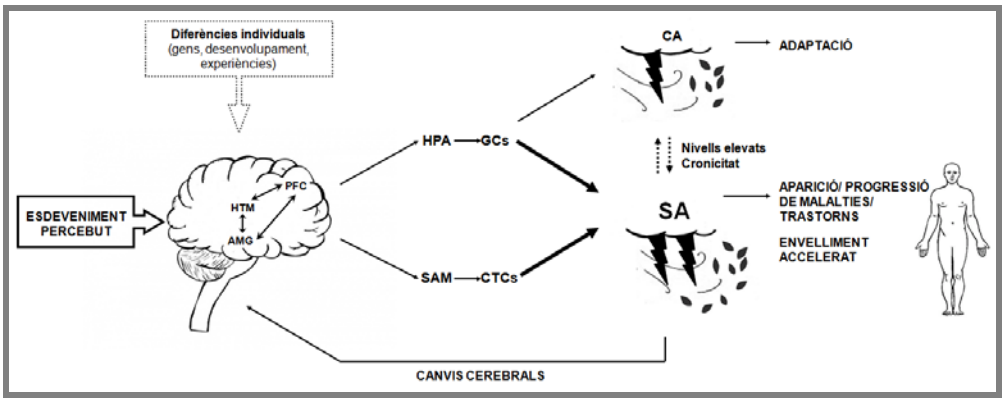


*Figura 2:* Resposta a l'estrès i alostasi: la percepció d'estrès es troba modulada per diferències personals que en últim punt, modulen la capacitat d'adaptació de l'individu a l'estrès. Quan el cervell percep un esdeveniment com a generador d'estrès, respostes fisiològiques es desencadenen alliberant, bàsicament, glucocorticoides (GCs) i catecolamines (CTCs) a partir dels eixos hipotalàmico-pituitari-adrenal (HPA) i simpàtico-adreno-medul.lar (SAM), respectivament en un procés adaptatiu d'alostasi. Si la càrrega alostàtica (CA) incrementa de forma dramàtica o és elevada d'una forma continuada, la capacitat adaptativa a l'estrès és insuficient i la CA esdevindrà sobrecàrrega alostàtica (SA), que no té cap finalitat per si mateixa i predisposa l'individu a un envelliment accelerat i a malalties[32]. AMG: amígdala, HTM: hipotàlem, PFC: còrtex prefrontal.

### 3.1.3. Biomarcadors

Els estats d'alostasi es desencadenen a partir de l'alliberació de diferents factors com les catecolamines, els glucocorticoides, factors relacionats amb els factors neurotròfics, els mediadors d'estrès oxidatiu i la inflamació com les citocines. Tots aquests factors interaccionen d'una manera no lineal (*Figura 3*)[33] intentant assolir un nivell estable d'alostasi.

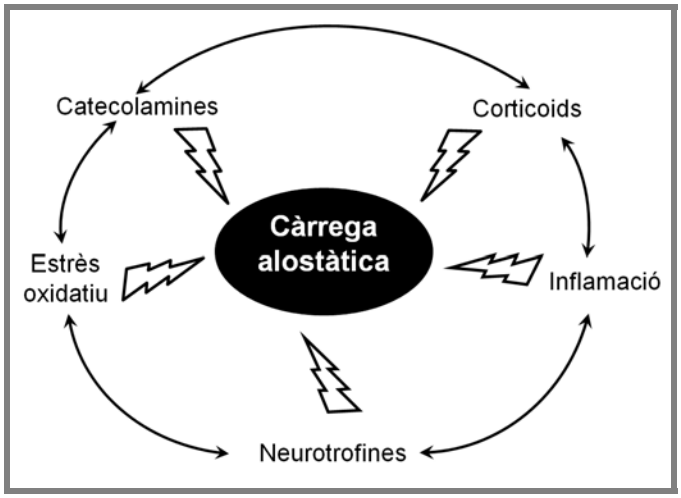


*Figura 3:* Biomarcadors involucrats en la càrrega alostàtica del trastorn bipolar.

En el camp de les neurotrofines, el factor neurotròfic derivat del cervell (BDNF) ha estat àmpliament estudiat en Neurociències. És un membre de la família dels factors de creixement i un agent clau en la promoció de l'eficiència sinàptica, col·laborant en l'arborització[2]. En el TB existeix una consistent evidència d'alteracions dels nivells de BDNF a nivell perifèric[34]. Durant els episodis afectius aguts, els nivells s'han descrit amb una tendència a disminuir i a retornar a nivells estables un cop assolida l'eutímia[34]. Aquests resultats s'han obtingut tant en pacients en episodi maníac[35, 36] com en episodi depressiu[35]. D'altra banda, els

nivells baixos de BDNF també s'han vist relacionats amb la cronicitat del TB presentant una correlació negativa amb la duració de la malaltia[37]. La secreció de BDNF pot ser constitutiva tot i que majorment es troba regulada per estímuls[2]. Determinats polimorfismes funcionals en el gen *BDNF,* junt a mecanismes de regulació epigenètics, determinen la secreció de BDNF[38, 39]. En concret, la substitució de la valina (Val) per metionina (Met) en la pro-regió 5' en el gen humà *BDNF* s'ha vist relacionada amb la secreció depenent d'estímuls[39]. Així mateix, aquest polimorfisme es troba associat al diagnòstic de TB[40], al patró de ciclació ràpida en el TB[41] i a un inici precoç de la malaltia bipolar[42].

En el TB s'han descrit alteracions en el metabolisme cerebral con delecions a nivell del DNA i disfuncions en el complex I mitocondrial que indueixen un increment de l'oxidació proteica i lipídica[4]. Així mateix, també s'han descrit alteracions en els enzims antioxidants com la superòxid dismutasa o la catalasa[43]. Al llarg de la malaltia, al igual que la resta de biomarcadors, els nivells de marcadors d'estrès oxidatiu varien[44]. S'ha mostrat un augment de la glutatió reductasa i la glutatió-S-transferasa en estadis avançats de la malaltia comparat a etapes més precoces[44]. A més a més, l'estrès oxidatiu s'ha trobat íntimament lligat a la neuroinflamació, ja que aquest activa la micròglia, afavorint la producció de citocines pro-inflamatòries.

Pertanyent al camp de la neuroinflamació, la resposta inflamatòria en el TB, moderada però crònica, ha conduït a un profund estudi de les diferents interleucines (IL), tant les relacionades amb la resposta inflamatòria primària com amb la resposta inflamatòria secundària. En el TB existiria una descompensació en el balanç entre les IL pro-inflamatòries i antiinflamatòries, sobretot en els episodis de descompensació aguda[45]. En la mania, aquesta diferència seria més marcada

que en la depressió respecte a l'eutímia[3]. Les IL més descrites en la bibliografia són la interleucina-6 (IL-6), la interleucina-4 (IL-4) i el factor de necrosi tumoral alfa (TNF-α).

Donades les alteracions descrites en aquestes tres àrees en el TB, Kapczinski i col.[46] van proposar un *Índex de Toxicitat Sistèmica* en episodis aguts que englobés els factors neurotròfics, l'estrès oxidatiu i la resposta inflamatòria. Es va evidenciar una marcada diferència d'aquest índex entre els episodis afectius i l'estat d'eutímia. De fet, la toxicitat assolida en els episodis s'aproximava a un estat de sèpsia, paradigma de la inflamació sistèmica aguda.

### *3.2. La neuroprogressió de la malaltia bipolar*

La noció del progressiu avenç de la malaltia bipolar ja fou evidenciada pel mateix Kraepelin[47]. Posteriorment, Post[48] assentà els fonaments dels coneixements actuals sobre la patofisiologia de l'evolució del TB basant-se en la teoria del *kindling* o sensibilització. Sota aquest concepte s'entén que l'estimulació repetida, utilitzada en el model experimental de l'epilèpsia, indueix canvis a nivell del sistema nerviós central que provoquen estimulacions pròpies, en aquest cas, crisis epilèptiques endògenes. El mateix succeiria amb els episodis afectius bipolars que, a l'inici de la malaltia es trobaven més vinculats a esdeveniments vitals mentre que, a mesura que la malaltia avança, serien cada cop més independents de factors externs. La repercussió dels episodis a nivell organitzatiu cerebral podria explicar fins a cert nivell, troballes a nivell més macroscòpic com la diferència descrita per Strakowski i col.[49] entre la major mida dels ventricles laterals en pacients amb TB

que han patit episodis maníacs repetits comparat amb pacients després d'un primer episodi o subjectes sans.

Aquest patró evolutiu del curs de la malaltia també és visible en altres àmbits més clínics de la malaltia, com la progressiva reducció del temps interepisòdic entre les recurrències[47, 50]. Així mateix, en el camp de les funcions cognitives, el nombre d'episodis maníacs s'ha vist associat a un pitjor rendiment en les avaluacions neuropsicològiques suggerint que la recurrència de mania podria tenir un efecte neuropsicològic a llarg terme. Quan a les estratègies farmacològiques, en el clàssic estudi de Swann i col.[51], l'efecte antimaníac del liti en pacients que havien sofert més de 10 episodis no presentava diferències respecte a placebo en contrast amb aquells pacients que havien patit menys episodis i presentaven millor resposta respecte placebo. Més recentment, Berk i col.[52] van descriure resultats similars amb un fàrmac amb diferent mecanisme d'acció com és l'olanzapina. Aquells pacients amb menys episodis al llarg de la malaltia van presentar una resposta més favorable a l'olanzapina com a tractament antimaníac amb un increment de resposta de fins el doble comparat amb aquells pacients amb més de 10 episodis al llarg de la seva malaltia. Quant al tractament de manteniment, la prevenció de recaigudes maníaques era també més manifesta en pacients amb menor número d'episodis. Considerant les teràpies psicològiques, Scott i col.[53] determinaren que la tècnica cognitivo-conductual era també comparativament més efectiva en aquells pacients amb pocs episodis. Les diferències quant a l'efectivitat del tractament es mantenien així mateix quan s'avaluava l'efectivitat de la teràpia psicoeducativa a cuidadors de familiars amb TB[54]. Els pacients en una etapa més primerenca presentaven un període més llarg d'estabilitat clínica fins a la recurrència comparat

amb el grup control. Aquest benefici no fou objectivat en aquells pacients en una etapa més avançada de la malaltia.

Berk[55] va encunyar el terme de *neuroprogressió* per englobar tots aquests canvis gradualment progressius que esdevenen en el TB al llarg dels successius episodis afectius visibles tant a nivell clínic, funcional, terapèutic, bioquímic i neuroestructural. Emprant termes afins descrits prèviament, la CA podria explicar el desgast en tot l'organisme. Aquest es mostraria a nivell cerebral en forma de deteriorament cognitiu i resistència al tractament, mentre que en la resta de l'organisme, amb les malalties comòrbides tant psiquiàtriques com mèdiques prèviament descrites.

Donada aquesta neuroprogressió de la malaltia bipolar, es creu que el TB seria tributari d'una classificació basada en l'estadificació on la situació concreta del malalt s'orienta atenent a un pronòstic i un abordatge terapèutic adient a cada etapa evolutiva del pacient amb TB.

### *3.3. El model d'estadificació en el trastorn bipolar*

La classificació en estadis s'ha utilitzat de forma generalitzada en medicina, però sols s'ha plantejat recentment la seva aplicació, des d'una vessant teòrica, en el camp de la psiquiatria. Fa 30 anys Fava i Kellner[56] van realitzar la primera proposta en l'àrea de la psiquiatria i en concret, pel que fa als trastorns psicòtics[57] i els afectius s'han plantejat propostes en els darrers 5 anys[58–60].

Un cop conegut el curs deteriorant de la malaltia bipolar, no és gens menyspreable considerar l'aplicabilitat de l'estadificació en el TB. En les etapes

inicials, el TB es pot presentar amb símptomes prodròmics amb pronòstic favorable i requerir estratègies terapèutiques més simples a diferència d'etapes més avançades on existeix un agreujament clínic, refractarietat al tractament al mateix temps que deteriorament cognitiu i funcional[61]. Donada aquesta evolució, s'argumenta que el TB podria classificar-se en un model d'estadificació segons els següents raonaments: a) el primer és que la història natural de trastorn es mou al llarg d'una progressió temporal; b) el segon, un tractament administrat en el moment i estadi apropiat pot modificar el patró de la progressió de la malaltia; c) el tercer, el pronòstic és més favorable en diagnòstics i tractaments més precoços deixant de banda, que els tractaments utilitzats de forma més precoç tenen una relació risc-benefici més favorable que els utilitzats en etapes més avançades; d) finalment, es creu que els efectes d'una intervenció precoç pot alterar la distribució dels estadis en la població al llarg del temps[59, 62].

Per classificar la gradació del TB, s'han formulat diferents propostes d'estadificació en un marc teòric. Entre les suggerides, destaca la realitzada pel grup de McGorry aplicada als trastorns psicòtics i afectius, que ha servit per a què altres autors com Berk i col.[59] (***Taula 1***) o Kapczinski col.[58] (***Taula 2***) hagin formulat propostes adaptades al TB. Berk i col. proposen un model que dona una rellevància marcada als estats de risc i es centra en la recurrència episòdica. En contraposició, Kapczinksi i col. es fonamenten en el rendiment funcional del pacient en l'interval interepisòdic de màxima estabilitat clínica. Ambdós models tenen les seves fortaleses i limitacions. Referent al primer, s'ha de tenir present que el deteriorament no sempre presenta una relació lineal predictiva amb el número d'episodis mentre que el segon pot caure en cert grau de circularitat tautològica a l'hora de predir un estadi funcional.

*Taula 1:* Model d'estadificació proposat per Berk i col[63].

| Estadi clínic | Definició |
|---|---|
| 0 | Risc incrementat de trastorn afectiu greu (història familiar, consum de tòxics...) No símptomes específics en l'actualitat |
| 1a | Símptomes lleus o no específics de trastorn afectiu |
| 1b | Trets prodròmics: pacients d'ultra elevat risc |
| 2 | Primer episodi afectiu |
| 3a | Recurrència de símptomes afectius subsindròmics |
| 3b | Primera recaiguda afectiva |
| 3c | Multiple recaigudes |
| 4 | Malaltia persistent no remitent |

*Taula 2:* Model d'estadificació proposat per Kapczinski i col[58].

| Estadi | Definició |
|---|---|
| Latent | En risc de desenvolpar TB (història familiar positiva, símptomes afectius o d'ansietat sense cumplir criteris de TB) |
| I | Episodis afectius amb períodes ben definits d'eutímia sense símptomes psiquiàtrics manifests |
| II | Símptomes en períodes interepisòdics (relacionats amb comorbilitats) |
| III | Franc deteriorament en cognició i funcionament |
| IV | Incapacitat per viure de forma autònoma a causa de dèficit cognitius i funcionals |

L'aplicabilitat en la pràctica clínica dels models descrits encara no s'ha dut a terme. D'aquí la importància d'operacionalitzar i validar aquests models a partir de variables clíniques, funcionals i bioquímiques per tal d'així, aproximar-nos a un diagnòstic i tractament, el més òptim possible en cada pacient, emfatitzant la necessitat de la intervenció precoç per frenar la progressió de la malaltia i poder modificar el pronòstic de la malaltia.

# 4. Hipòtesis

## 4. Hipòtesis

Les hipòtesis en aquesta tesi doctoral són:

1. Donat el curs progressiu del TB, existirien variables clíniques i funcionals que podrien ser d'ajuda a l'hora d'establir una classificació segons un model d'estadificació en el TB.

2. Un cop conegudes les vies de neuroprogressió vinculades al TB, la determinació de biomarcadors relacionats en aquestos processos, com les neurotrofines, els reactants d'estrès oxidatiu i les citocines inflamatòries, podria reforçar la distinció entre aquells pacients en un estadi més avançat de la malaltia respecte aquells pacients en etapes més primerenques.

3. Considerant el paper clau que juguen les neurotrofines i els seus polimorfismes funcionals en la neuroplasticitat i la regeneració neuronal, aquells pacients amb TB en episodi agut tant, homozigots per Val en el polimorfisme BDNF Val66Met com responedors al tractament, mostrarien increment dels nivells de BDNF, afavorint, en conseqüència, la remodelació sinàptica.

# 5. Objectius

# 5. Objectius

## 5.1. Objectius principals

**5.1.1.** Avaluar una proposta de model d'estadificació en el TB basada en variables clíniques i funcionals.

**5.1.2.** Identificar un correlat biològic d'un model d'estadificació a partir de la determinació de biomarcadors involucrats en les vies de neuroprogressió.

**5.1.3.** Determinar de forma prospectiva els nivells del biomarcador neurotròfic, BDNF, en una mostra de pacients amb TB en episodi afectiu agut al llarg de 16 setmanes de tractament.

## 5.2. Objectius secundaris

**5.2.1.** Objectivar la possible interacció del polimorfisme funcional BDNF Val66Met amb els nivells de BDNF en una mostra de pacients amb TB en episodi afectiu agut al llarg de 16 setmanes de tractament.

**5.2.2.** Determinar el pes que pot tenir la polaritat de l'episodi afectiu en els nivells de BDNF en una mostra de pacients amb TB en episodi afectiu agut al llarg de 16 setmanes de tractament.

# 6. Mètodes

## 6. Mètodes

Aquesta tesi doctoral està formada per tres articles que s'han desenvolupat al llarg de la formació del Programa de Doctorat Medicina i són fruït d'estudis col·laboratius amb l'equip del Prof. Flávio Kapczinski. La metodologia emprada quant a la descripció detallada de les característiques de la mostra, de les escales psicomètriques emprades així com dels passos realitzats en les determinacions de biomarcardors, de la resta de procediments i de l'anàlisi estadística es troben en cada un dels articles corresponents.

Els treballs que conformen aquesta tesi doctoral corresponen amb les hipòtesis d'estudi segons es detalla a continuació:

***Hipòtesi 1:***

*"Donat el curs progressiu del TB, existirien variables clíniques i funcionals que podrien ser d'ajuda a l'hora d'establir una classificació segons un model d'estadificació en el TB."*

- ***Estudi 1:*** **I Grande**, PVS Magalhaes, I Chendo, L Stertz, B Panizutti, GD Colpo, AR Rosa, CS Gama, F Kapczinski, E Vieta, Staging bipolar disorder: clinical, biochemical, and functional correlates (en revisió).

***Hipòtesi 2:***

*"Un cop conegudes les vies de neuroprogressió vinculades al TB, la determinació de biomarcadors relacionats en aquestos processos, com les neurotrofines, els reactants d'estrès oxidatiu i les citocines inflamatòries, podria reforçar la distinció entre aquells pacients en un estadi més avançat de la malaltia respecte aquells pacients en etapes més primerenques."*

- ***Estudi 1:*** **I Grande**, PVS Magalhaes, I Chendo, L Stertz, B Panizutti, GD Colpo, AR Rosa, CS Gama, F Kapczinski, E Vieta, Staging bipolar disorder: clinical, biochemical, and functional correlates (en revisió).

***Hipòtesi 3:***

*"Considerant el paper clau que juguen les neurotrofines i els seus polimorfismes funcionals en la neuroplasticitat i la regeneració neuronal, aquells pacients amb TB en episodi agut tant, homozigots per Val en el polimorfisme BDNF Val66Met com responedors al tractament, mostrarien increment dels nivells de BDNF, afavorint, en conseqüència, la remodelació sinàptica."*

- ***Estudi 2:*** **I Grande**, PVS Magalhaes, I Chendo, L Stertz, GR Fries, KM Cereser, ABM Cunha, P Goi, M Kunz, M Udina, R Martín-Santos, BN Frey,

E Vieta, F Kapczinski, Val66Met polymorphism and serum brain-derived neurotrophic factor in bipolar disorder: an open-label trial, *Acta Psychiatrica Scandinavica (on-line)*.

- ***Estudi 3:*** **I Grande**, F Kapczinksi, L Stertz, GD Colpo, M Kunz, KM Cereser, M Kauer-Sant'Anna, B Frey, E Vieta, PVS Magalhaes, Peripheral brain-derived neurotrophic factor changes along treatment with extended release quetiapine during acute mood episodes: An open-label trial in drug-free patients with bipolar disorder, *Journal of Psychiatric Research* 2012;46:1511-4.

En l'***estudi 1*** es pretenen complir els objectius ***5.1.1.*** i ***5.1.2***, en l'***estudi 2*** els objectius ***5.1.3.*** i ***5.2.1.*** i en l'estudi 3 els objectius ***5.1.3.*** i ***5.2.2.***

# 7. Resultats

# 7. Resultats

Aquesta tesi doctoral està conformada per tres articles, dos d'ells publicats en revistes indexades i amb factor d'impacte en el primer quartil de la categoria de psiquiatria i l'altre es troba, a dia d'avui, en revisió.

## 7.1. Estudi 1

***I Grande, PVS Magalhaes, I Chendo, L Stertz, B Panizutti, GD Colpo, AR Rosa, CS Gama, F Kapczinski, E Vieta, Staging bipolar disorder: clinical, biochemical, and functional correlates (en revisió).***

### 7.1.1. Resum de l'estudi 1

**Introducció:** Existeixen diversos models d'estadificació en el TB però encara cap ha estat validat. L'objectiu d'aquest estudi és investigar empíricament si hi ha variables clíniques i funcionals que poguessin ser útils a l'hora de classificar els pacients en un model d'estadificació i estudiar l'associació d'aquesta proposta amb biomarcadors relacionats amb els factors neurotròfics, l'estrès oxidatiu i els processos inflamatoris, que poguessin servir com a validadors biològics.

**Mètodes:** Es va realitzar un estudi cas-control aparellat per edat i sexe. S'inclogueren pacients diagnosticats de TB que no havien presentat cap episodi afectiu agut en el darrer mes, segons *Structured Clinical Interview for DSM-IV* (SCID-I). S'exclogueren aquells pacients amb altres malalties neuropsiquiàtriques, càncer, infecció aguda o crònica i tractament amb glucocorticoides. Els controls foren familiars de primer grau de pacients diagnosticats de TB que no havien rebut mai cap diagnòstic psiquiàtric segons SCID-I/NP. Es van recollir dades sociodemogràfiques, clíniques i funcionals i es determinaren els nivells sèrics de

BDNF, biomarcadors d'oxidació lipídica (substàncies reactives a l'àcid tiobarbitúric (TBARS)) i proteica (contingut carbonil proteic (PCC)) i de citocines (IL-2, IL-4, IL-6, IL-10, IL-17A, interferó gamma (*INF-*□)). Una anàlisi de *cluster* es va dur a terme per construir un model d'estadificació i una regressió logística per a estudiar les associacions entre el model i els biomarcadors.

**Resultats:** L'anàlisi de *cluster* va dividir la mostra entre dos grups equitatius, que denominàrem "*primerenc*" i "*tardà*" a partir de talls quantitatius empírics en les variables: a) funcionament avaluada segons *Functioning Assessment Short Test* (FAST); b) número d'episodis; c) edat a l'inici de la malaltia i d) temps d'evolució de la malaltia (***Figura 4***). En la regressió logística, IL-6 es va associar a un estadi tardà.

**Conclusions:** L'estudi aquí presentat dona suport a la distinció entre dos *clusters* de pacients bipolars, denominats *primerenc* i *tardà* en termes de correlats clínics, funcionals i bioquímics.

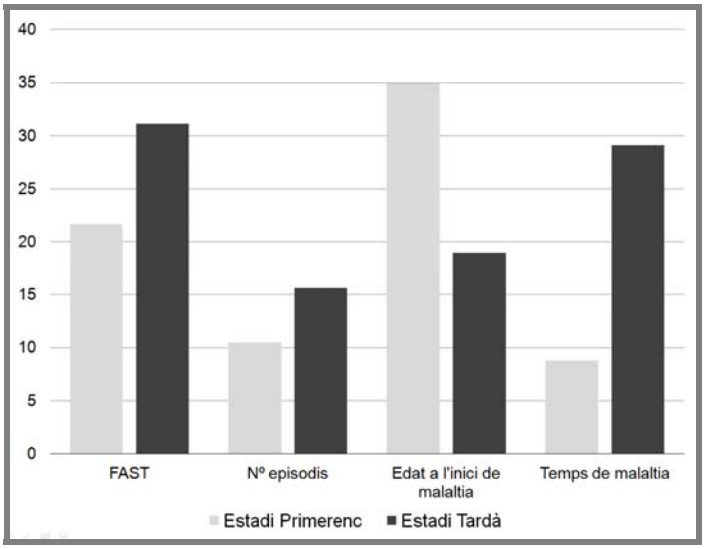


***Figura 4:*** Centre dels *clusters* de les variables que delimiten els dos estadis (*Primerenc* i *Tardà*).

**Title:** Staging bipolar disorder: clinical, biochemical, and functional correlates

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**Short title:** BD staging: clinical & biochemical correlates

**Source of financial and material support:** Research grant Río Hortega Contract (CM12/00062), Instituto de Salud Carlos III, Spanish Ministry of Economy and Competiveness.

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**Word Count of the abstract:** 245

**Word Count of the body of the manuscript:** 2694

**Number of figures:** 1

**Number of tables:** 3

**ABSTRACT**

**Background**: There are several models of staging in bipolar disorder (BD), but none has been validated. The aims of this study were to investigate empirically if there are clinical variable that may be useful to classify patients in different cluster according to stage and study the association with biomarkers as biological validators. **Methods:** This was a case-control study matched for age and gender performed in the Bipolar Disorders Program of Hospital de Clínicas de Porto Alegre from April 2009 to September 2011. Patients (n=115) were diagnosed with BD and had not had an acute episode during the previous month. Exclusion criteria included neuropsychiatric or medical disorders. Controls (n=25) were first degree relatives of patients diagnosed with BD and never diagnosed with a psychiatric disorder. Socio-demographic, clinical and functional data were collected. Serum cytokines, brain-derived neurotrophic factor and biomarkers of lipid and protein oxidation were assessed. Cluster analysis was carried out to build a model of staging and a logistic regression was conducted to study any associations between the model and biomarkers. **Results:** Cluster analysis divided the sample into two equitable groups, denominated early- and late-stage, with empirical cut-offs for the Functioning Assessment Short Test score, number of episodes, age at onset of the disorder and time elapsed since first episode. In the logistic regression, IL-6 was associated with late-stage (p=0.029). **Conclusion:** The present study supports the distinction between two clusters in bipolar patients, namely early- and late-stage, in terms of clinical, functional and biochemical correlates.

**Keywords:** staging; early-stage; late-stage; bipolar disorder; interleukin 6; functional outcome.

## 1. - INTRODUCTION

Despite the wide implementation of the staging model in general medicine, it is still a heuristic concept in the field of psychiatry[1]. In 1993, Fava and Kellner[2] encouraged the integration of the staging model in psychiatry and since then, a wide range of proposals have come to light. Within the frame of psychosis, McGorry et al. developed a model focused on the risk and prodromal phases of this entity[3] and within the scope of depression, several groups have attempted to systematize a treatment-resistant classification[4]. In the field of bipolar disorder (BD), some models have been proposed each emphasizing the progressive features of the disorder[5,6]. Berk et al. mainly underscored symptomatic severity from the risk to refractory stages of BD, while Reinares et al. highlighted the relationship between functioning, clinical and, neuropsychological variables and finally, Kapczinski et al. studied in depth the underpinning biological correlates[7–9].

There is increasing awareness of the limitations of the current diagnostic systems and concern about future classifications of the Diagnostic and Statistical Manual of Mental Disorders and the International Classification of Diseases since these customary clinical taxonomies do not include characteristics that delimit major prognostic and therapeutic differences among patients receiving the same diagnosis[10]. The staging model regards these special features of the patients and therefore draws a route to define the prognosis and treatment as well as the neurobiological background of the disorder. The knowledge acquired in the latter identifying pathways associated with inflammation, neurotrophin expression, and oxidative stress may furthermore prove the validity and operability of this classification[11]. In previous studies regarding the model of staging, the levels of brain-derived neurotrophic factor, a neurotrophin, was decreased in the patients in the late stage of BD compared to controls while the levels of interleukin 6 and 10 were increased in the early stages compared to controls.[12]

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Although different theoretical proposals have been put forward in BD, there is still a lack of an operative, valid staging model. In this line, the aim of this study was to investigate empirically if clinical variables such as number of episodes, age at onset of the disorder, time elapsed since first episode and functioning assessed by the Functional Assessment Short Test (FAST) may be useful to classify patients in different cluster according to stage since the complexity of a psychiatric disorder such as BD and its neuroprogression can be hardly summed up in a single variable. We further investigated the association between biomarkers related to inflammation (interleukin 2, 4, 6, 10, 17 A, interferon-gamma (INF-g)), neurotrophins (brain-derived neurotrophic factor) and oxidative stress (thiobarbituric acid reactive substances (TBARS) and protein carbonyl content (PCC), and the proposed clinical staging as biological validators.

## 2. – METHOD

### *2.1. - Study population and participants*

Recruitment of patients was performed in the Bipolar Disorders Program of Hospital de Clínicas de Porto Alegre and the recruitment of controls was carried out within the hospital catchment area. Data were collected from April 2009 to September 2011. Inclusion criteria were: age from 18 to 65 years old, BD diagnosis, according to DSM-IV-TR criteria[13] and absence of an acute episode during the previous month assessed by means of the Hamilton Rating Scale score (HDRS) <17 or/and the Young Mania Rating Scale score (YMRS) <20[14,15]. The confirmation of the diagnosis was established by board certified psychiatrists based on the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I)[16]. The exclusion criteria were: history of neurodegenerative disorders, mental retardation, current cancer, chronic or acute infection and glucocorticoid treatment.

Controls were 18 years of age or older first-degree relative of patients diagnosed with BD. Absence of psychiatric morbidity was confirmed by the non-patient version of the SCID (SCID/NP)[17]. They were frequency-matched to the patient group for age and sex. Such control group is a more realistic comparison to patients than healthy subjects, since it will be less confounded by group differences.

### *2.2. - Procedure*

A baseline assessment was carried out to obtain socio-demographic, clinical, therapeutic and functional data using a semi-structured interview. Functioning was evaluated with the Functional Assessment Short Test (FAST)[18–20].

All participants provided written informed consent after receiving a complete description of the study. Procedures were approved by the Ethical and Research Committee of the Hospital de Clínicas de Porto Alegre.

*2.3. - Biochemical assays*

Five milliliters of blood were withdrawn from each subject by venipuncture and were introduced into a free-anticoagulant vacuum tube for serum analysis. Serum samples were separated and frozen in -80ºC until analyses. BDNF levels in serum were determined by sandwich-ELISA using monoclonal antibodies specific for BDNF as described previously (R&D Systems, Minneapolis, MN, USA) [21]. Briefly, microtiter plates (96-well flat-bottom) were coated overnight at room temperature with the monoclonal anti-BDNF antibody at 4 µg/ml in phosphate buffer solution (PBS, Laborclin, Paraná, Brazil). Then, plates were washed three times with wash buffer [PBS, pH 7.4, with 0.05% Tween 20 (Nuclear, São Paulo, Brazil)] and were blocked for 1 h at room temperature with PBS containing 5% nonfat milk powder. After another washing, plates were coated for 2 h at room temperature with the samples diluted 1:200 in sample diluents [PBS with 1% bovine serum albumin (BSA) (Sigma-Aldrich, St. Louis, MO, USA)], and standard curve ranged from 7.8 to 500 pg/ml of BDNF. Plates were washed again, 0.2 µg/ml of a biotinylated anti-BDNF antibody in PBS was added, and new incubation was performed for 2 h at room temperature. After washing, the incubation with streptavidin-peroxidase conjugate (diluted 1:200 in sample diluents) for 20 min at room temperature was performed. Plates were then washed and incubated with the substrate for 20 min at room temperature. Finally, the stop solution [H2SO4, 1 M (Nuclear, São Paulo, Brazil)] was added, and the amount of BDNF was determined by absorbance at 450 nm with correction at 540 nm. The standard curve demonstrated a direct relationship between optical density (OD) and BDNF concentration.

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Oxidative damage to proteins was measured by the determination of carbonyl groups by the protein carbonyl content (PCC) method, as previously described[22]. Briefly, 2,4-dinitrophenylhydrazine (DNPH) was added to the plasma samples followed by an incubation at room temperature for 1 h, with subsequent addition of 20% trichloroacetic acid. After centrifugation, the supernatant was subjected to two washings with ethanol: ethyl acetate (1:1). Afterwards, urea was added to the samples. Incubation at 60 °C for 30 minutes was follow by and overnight incubation at room temperature. For each sample, a respective control tube was used. Absorbance was determined at 370 nm and results were expressed as nmol/mg of protein.

The levels of lipid peroxidation were measured using the commercial TBARS kit according to the manufacturer's instructions (Cayman, USA).[23] In this method, the quantification of lipid peroxidation products is performed by serum formation of substances reacting to thiobarbituric acid, which is the analysis of the final products of lipid peroxidation, like malondialdehyde (MDA) that react with 2-thiobarbituric acid (TBA) form Schiff bases. These complexes exhibit color and its concentration can be determined spectrophotometrically at 535 nm. The results are expressed in µM of MDA.

The concentration of serum cytokines was determined by flow cytometry using the BD™ Cytometric Bead Array (CBA) Human Th1/Th2/Th17 (BD Biosciences, San Diego, CA). The CBA kit employed allows the discrimination of the following cytokines: IL-2, IL-4, IL-6, IL-10, INF-α and IL-17A. Sample processing and data analysis were performed according to the manufacturer's instructions. Briefly, serum samples were incubated with the six cytokine capture beads for 1.5 h, then washed and incubated for more 1.5 h with PE-conjugated detection antibodies, both incubations at room temperature and protected from light. Afterwards, samples were washed and sample data were acquired using a FACSCalibur flow cytometer (BD Biosciences, San Diego, CA). Results were generated in graphical and

tabular format using the BD CBA Analysis Software FCAP Array™ (BD Biosciences, San Diego, CA).

The investigators who performed the laboratory assays were blinded to the clinical status of the subjects.

*2.4. - Data analysis*

Categorical variables were analyzed using $\chi^2$ analysis. We performed a cluster analysis by 2-means[24] since previous bibliography and task force to this effect has put forward a division between two groups, "early-stage" and "late-stage", apart from latent-stage.[12] Clinical variables such as number of episodes, age at onset of the disorder, time elapsed since the first episode, and functioning assessed by FAST were regarded.[8,12,25] Data were standardized so as to control the contributional effect of the variables to the model since variables with high values contribute more to the model than those with low values.

After having determined a model of staging in BD we built a logistic regression model to ascertain which biomarkers were associated with the stages defined. The election of biomarkers was carried out according to the described pathways underlying neuroprogression in BD in which inflammation, oxidative stress and neurotrophins play a main role[11]. The model was created according to Hosmer and Lemeshow[26]. Regarding inflammatory, neurotrophic and oxidative biomarkers, the values were Box-cox transformed for parametric analysis and subsequently z-scores were generated[27].

Statistical analyses were conducted using the software Statistical Package for Social Sciences version 18.0 (SPSS Inc., Chicago, IL, U.S.A.) for Windows (Microsoft Corporation, Redmond, WA, USA). All statistical tests were two-tailed and were performed using a significance level of alfa $\leq 0.05$.

**3.- RESULTS**

*3.1- Demographic and clinical characteristics of the sample*

The group of patients and the control group did not differ in age, gender, marital status, time of education or occupational status (table 1). There was a significant deterioration in the functional outcome in patients with BD compared to positive controls. The clinical profile of the patients is depicted in table 1.

*3.2. –Characterization of the stages*

Empirical cut-offs for the variables elected to describe the model of staging were determined for the early and late stages (figure 1). ANOVA analysis showed significant differences between the values of the two clusters centers ($F(1)= 7.32$, $p= 0.008$ for the FAST score; $F(1)=7.34$, $p=0.008$ for the number of episodes; $F(1)= 62.38$, $p= 0.0001$ for the age at onset and; $F(1)= 127.98$, $p= 0.0001$ for the time elapsed since first episode). The sample was divided into two relatively equitable groups (61.6% and 38.4% of the sample).

*3.3. -Biomarker analysis*

In table 2, we present the comparison between levels of the mentioned biomarkers related to inflammation, neurotrophins and oxidative stress between patients and controls. Interleukin 6 (IL-6) levels were significantly higher in the group of patients compared to controls. When we performed the logistic regression model, on assessing the association of the inflammatory, neurotrophic and oxidative pathways in the proposed staging model, IL-6 showed significant differences between the early- and late-stages (table 3).

**4. - DISCUSSION**

The present study supports the distinction between two clusters in bipolar patients, namely early- and late-stage. Early-stage bipolar patients typically have better functioning, fewer episodes, older age at onset of the disorder, as well as lower levels of IL-6. Conversely, late-stage bipolar patients present a higher number of episodes and clinical correlates of a more severe disorder in terms of ability to function as well as in terms of bodily changes such as increased inflammatory states.

From our point of view, it seems an arduous task to encompass the complexity of a psychiatric disorder such as BD and its neuroprogression in a single variable. Thus, we selected several clinical items described in previous studies focused on staging dimensions and quantified these items in order to more objectively define a model of staging compared to pure clinical judgement. Reinares recently put forward a proposal of staging based on functioning outcome assessed by the World Health Organization Dissability Assessment Schedule[8]. We preferred to assess functioning according to a scale especially designed to evaluate functioning in psychiatric disorders such as the FAST score[18,19]. Regarding the number of episodes, this feature has repeatedly been used in the field of staging either for studying functioning[19,25] or treatment response[28]. Considering the length of the disorder, Kauer-Sant'Anna et al.[12] defined the period within the first 3 years of a first manic episode as early-stage while the late-stage was defined as at least 10 years after the diagnosis of BD. In addition, age at onset was also taken into account regardless of the length of the disorder, since in several studies, early onset *per se* has been suggested to be associated with greater illness severity and poorer outcome[29,30]. In fact, early onset has been proposed as a valid specifier for the recent DSM-5[31].

The shortening of the inter-episode interval within each recurrence and the reduced probability of treatment response as the illness progresses results from neurobiological

interrelated processes which take place in the brain of patients with BD[11,32]. Deeper understanding of the neurobiology of BD may shed some light into the neuroprogression of BD and new pharmacological targets[33]. No differences were found in the neurotrophins or in the oxidative stress fields in our sample. In a recent study with participants from the general population, only protein oxidative damage was demonstrated in young adults with BD compared with the healthy controls[34]. Indeed, Kauer-Sant'Anna depicted differences in peripheral BDNF levels between patients in late-stage compared to those in early-stage and between patients in late-stage compared to controls[12]. In our study, IL-6 was considered to be a feasible biomarker to characterize the progression of BD. Peripheral IL-6 levels increased along the neuroprogression of BD from first degree relatives of patients diagnosed with BD to patients categorized in the late-stage. In the staging model based on the length of the disorder, Kauer-Sant'Anna[12] also determined differences in IL-6 levels between early- and late-stage as well as between early- and late-stage compared to controls. Considering the role of IL-6 in the neuroprogression of BD, the therapeutic potential of IL-6 antagonism has been previously suggested as well as the possible use of minocycline[35,36]. Regarding autoimmunity, data from animal models have shown a pivotal role of IL-6 in the induction of chronic autoimmune inflammation as IL-6 appears to be crucial in driving the differentiation of specific T helper (Th) lymphocytes as well as inhibiting the differentiation of T regulatory lymphocytes[35].

In this study some limitations should be taken into account. A possible caveat is that patients with subsyndromal symptoms were not excluded from the sample. Nevertheless, along the neuroprogression of BD and in particular, in late-stages, clinical remission is barely achieved[37,38]. Therefore, excluding these patients would have not been representative of patients in late-stage. Moreover, the biomarker assessment was carried out peripherally. Despite evidence of a correlation between cortical biomarkers and serum biomarkers in basic

neuroscience, the presumption that such findings might occur in the human brain needs to be confirmed. In addition, the control group consisted in a control positive including first degree relatives of patients diagnosed with BD without medical comorbidities. We preferred this positive control group so as to better depict the longitudinal dimension of BD. Finally, it could be argued that the findings related to IL-6 could be just the result of multiple comparisons; however, the logistic regression analysis showed consistent results with the comparison between early- and late-stage patients, and as discussed IL-6 was already pointed out as primary candidate for biomarkers of inflamatory changes according to staging.

Advocating for a model of staging in bipolar disorder that can group the patients according to quantitative cut-offs of common practice clinical variables as well as defining a biochemical correlation is a further step towards establishing a model of staging in bipolar disorder. The implementation of a valid staging classification in bipolar disorder may facilitate the identification of rational therapeutic targets and the potential benefit from providing the most effective and less toxic intervention in a time-sensitive manner. For instance, patients who are identified as late-stage may not benefit from first line treatments for bipolar disorders such as lithium, family psychoeducation or cognitive-behavioral therapy which may be more suitable for early-stage to mitigate the course of the disorder.[39–42] Conversely, recent studies suggest that for a subset of bipolar patients, who would mostly qualify for late stage illness, therapies focused on functional remediation may be indicated[43]. Therefore, the staging model stresses specific intervention and its possibilities to modify the course of the disorder by preventing the progression to a malignant and treatment-resistant pattern. Longitudinal and clinical trials may help to clarify the potential use of staging systems in bipolar disorder.

**ACKNOWLEDGMENTS**

Dr I Grande would like to thank the Instituto de Salud Carlos III, Spanish Ministry of Economy and Competiveness for her research grant Río Hortega Contract (CM12/00062), Barcelona, Spain.

**Disclaimer statement:**

Dr. I. Grande has received a research grant Río Hortega Contract (CM12/00062), Instituto de Salud Carlos III, Spanish Ministry of Economy and Competiveness, Barcelona, Spain and has served as a speaker for AstraZeneca.

Dr. Clarissa S. Gama has received grant/research support from CNPq, FIPEeHCPA, and FAPERGS, and has been a speaker/advisory for AstraZeneca, Lundbeck, Pfizer, Actelion.

Prof. Kapczinski has received grant/research support from Astra-Zeneca, Eli Lilly, the Janssen-Cilag, Servier, CNPq, CAPES, NARSAD and the Stanley Medical Research Institute; has been a member of the speakers' boards for Astra-Zeneca, Eli Lilly, Janssen and Servier; and has served as a consultant for Servier.

Prof. E. Vieta has received research grants, and served as consultant, advisor or speaker for the following companies: AstraZeneca, Bristol-Myers Squibb, Eli Lilly, Forest Research Institute, Geodon Richter, GlaxoSmithKline, Janssen-Cilag, Jazz, Lundbeck, Merck Sharp & Dohme, Novartis, Otsuka, Pfizer Inc, Sanofi-Aventis, Servier, Takeda, and UBC.

All other authors declare that they have no conflicts of interest.

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**TABLES**

**Table 1:** Sociodemographic and clinical characteristics of patients diagnosed with bipolar disorder and controls.

| | **Patients with BD (n= 115)** | **Controls (n=25)** | ***Stats*** [a] | ***p-value*** |
|---|---|---|---|---|
| ***Demographic variables*** | | | | |
| **Age at enrolment (years)** [c] | 44 (34-54) | 41 (25.5-58.0) | 0.499 | 0.622 |
| **Gender (female)** [b] | 81 (70.4) | 19 (76.0) | 0.312 | 0.635 |
| **Marital status** [b] | | | 1.287 | 0.732 |
| **Stable partner** | 57 (50.0) | 13 (52.0) | | |
| **Single** | 35 (30.7) | 6 (24.0) | | |
| **Divorced** | 18 (15.7) | 6 (24.0) | | |
| **Widow** | 4 (3.5) | 2 (8.0) | | |
| **Time of education (years)** [c] | 9.95 (4.17) | 9.88 (4.46) | -0.8 | 0.937 |
| **Occupational status** [b] | | | 2.421 | 0.298 |
| **Student** | 8 (7.8) | 2 (8.0) | | |
| **Employed** | 36 (35.0) | 12 (48.0) | | |
| **Retired** | 59 (51.3) | 11 (44.0) | | |
| **FAST** [c] | 21.06 (17.0) | 4 (.50-7.50) | 10.354 | 0.0001* |
| ***Clinical variables*** | | | | |
| **Number of episodes** [c] | 13.17 (16.49) | - | - | - |
| **Age at first episode (years)** [c] | 27.34 (12.12) | - | - | - |
| **Age at diagnosis** [c] | 33.39 (12.39) | - | - | - |
| **Time elapsed since first episode (years)** [c] | 16.4 (13.13) | - | - | - |
| **Bipolar Disorder** [b] | | - | - | - |
| **Type I** | 79 (68.7) | | | |
| **Type II** | 18 (15.7) | | | |
| **Unspecified** | 18 (15.7) | | | |
| **Psychiatric comorbidities** [b] | 31 (0.27) | - | - | - |

*p<0.05

a Stats: statistical analysis: Student's-t or $\chi 2$, as appropriate.

b: values are indicated as n (%).

c: values are indicated as mean (SD).

**Table 2:** Serum inflammatory, neurotrophic and oxidative biomarkers according to patient and control positive condition. IL-6 significantly discerned between groups.

| Markers | Patients with BD | Controls | Student's-*t* | *p*-value |
|---|---|---|---|---|
| **IL-2** | 0.007 (0.765) | 0.032 (0.676) | 0.14 | 0.888 |
| **IL-4** | 0.017 (0.847) | 0.068 (0.596) | 0.28 | 0.780 |
| **Il-6** | 0.066 (0.860) | -0.335 (0.801) | -2.13 | 0.035* |
| **Il-10** | 0.003 (.817) | -0.203 (0.543) | -1.12 | 0.266 |
| **Il-17 A** | -0.065 (0.882) | 0.230 (0.708) | 1.47 | 0.145 |
| **INF-gamma** | -0.003 (0.908) | -0.175 (0.940) | -0.82 | 0.412 |
| **BDNF** | -0.046 (0.797) | 0.151 (0.727) | 1.09 | 0.276 |
| **TBARS** | 0.024 (0.980) | -0.232 (0.793) | -1.22 | 0.224 |
| **PCC** | 0.021 (0.773) | 0.057 (0.708) | 0.20 | 0.846 |

*p<0.05

Values were first normalized following the Box-cox transformation and then standardize.

BD: bipolar disorder, IL-2: interleukin 2; IL-4: interleukin 4; IL-6: interleukin 6; IL-10: interleukin 10; IL-17: interleukin 17; INF-gamma; interferon gamma:, BDNF: brain-derived neurotrophic factor; TBARS: thiobarbituric acid reactive substances; PCC: protein carbonyl content.

**Table 3:** Logistic regression assessing the utility of inflammatory, neurotrophic and oxidative biomarkers in a model of staging. IL-6 significantly discerned between patients in early and late stages.

| | Beta | OR | 95% CI | p-value |
|---|---|---|---|---|
| **IL-6** | **0.710** | **2.033** | **1.076-3.843** | **0.029*** |
| **BDNF** | **-0.177** | **0.838** | **0.432-1.624** | **0.600** |
| **TBARS** | **-0.009** | **0.991** | **0.576-1.704** | **0.974** |

*p<0.05

OR: Odds ratio, CI: Confidence interval.

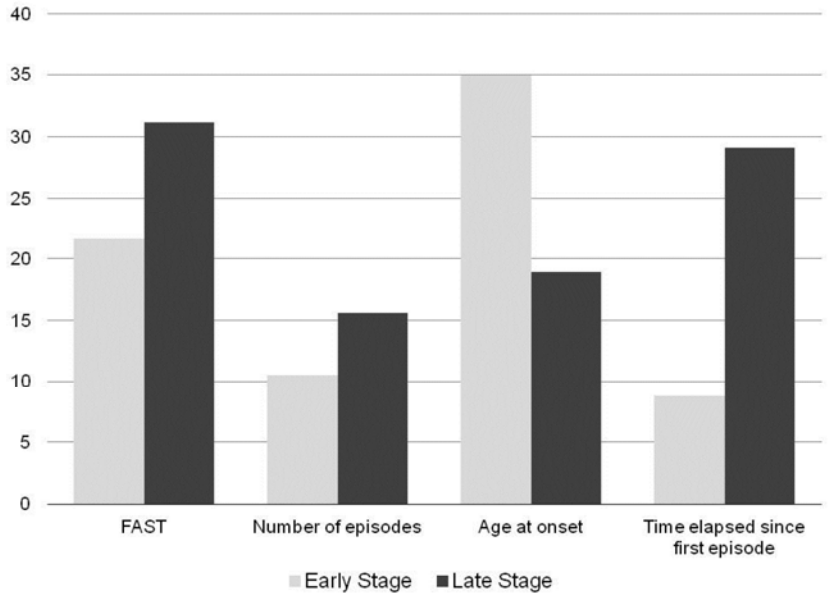
IL: interleukine, BDNF: brain-derived neurotrophic factor, TBARS: thiobarbituric acid reactive substances.

Hosmer and Lemeshow´s test (p=0.784).

**FIGURES**

**Figure 1:** Cluster centers of the variables delimiting early- and late- stages. According to these centers the sample of the study was homogeneously divided into two groups.

FAST: Functioning Assessment Short Test.



## 7.2. Estudi 2

***I Grande, PVS Magalhaes, I Chendo, L Stertz, GR Fries, KM Cereser, ABM Cunha, P Goi, M Kunz, M Udina, R Martín-Santos, BN Frey, E Vieta, F Kapczinski, Val66Met polymorphism and serum brain-derived neurotrophic factor in bipolar disorder: an open-label trial, Acta Psychiatrica Scandinavica (on-line).***

### *7.2.1. Resum de l'estudi 2*

**Introducció:** Els nivells del BDNF es troben disminuïts durant els episodis afectius en el TB. Tanmateix aquesta associació només s'ha evidenciat pràcticament en exclusiva en estudis transversals. En aquest assaig clínic obert a 16 setmanes, preteníem valorar de forma longitudinal la relació entre els nivells sèrics de BDNF i la resposta clínica així com el possible paper predictor del polimorfisme BDNF Val66Met en pacients amb TB que presentaven un episodi afectiu.

**Mètodes:** Es van estudiar 64 pacients diagnosticats de TB lliures de medicació durant un episodi afectiu agut aparellats amb 64 controls sans segons sexe, edat i ètnia. Es va realitzar una avaluació clínica (*Hamilton Depresion Rating Scale* (HDRS), *Young Mania Rating Scale* (YMRS), *Clinical Global Impression scale* (CGI), *Global Assessment of Functioning scale* (GAF)) i la determinació de nivells sèrics de BDNF per ELISA (Millipore, Temecula, USA) a les setmanes 2, 4, 8, i 16. Així mateix, es determinà el polimofisme BDNF Val66Met en el moment basal (5'nucleasa TaqMan Applied Biosystems 7500 Real-Time PCR Systems, Carlsbad, USA). Les anàlisis es realitzaren a partir d'un model d'efecte mixt.

**Resultats:** No es trobaren diferències en els nivells sèrics de BDNF ni en les freqüències genotípiques del polimorfisme BDNF Val66Mel entre el grup de pacients i controls. En el model multivariable es determinà com a predictor de nivells inferiors de BDNF, el fet de ser portador de l'al·lel Met en el polimorfisme BDNF Val66Met comparat amb els pacients homozigots per Val. El fet de no assolir la resposta completa o la remissió es va veure associat a nivells inferiors de BDNF de forma prospectiva (***Figura 5***).

**Conclusions:** Aquest estudi és la primera evidència longitudinal de que tant el polimorfisme BDNF Val66Met així com l'estat de resposta clínica estarien relacionats amb nivells perifèrics de BDNF.

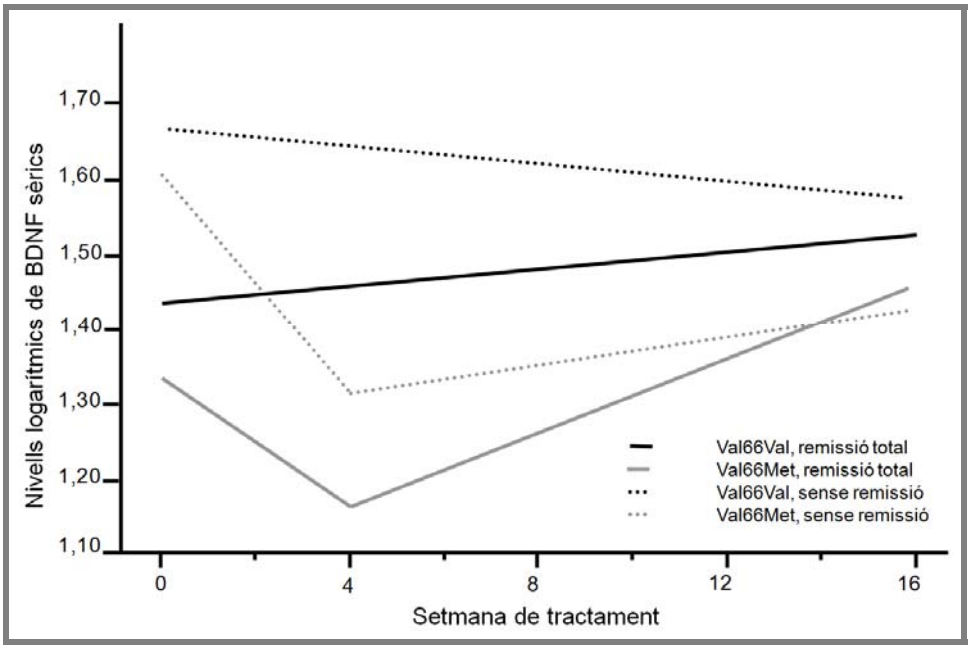



***Figura 5:*** Nivells de BDNF al llarg del seguiment a 16 setmanes segons la resposta clínica i el polimorfisme BDNF Val66Met.

Acta Psychiatrica Scandinavica

Acta Psychiatr Scand 2013: 1–8
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DOI: 10.1111/acps.12192


ACTA PSYCHIATRICA SCANDINAVICA

# Val66Met polymorphism and serum brain-derived neurotrophic factor in bipolar disorder: an open-label trial

Grande I, Magalhães PVS, Chendo I, Stertz L, Fries GR, Cereser KM, Cunha ÂBM, Gói P, Kunz M, Udina M, Martõn-Santos R, Frey BN, Vieta E, Kapczinski F. Val66Met polymorphism and serum brain-derived neurotrophic factor in bipolar disorder: an open-label trial.

**Objective:** Brain-derived neurotrophic factor (BDNF) is consistently associated with acute mood episodes in bipolar disorder, but there is a lack of longitudinal data to support this hypothesis. In this 16-week open-label clinical trial, we tested the predictive role of BDNF Val66Met polymorphism on serum BDNF levels and the relationship of serum BDNF and clinical response in people with bipolar disorder during an acute illness episode.
**Method:** Sixty-four people with bipolar disorder who were medication-free at baseline and in an acute mood episode were recruited. They were matched with 64 healthy controls. Clinical evaluation, serum BDNF, and BDNF Val66Met polymorphism were determined at baseline, and change in serum BDNF was assessed in patients at weeks 2, 4, 8 and 16.
**Results:** There were no differences between patients and controls in serum BDNF or in frequencies of the BDNF Val66Met polymorphism genotype at baseline. The multivariable model showed that Met carriers had a significantly different change in BDNF levels compared with Val homozygotes. Not achieving a complete remission was also associated with lower prospectively assessed BDNF levels.
**Conclusion:** This study provides the first longitudinal evidence that both the BDNF Val66Met polymorphism and remission status predict change in circulating BDNF levels.

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Key words: bipolar disorder; brain-derived neurotrophic factor; biomarkers; treatment response; quetiapine

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Accepted for publication July 11, 2013

**Significant Outcomes**

- People with bipolar disorder had a differential change in peripheral levels of brain-derived neurotrophic factor along the treatment of mood episodes depending on their Val66Met genotype.
- Met carriers of Val66Met genotype had a significantly different trajectory and a tendency for a less substantial increase in peripheral levels than Val homozygotes.
- Change in brain-derived neurotrophic factor levels was associated with treatment response.

**Limitations**

- There were no differences in brain-derived neurotrophic factor levels between patients and controls at baseline; this is possibly due to a floor effect.
- Owing to the naturalistic design of the study, we could not control for the effect of each particular treatment.

## Introduction

Synaptic plasticity and resilience are increasingly recognized as a dimension of vital importance in mood disorders (1, 2). Neuronal survival and apoptosis are regulated by key molecules, among them the class of neurotrophins (3). A neurotrophic model of mood disorders has been actively investigated in the last decade (4). Brain-derived neurotrophic factor (BDNF) has enjoyed particular prominence, mediating neural processes involved in synaptic efficacy and neuroplasticity (5). Consistent study data indicate that BDNF is associated with mood disorders and with the mechanism of action of antidepressants, mood stabilizers, and antipsychotics (6, 7).

Secretion of the BDNF protein can be constitutive, but it is mainly regulated by stimuli (8). Certain functional polymorphisms on *BDNF* gene and epigenetic regulation may determine BDNF secretion (9, 10). Specifically, the valine (Val) to methionine (Met) substitution in the 5' proregion of the human *BDNF* gene has been shown to influence the activity-dependent BDNF secretion and signaling (11). This same polymorphism has been associated with the diagnosis of bipolar disorder (12), rapid cycling (13), and early age of onset (14).

In bipolar disorder, available data largely indicate an association between circulating BDNF levels and acute mood episodes. There is consistent cross-sectional evidence, including meta-analytic data, that episodes of mania and depression are associated with low peripheral BDNF levels (15–17). Considering that serum BDNF levels are decreased during manic and depressive episodes and that the normalization of BDNF levels may be associated with clinical stabilization, we have argued that BDNF is a potentially relevant biomarker of illness activity in BD (18). Nevertheless, these hypotheses have been largely based on case–control studies. To date, only three longitudinal studies to our knowledge have investigated longitudinal BDNF levels in BD. In two studies (19, 20), BDNF levels of patients with BD were lower than those of healthy controls, and the differences in baseline BDNF levels vanished after successful treatment. In the other study (21), the trajectory of BDNF levels during treatment seemed to depend on the polarity of the mood episode.

It is clear from the extant information that longitudinal studies are needed to understand how BDNF changes during treatment and what factors influence this change. Such knowledge is relevant given the effects of neurotrophins on neuroplasticity and ultimately on cognition and functioning (18).

### Aims of the study

In this study, we prospectively investigated the effects of treatment and Val66Met polymorphism on serum brain-derived neurotrophic factor levels in a sample of drug-free patients with BD during an acute mood episode along 16 weeks.

## Material and methods

### Participants

This is an open-label, longitudinal trial with individuals diagnosed with BD and a group of healthy control subjects matched for age, gender, and ethnicity. Recruitment of patients was performed in the Bipolar Disorders Program of Hospital de Clínicas de Porto Alegre and the Hospital Universitario de Santa Maria in Brazil. The recruitment of controls was carried out in the hospital catchment area of Hospital de Clínicas de Porto Alegre. The study was conducted from April 2009 to December 2011. Inclusion criteria were as follows: 18 years of age or older, BD diagnosis, and current manic, mixed, or depressive episode according to DSM-IV-TR criteria. The confirmation of the diagnosis was established by board-certified psychiatrists based on the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I). Patients additionally had to be medication-free, except for benzodiazepines, for at least 2 weeks (6 weeks in the case of fluoxetine or depot antipsychotics). The exclusion criteria were as follows: any DSM-IV disorders not defined in the inclusion criteria comprising drug abuse, history of neurodegenerative disorders and mental retardation, as well as current cancer, chronic or acute infection, unstable medical illness, glucocorticoid treatment, pregnancy or lactation, and participation in a drug trial within 4 weeks prior to enrollment. Controls were 18 years of age or older and were never diagnosed with a psychiatric disorder. Absence of psychiatric morbidity was confirmed by the non-patient version of SCID-I (SCID/NP). Subjects on current pharmacological treatment, smokers, or those with a family history of psychiatric disorder in first-degree relatives were ruled out as controls.

All participants provided written informed consent after receiving a complete description of the study and adequately understanding it. Procedures were approved by the Institutional Review Board of Hospital de Clínicas and were conducted in accordance with the Declaration of Helsinki.

A total of 64 patients with BD diagnosis and 64 healthy controls were enrolled. Patients were

recruited from two similar, but distinct treatment protocols. Patients in an open-label treatment group ($n$ = 31) were medication-free at intake and were medicated at the discretion of the therapist. Patients recruited from a quetiapine extended-release monotherapy open-label trial ($n$ = 33) were also medication-free at intake and received exclusively 300 mg/day of quetiapine extended release. In the event that patients developed severe adverse effects or were clinically deemed to need other treatment, they were withdrawn from the study (ClinicalTrial.gov identifiers: NCT00879632 and NCT00879307).

### Procedure and outcome

A baseline assessment was carried out to obtain sociodemographic, clinical, therapeutic, and functional data using semistructured interview. Symptomatology was assessed with clinical rating scales: the Young Mania Rating Scale (YMRS) for mania, the Hamilton Depression Rating Scale 21-item version (HDRS-21) for depression, and the Clinical Global Impression (CGI) severity scale for overall clinical judgment. Functioning was evaluated with the Global Assessment of Functioning (GAF) scale (22–25). A register of pharmacological treatment was conducted as well.

Longitudinal assessments were performed at weeks 2, 4, 8, and 16. We classified patients as responders if they showed a ≥50% reduction in YMRS and/or HDRS scores from baseline to the final assessment and as remitters those with YMRS and/or HDRS scores ≤8 at the final assessment (26).

### Blood and genetic analysis

Ten milliliters of blood was collected from each subject by venipuncture at baseline and weeks 2, 4, 8, and 16 of the treatment. Five milliliters was introduced into a free-anticoagulant vacuum tube for serum BDNF level analysis and immediately centrifuged at 2000 × $g$ for 10 min. Five milliliters was placed into an ethylenediaminetetraacetic acid (EDTA) vacuum tube for DNA analysis. Serum and total blood were kept frozen at −80°C until further procedures.

Serum BDNF levels were measured with sandwich ELISA, using a commercial kit according to the manufacturer's instructions (Millipore, Temecula, CA, USA). Briefly, 96-well, flat-bottomed microtiter plates were coated for 24 h with the samples diluted 1 : 100. Plates were then washed four times with wash buffer, and monoclonal anti-BDNF rabbit antibody was added (diluted 1 : 1000 with sample diluents) and incubated for 3 h at room temperature. After washing, a second incubation with peroxidase-conjugated anti-rabbit antibody (diluted 1 : 1000) was carried out for 1 h at room temperature. After addition of streptavidin enzyme, substrate, and stop solution, the amount of BDNF was determined (absorbance set at 450 nm). The standard curve demonstrated a direct relationship between optical density and BDNF concentrations. The assay sensitivity for BDNF was 7.8 pg/ml. BDNF values are presented as ng/ml.

Genomic DNA was extracted using standard procedures (27). The genotyping of the BDNF Val66Met polymorphism was performed using a 5' nuclease TaqMan allelic discrimination assay on the Applied Biosystems 7500 Real-Time PCR Systems (Applied Biosystems, Carlsbad, CA, USA). We grouped Met allele carriers (Val/Met and Met/Met genotypes) together for analyses because the rarity of the Met/Met genotype prevents meaningful analysis.

The investigators who performed the laboratory assays were blinded to the clinical status of the subjects.

### Statistical analysis

Twenty-seven patients failed to complete all assessments. All but one had at least one postbaseline BDNF level, with an average of 3.6 observations. BDNF levels were log-transformed. Categorical variables were analyzed using chi-square analysis. The distribution of continuous variables was examined using univariate procedures. We constructed random slope and random intercept mixed effect models to investigate longitudinal changes in BDNF levels and longitudinal clinical variations (28). Residuals were investigated for normality. Mixed models with BDNF polymorphism as a predictor of clinical change in depressive and manic symptoms were used to test these effects controlling for baseline episode. All $P$ values reported were two-tailed. Statistical significance was defined as $P < 0.05$.

## Results

### Demographic and clinical data

Sixty-four patients were included in this trial, of which 48% were in the open-label treatment protocol and other 52% were in the quetiapine extended-release monotherapy protocol. Thirty per cent were in a manic episode, 45% in a depressive episode, and 25% in a mixed episode. Table 1 depicts the demographic profile of the

participants at baseline. No differences were found between patients and controls according to age, gender, and ethnicity. Two patients were dropped out immediately after baseline and were excluded from posterior analyses. Mean body mass index at endpoint was 27.88 with a standard deviation of 4.76 kg/m$^2$.

### Treatment response

There was a significant clinical improvement from baseline to endpoint according to the CGI; that was seen in the whole sample ($Z = 6.10$, $P < 0.001$) and divided according to the polarity of the index episode: mania ($Z = 3.78$, $P < 0.001$), depression ($Z = 3.36$, $P < 0.001$), and mixed ($Z = 3.30$, $P = 0.001$). Figure 1 shows symptom improvement according to the polarity of the index episode. Seventy-seven per cent of those on treatment and 53% of those on quetiapine extended-release monotherapy were responders. Seventy per cent of those on treatment and 38% of those on quetiapine extended-release monotherapy achieved clinical remission.

The BDNF Val66Met genotype frequencies were in Hardy–Weinberg equilibrium in the patient and control groups ($\chi^2 = 1.57$, $P = 0.2109$; $\chi^2 = 0.33$, $P = 0.565$ respectively). No significant differences were found in the frequencies of the BDNF Val66-Met genotypes, in the allele distribution, or in the serum BDNF levels between patients and controls (Table 2). Considering the clinical response, serum BDNF levels were significantly higher at baseline in non-responders compared with controls ($t = 2.11$, $P = 0.044$) and in non-responders compared with responders ($t = 2.76$, $P = 0.008$).

Along the follow-up, no general changes were detected in serum BDNF levels ($Z = 0.67$, $P = 0.501$) (Fig. 2). BDNF levels were consistently lower in patients under the naturalistic protocol compared with the quetiapine extended-release protocol ($Z = 4.32$, $P < 0.001$) and in the Met carrier patients compared with patients with the BDNF Val66Val genotype ($Z = 2.35$, $P = 0.019$). These were the baseline predictors found for BDNF levels. We further tested whether treatment response and remission were associated with changes in BDNF levels. Interestingly, in addition to both being associated with serum BDNF levels, remission was more strongly associated, and the use of remission as a predictor yielded a better model, with significant effects for time and time vs. remission interaction (Table 3). We built a final multivariate model that included BDNF Val66Met polymorphism, protocol followed, remission status, time on treatment, time vs. remission status interaction, and polarity of the index episode. This model revealed significant effects for the BDNF Val66Met genotype ($Z = 2.35$, $P = 0.019$), the protocol followed ($Z = 2.85$, $P = 0.004$), the remission status ($Z = 2.85$, $P = 0.004$), the time ($Z = 2.08$, $P = 0.038$), as well as the remission vs. time interaction ($Z = -2.34$, $P = 0.019$), but not for the polarity of the index episode ($Z = 0.30$, $P = 0.766$).

Table 1. Baseline characteristics of patients

| Variable | Patients with BD ($n = 64$) | |
|---|---|---|
| *Sociodemographic features* | | |
| Gender (woman)* | 40 | 62.5 |
| Age at enrolment (years)† | 37.2 | 11.5 |
| Ethnicity (Caucasian)* | 56 | 87.5 |
| Marital Status* | | |
| Married/Stable partner | 32 | 50.0 |
| Single | 19 | 29.7 |
| Other situation | 12 | 18.8 |
| Educational level (years of study)† | 11.8 | 4.3 |
| Occupational status* | | |
| Working | 32 | 50.0 |
| Student | 7 | 10.9 |
| Unemployed | 25 | 39.1 |
| Body Mass Index (kg/m$^2$)† | 26.5 | 5.0 |
| *Clinical features* | | |
| Bipolar disorder* | | |
| Type I | 46 | 71.9 |
| Type II or NOS | 18 | 28.1 |
| Any psychiatric comorbidity* | 25 | 39.1 |
| Family history* | 38 | 59.4 |
| First episode | | |
| Age (years)† | 23.4 | 10.5 |
| Psychotic features* | 18 | 28.1 |
| Substance-induced mood episode* | 4 | 6.3 |
| Duration of disorder (years)† | 13.5 | 10.3 |
| Total number of episodes† | 10.1 | 10.5 |
| Depressive episodes | 5.5 | 7.9 |
| Manic episodes | 4.6 | 5.2 |
| Scale assessment† | | |
| YMRS | 13.9 | 13.0 |
| HDRS | 16.3 | 10.4 |
| CGI-BD general | 4.5 | 1.5 |
| GAF | 48.8 | 18.9 |
| Episode at study entry* | | |
| Depressive type | 29 | 45.3 |
| Manic type | 19 | 29.7 |
| Mixed type | 16 | 25.0 |
| Protocol followed* | | |
| Treatment as usual | 31 | 48.4 |
| Quetiapine monotherapy | 33 | 51.6 |
| Treatment* | | |
| Mood stabilizers | | |
| Lithium | 20 | 31.3 |
| Valproate | 10 | 15.6 |
| Lamotrigine | 1 | 1.6 |
| Antipsychotics | | |
| Quetiapine extended release | 33 | 51.6 |
| Antidepressants | 4 | 6.3 |
| Benzodiazepines | 26 | 40.6 |

*Values are indicated as *n* (%).
†Values are indicated as mean (SD).
BD, Bipolar disorder; NOS, not otherwise specified; YMRS, Young Mania Rating Scale; HDRS, Hamilton Depression Rating Scale; CGI, Clinical Global Impression scale; GAF, Global Assessment of Functioning scale; ECT, electroconvulsive therapy.

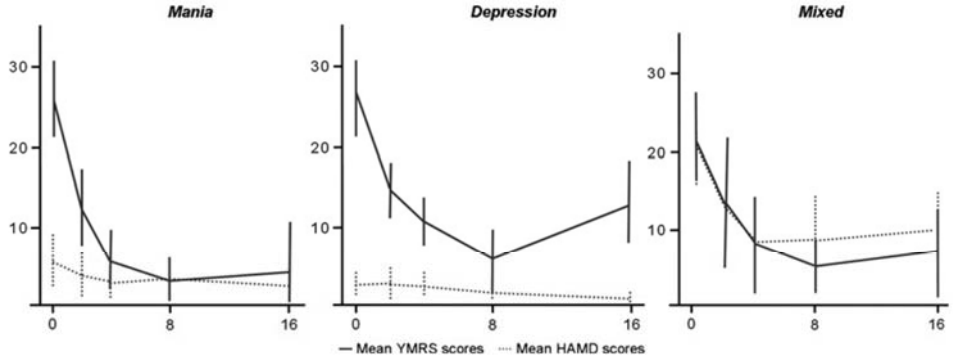


*Fig. 1.* Clinical evolution of the patients according to the polarity of the index episode. YMRS, Young Mania Rating Scale, HAMD, Hamilton Depression Rating Scale.

Table 2. Baseline peripheral brain-derived neurotrophic factor (BDNF) levels and BDNF Val66Met polymorphism in patients with BD and control subjects

| Variable | Patients with BD ($n$ = 62)* | | Controls ($n$ = 62)* | | *Stat*† | *P*-value |
|---|---|---|---|---|---|---|
| *BDNF Val66Met polymorphism* | | | | | | |
| Genotype distribution‡ | | | | | 1.074 | 0.584 |
| Val66Val | 45 | 72.6 | 43 | 67.2 | 1.074 | 0.584 |
| Val66Met | 17 | 27.4 | 18 | 28.1 | | |
| Met66Met | – | – | 1 | 1.6 | | |
| Allele distribution‡ | | | | | 0.127 | 0.722 |
| Val allele | 107 | 86.3 | 104 | 83.9 | | |
| Met allele | 17 | 13.7 | 20 | 16.1 | | |
| Serum BDNF levels§ | 41.5 | 29.8 | 36.4 | 21.2 | 1.243 | 0.219 |

*Two patients and two controls have missing BDNF genotype because of difficulties in the technique.
†*Stats*: statistical analysis: *t*-test or chi-square, as appropriate.
‡Values are indicated as *n* (%).
§Values are indicated as mean (SD).
BD, Bipolar disorder; BDNF, brain-derived neurotrophic factor; Val, valine; Met, methionine.

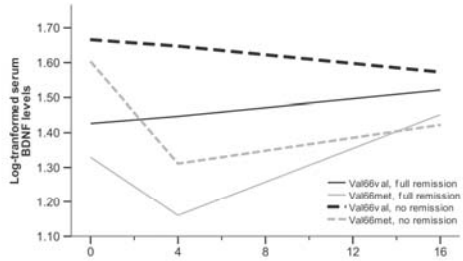


*Fig. 2.* Log-transformed serum brain-derived neurotrophic factor (BDNF) levels along the 16-week follow-up according to clinical remission and BDNF Val66Met polymorphism.

Finally, we tested the effect of the BDNF Val66-Met polymorphism on clinical change. In the multivariate models, BDNF Val66Met polymorphism was not associated with changes in depressive ($Z$ = 0.81, $P$ = 0.416) or manic symptoms ($Z$ = 0.51, $P$ = 0.613) or with significant time interactions.

## Discussion

People with bipolar disorder had a differential change in peripheral levels of BDNF along the treatment of mood episodes depending on their genotype. Met carriers had a significantly different trajectory and a tendency for a less substantial increase in peripheral levels than Val homozygotes. This is the first clinical trial with frequent assessments and a relatively large sample demonstrating such effects in BD.

The BDNF Val66Met polymorphism plays a critical role in the expression of BDNF and prompts cerebral modifications. In a recent study, Matsuo et al. (29) studied the differences between patients with BD and healthy volunteers according to memory function, diagnosis, and BDNF Val66-Met polymorphism. Patients with BD showed smaller regional brain volumes than healthy controls, and Met carriers within the same population were likely to have smaller regional brain volumes as compared with Val homozygotes. In our study, the Met carriers had lower serum BDNF levels at baseline compared with Val homozygotes. This is in line with a study of Rybakowski et al. (30) who assessed the neurocognitive performance in patients with BD compared with schizophrenic and control subjects. Interestingly, the only difference related to the BDNF Val66Met polymorphism in the performance of the Wiscon-

Table 3. Fixed effects and interactions with time of selected predictors of change in serum brain-derived neurotrophic factor (BDNF) levels

| | Fixed effect | Time interaction |
|---|---|---|
| Treatment response | $Z = 2.99$, $P = 0.003$ | $Z = -2.07$, $P = 0.038$ |
| Full remission | $Z = 3.82$, $P < 0.001$ | $Z = -2.19$, $P = 0.028$ |
| Treatment protocol | $Z = 3.51$, $P < 0.001$ | $Z = -0.17$, $P = 0.862$ |
| Antidepressants* | $Z = 0.23$, $P = 0.816$ | $Z = -0.58$, $P = 0.563$ |
| Lithium* | $Z = 0.30$, $P = 0.761$ | $Z = 0.32$, $P = 0.746$ |
| BDNF Val66Met polymorphism | $Z = 2.11$, $P = 0.035$ | $Z = 0.28$, $P = 0.776$ |
| Bipolar disorder type I | $Z = -0.97$, $P = 0.330$ | $Z = -0.25$, $P = 0.800$ |
| Sex | $Z = 0.27$, $P = 0.788$ | $Z = -1.25$, $P = 0.210$ |
| Polarity of index episode | $Z = 0.99$, $P = 0.324$ | $Z = -0.11$, $P = 0.914$ |

*Controlled for treatment protocol.

sin Card Sorting Test was determined in patients with BD, depicting a significantly better accomplishment in subjects with the BDNF Val66Val genotype compared with the BDNF Val66Met genotype. These results may be caused by the effect of the BDNF Val66Met polymorphism on the activity-dependent secretion of BDNF (11), which might be particularly evident during acute affective episodes rather than in euthymia. The dysregulation of BDNF release may also help to explain the evolution of the serum BDNF levels in our study. They varied notably showing an initial slump in the group of Met carriers. All in all, activity-dependent secretion on BDNF might be crucial for clinical response in BD unlike what has been described in unipolar depression in a recent meta-analysis (31).

Previous pilot longitudinal studies of BDNF levels in BD reported an increase in BDNF levels after the patients had received pharmacological treatment. Tramontina et al. (20) assessed serum BDNF levels in 10 manic in-patients before and after a mean of 52 days of treatment. Palomino et al. (19) studied plasma BDNF levels in 14 patients with BD undergoing their first psychotic episode. Both studies reported lower baseline BDNF levels during acute episode compared with controls, with no differences in BDNF levels between patients and controls after acute treatment and after treatment for 6 months respectively. These results are consistent with the literature on major depression, in which an increase in serum BDNF is usually seen with antidepressant treatment (32). In our study, we did not find initial differences in serum BDNF levels between patients and controls as in the study of Rybakowski et al. (33).

Despite the advances achieved in the field of biomarkers in BD, their usefulness related to clinical improvement and treatment response still remains to be elucidated. In a recent open-label study, Grande et al. (21) found a time per mood polarity interaction. Increasing BDNF levels were associated with clinical improvement from a depressive episode, and decreasing BDNF levels were related to clinical improvement from a manic/mixed episode. A dose effect could have influenced outcome as patients were treated with quetiapine extended-release 300 mg/day monotherapy. Higher quetiapine doses may need to be used for manic or mixed episodes. In the present study, achieving both treatment response and full remission determined an increase in serum BDNF levels, regardless of the polarity of the index episode. Similar results have been described in individuals with major depressive disorder. In a 12-week follow-up study, Kurita et al. (34) found increased plasma BDNF levels in patients with depressive syndrome who underwent remission in contrast with non-responders, showing a decrease in plasma BDNF levels during monitoring. Likewise, in a cross-sectional study, Molendijk et al. (35) compared the close link between clinical response and serum BDNF levels in patients with major depressive disorder. Serum BDNF levels were low in patients with a current depressive episode regardless of receiving antidepressant treatment compared with healthy controls. Nevertheless, serum BDNF levels were higher in patients in an acute depression with antidepressants compared with those without. Remarkably, the difference between patients in a current depressive episode and controls became imperceptible after 6 months of having attained sustained full remission, which may indicate the prolonged biological impact of affective symptomatology on BDNF.

Different variables that could have confounded the presented results were inspected. As it has been reported that BDNF decreases along the neuroprogression of the disorder (36), chronicity of the disorder was taken into account. Considering the systemic toxicity of the affective episodes, the total number of episodes was evaluated (37, 38). The high affinity of quetiapine for the serotonin-2A receptor and a subsequent increase in BDNF levels could also be argued as an explanation for the higher BDNF levels in this group (35, 39, 40). Considering the influence of other treatments on the BDNF levels in our study, it is worth mentioning that neither lithium nor antidepressants showed any direct repercussion on these levels. The limited sample size is a plausible cause.

In this study, some limitations should be taken into account. A possible caveat is related to the neuroprogression hypothesis. At this time, there are data suggesting that the biology of BD may vary according to illness stage (41, 42). This may

signify that BDNF levels only drop significantly in certain stages. This may have acted as a floor effect in the current trial, as BDNF levels were not different from healthy controls at baseline. Moreover, assessment of BDNF levels was carried out in the peripheral blood. Despite evidence of correlation between cortical BDNF and serum BDNF and that BDNF crosses the blood–brain barrier, the presumption that such findings might occur in the human brain needs to be confirmed (43). In this regard, studies about postmortem brain tissue and cortical integrity are providing more evidence in this framework. As this is a naturalistic study, diversity in treatment may have had an effect on BDNF levels. Mood stabilizers, antidepressants, and atypical antipsychotics are reported to interfere with pathways related to BDNF. Despite having assessed the treatment with quetiapine extended release, lithium, and antidepressants, other pharmacological strategies were difficult to evaluate due to the reduced sample under these treatments. Nevertheless, the fact that patients were medication-free at baseline mitigates chronic treatment effects, which strengthens the results presented here.

There is growing evidence linking circulating BDNF and systemic toxicity, and perhaps as one of the most relevant mediators of allostatic overload in BD (2, 18, 44). There are several interesting competing hypotheses of how clinical improvement and serum BDNF levels are linked and how polymorphisms can influence these levels. This study suggests that assessment of peripheral BDNF levels may be useful as biomarkers of treatment response. A deeper understanding of the molecular determinants involved in BDNF-signaling cascades may provide a means for monitoring treatment response. Finally, the finding that Met carriers have less pronounced increase in BDNF levels is consistent with previous biological hypotheses and may provide future pharmacogenetic opportunities.

#### Acknowledgements

This study was sponsored by NARSAD and AstraZeneca.

#### Declaration of interest

Dr. I. Grande has received a research grant Río Hortega Contract (CM12/00062), Instituto de Salud Carlos III, Spanish Ministry of Economy and Competiveness, Barcelona, Spain, and has served as a speaker for AstraZeneca.

G.R. Fries has a doctoral scholarship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil.

Dr. B.N. Frey has received research support from CIHR, Father Sean O'Sullivan Research Centre, Hamilton Health Sciences Foundation, Stanley Medical Research Foundation, Eli Lilly, Pfizer, and Bristol-Myers Squibb; has received speaker fees/travel support from AstraZeneca, Lundbeck, and Pfizer.

Professor E. Vieta has received research grants and served as consultant, advisor, or speaker for the following companies: Alexza, AstraZeneca, Bristol-Myers Squibb, Cephalon, Eli Lilly, Forest Research Institute, Gedeon Richter, Glaxo-SmithKline, Janssen-Cilag, Jazz, Lundbeck, Merck Sharp & Dohme, Novartis, Otsuka, Pfizer Inc, Sanofi-Aventis, Servier, Takeda, and UBC.

Professor Kapczinski has received grant/research support from Astra-Zeneca, Eli Lilly, Janssen-Cilag, Servier, CNPq, CAPES, NARSAD, and Stanley Medical Research Institute; has been a member of the speakers' boards for Astra-Zeneca, Eli Lilly, Janssen, and Servier; and has served as a consultant for Servier.

The other authors declare no conflict of interests.

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## 7.3. Estudi 3

***I Grande, F Kapczinksi, L Stertz, GD Colpo, M Kunz, KM Cereser, M Kauer-Sant'Anna, B Frey, E Vieta, PVS Magalhaes, Peripheral brain-derived neurotrophic factor changes along treatment with extended release quetiapine during acute mood episodes: An open-label trial in drug-free patients with bipolar disorder, Journal of Psychiatric Research 2012;46:1511-4.***

### 7.3.1. Resum de l'estudi 3

**Introducció:** Les molècules involucrades en la comunicació interneuronal i l'adaptabilitat de les xarxes neuronals, com el BDNF, són objecte d'estudi en el TB. La quetiapina s'ha demostrat com una efectiva estratègia terapèutica en aquest trastorn tant en el tractament agut com el de manteniment. Donat aquests coneixements, l'objectiu d'aquest estudi fou estudiar de forma prospectiva els canvis en els nivells de BDNF en pacients lliures de mediació que es troben en un episodi afectiu agut i se'ls administra tractament amb quetiapina d'alliberació perllongada al llarg de 16 setmanes de seguiment.

**Mètodes:** Es van estudiar 25 pacients diagnosticats de TB lliures de medicació durant un episodi afectiu agut i aparellats amb controls sans segons sexe, edat i ètnia. Es va realitzar una avaluació clínica (HDRS, YMRS, CGI, GAF) i la determinació de nivells sèrics de BDNF per ELISA (Millipore, Temecula, USA) a les setmanes 2, 4, 8 i 16. Les anàlisis es realitzaren a partir d'un model d'efecte mixt.

**Resultats:** Dels 25 pacients, 17 presentaren a l'inici de l'estudi un episodi agut depressiu i 8, un episodi agut maníac o mixt. Una millora clínica significativa es va observar al llarg de l'estudi. En el model d'efecte mixt, es va determinar un efecte de l'episodi al llarg del temps i una interacció temps per episodi evidenciant que aquells pacients amb un episodi depressiu presentaven un increment dels nivells de BDNF comparat amb els pacients amb un episodi maníac o mixt que mostraven un decreixement (***Figura 6***).

**Conclusions:** Els nivells de BNDF poden ser un biomarcador amb resposta diferencial en funció de la polaritat de l'episodi afectiu.

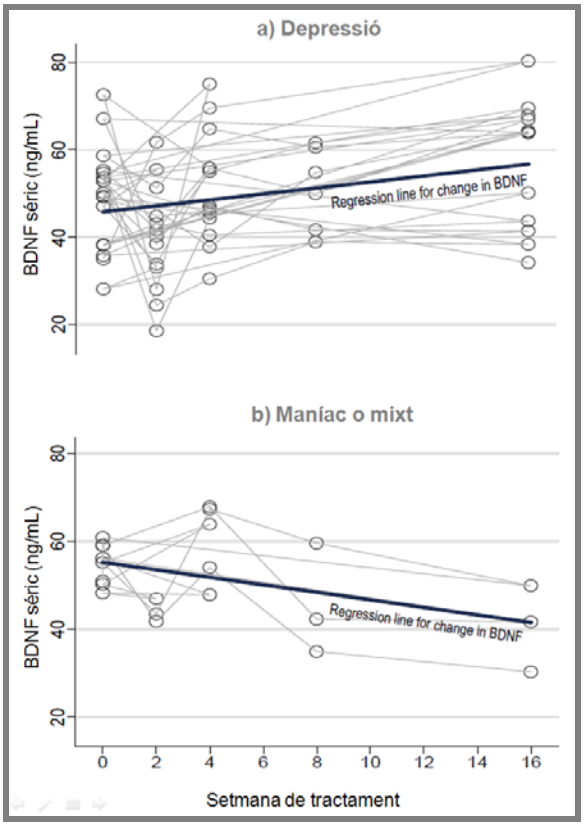


***Figura 6:*** Nivells de BDNF al llarg del seguiment a 16 setmanes en pacients en episodi agut ***a)*** depressiu, ***b)*** maníac o mixt.

Journal of Psychiatric Research 46 (2012) 1511–1514

Contents lists available at SciVerse ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/psychires

# Peripheral brain-derived neurotrophic factor changes along treatment with extended release quetiapine during acute mood episodes: An open-label trial in drug-free patients with bipolar disorder

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ARTICLE INFO

*Article history:*
Received 11 May 2012
Received in revised form
13 July 2012
Accepted 16 August 2012

*Keywords:*
Bipolar disorder
Brain-derived neurotrophic factor
BDNF
Biomarkers
Treatment response
Mania
Depression

ABSTRACT

Molecules that are involved in neuronal intercommunication and adaptability of neural networks, such as brain-derived neurotrophic factor (BDNF), are targets of pathophysiological investigation in bipolar disorder (BD). Quetiapine is an attested treatment in this disorder, used in acute mood episodes. The aim of this study was to report prospective changes in serum BDNF levels in drug-free patients in acute mood episodes of BD who received treatment with extended-release quetiapine along a 16 week follow-up. Assessments were performed at baseline and weeks 2, 4, 8 and 16 with the Young Mania Rating Scale, the Hamilton Depression Rating Scale and the Clinical Global Impression severity scale. In these visits, serum BDNF levels were measured. Mixed effect models were used to investigate longitudinal changes. Twenty-five patients were included for this analysis, seventeen in a current depressive episode and eight in a manic/mixed episode. A significant improvement from baseline to endpoint was displayed. In the mixed model, significant main effects for episode and time appeared, and a time versus episode interaction showing increasing BDNF levels with time in those with a depressive episode, but a decrease in BDNF levels with time in those with a manic/mixed episode. BDNF may be a biomarker with differential response according to the polarity of mood episodes.

Clinical trial: Brain Derived Neurotrophic Factor as a Predictor of Response to Treatment in Bipolar Depression and Mania: 16-weeks Follow-up with Quetiapine XR NCT00879307 http://clinicaltrials.gov/ct2/show/NCT00879307?term=bdnf+quetiapine&rank=1.



## 1. Introduction

In the last decade, neuroplasticity has been put in the central stage in mood disorder research (Duman, 2002; Post, 2007; Manji et al., 2012). Molecules that are involved in neuronal intercommunication and adaptability of neural networks, such as the family of neurotrophins, have become major targets of pathophysiological biomarkers and possible future targets of treatment (Berk et al., 2011a). Among these proteins, brain-derived neurotrophic factor (BDNF) has had special prominence in the study of neuropsychiatric disorders because it is the best characterized member in terms of its role in synaptic plasticity (Grande et al., 2010; Autry and Monteggia, 2012).

In bipolar disorder (BD), there is consistent evidence pointing to impaired BDNF regulation. During acute mood episodes, BDNF levels are low, with a trend to return to normal levels during euthymia (Fernandes et al., 2011). Nevertheless, low BDNF levels have also been associated with a chronic illness course. After multiple episodes, cross-sectional data have revealed significantly low BDNF levels even in the interepisode period (Kauer-Sant'Anna et al., 2009), which is consistent with impaired functioning (Rosa et al., 2012). Preliminary data also indicate that young individuals with bipolar disorder from the general population have normal BDNF levels (Magalhaes et al., 2012).

Notwithstanding the extant evidence, one major caveat in the literature is that most studies are cross-sectional in nature.

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http://dx.doi.org/10.1016/j.jpsychires.2012.08.017

Longitudinal data are needed to demonstrate individual change, and in bipolar disorder this evidence is very limited (Palomino et al., 2006; Tramontina et al., 2009). In addition to that, little is known about the role that pharmacological treatment may play. Quetiapine is an antipsychotic widely used for acute mood episodes in bipolar disorder because of convincing evidence of its efficacy (Tamayo et al., 2010; Vieta et al., 2010). Most recent guidelines recommend its use as first line treatment (Yatham et al., 2009). Relevant to the issue here, quetiapine has been reported, in pre-clinical investigations, to increase BDNF mRNA levels in the gyrus dentatus and the hippocampus (Fumagalli et al., 2004; Park et al., 2006) and to enhance hippocampal cell proliferation in monotherapy and combination with antidepressants, suggesting neuroprotective potential (Luo et al., 2005; Xu et al., 2006). In patients with treatment-resistant depression, increasing levels of peripheral BDNF have also been determined after addition of a low dose of quetiapine to an antidepressant (Yoshimura et al., 2010). Furthermore, quetiapine may be safer than other antipsychotics as regards to its effects on neurocognition (Torrent et al., 2011).

In this study, we report prospective changes in serum BDNF levels in patients with acute mood episodes of bipolar disorder. All patients were medication-free with the exception of as needed use of benzodiazepines at baseline. The standardized quetiapine treatment allowed us to investigate the evolution of this important neurotrophin's levels over the course of 16 weeks.

## 2. Methods

This is an open-label longitudinal trial, conducted from April 2009 to September 2011. Recruitment of outpatients diagnosed with BD was performed at a tertiary care facility, the Bipolar Disorders Program (Hospital de Clínicas in Porto Alegre, Brasil). Diagnosis was confirmed according to the Structured Clinical Interview for DSM-IV-TR Axis I (SCID-I) (First et al., 2002) by board-certified and trained psychiatrists. Patients had to be suffering from an acute mood episode, also confirmed with the SCID. Exclusion criteria were: history of neurodegenerative disorders, mental retardation, current cancer, chronic or acute infection, glucocorticoid treatment and participation in another drug trial within 4 weeks prior to enrollment. Patients additionally had to be off psychotropics (except for benzodiazepines) for at least 2 weeks (6 weeks for fluoxetine or depot antipsychotics). All participants provided written informed consent after receiving a complete description of the study. Procedures were approved by the Ethical and Research Committee of Hospital de Clínicas. Current symptoms were assessed with the Young Mania Rating Scale (YMRS) (Young et al., 1978) for mania and the Hamilton Depression Rating Scale (HDRS) (Williams, 1988) for depression and the Clinical Global Impression (CGI) severity scale (Guy, 1976) for overall clinical judgment. Follow-up assessments were performed at weeks 2, 4, 8 and 16 after baseline. Patients were treated with quetiapine extended-release 300 mg/day monotherapy. Pharmacological treatment was revised and reassessed in each visit and modified when required. If patients developed severe adverse effects or were clinically deemed to need other treatments, they were withdrawn from the study.

Patients were classified as responders if they showed a reduction from baseline in YMRS and/or HDRS score $\geq$ 50% at the final assessment (Tohen et al., 2009). Number of previous manic and mixed episodes was assessed; an effect of multiple episodes was tested as having less than 10 or ten or more episodes. Although imperfect, this classification has demonstrated previous prognostic validity in clinical studies as a marker of stage of illness in BD (Berk et al., 2011b).

Ten milliliters of blood were withdrawn from each subject by venipuncture at baseline and weeks 2, 4, 8 and 16 of the treatment. Serum BDNF levels were measured with sandwich-ELISA, using a commercial kit according to the manufacturer's instructions (Millipore, Temecula, USA). The standard curve demonstrated a direct relationship between optical density and BDNF concentrations. The assay sensitivity for BDNF was 7.8 pg/ml. BDNF values were presented as ng/ml. The investigators who performed the analysis were blind to the clinical status of the subjects.

Eight patients failed to complete all assessments (two due to significant sedation, five due to inefficacy and one due to poor adherence). We use the nonparametric Wilcoxon signed-rank test to test for baseline to endpoint differences in rating scale scores. BDNF levels were normally distributed according to the Shapiro–Wilk test ($p = 0.99$). To investigate longitudinal changes in BDNF levels, random-slope and random-intercept mixed effect models were constructed (Willett et al., 1998). Because of the limited sample size, only a few predictors were tested, one at a time. We also report interactions with time for these same predictors.

## 3. Results

Twenty-five patients were included for this analysis, seventeen in a current depressive episode and eight in a manic or mixed episode (Table 1). Four patients, three in a depressive episode and one in a mixed episode, were taking benzodiazepines in combination with quetiapine. Patients displayed significant improvement from a median of 4 to a median of 2 on the CGI from baseline to endpoint ($Z = 2.92$, $p = 0.004$); for those with an index episode of depression, CGI dropped from 4 to 2, and for those on manic or mixed episodes from 4.5 to 3.5. Eight patients with an index depressive episode and three patients with a manic or mixed episode had a positive treatment response as defined above.

Mean baseline and endpoint BDNF levels were 49.33 (SE 2.81) ng/mL and 57.02 (SE 4.27) ng/mL, for those with an initial depressive episode and 54.93 (SE 1.66) ng/mL and 40.60 (SE 5.67) ng/mL for those with an initial manic or mixed episode. The initial mixed model did not reveal a significant change in BDNF levels related to week of treatment ($B = 0.32$, SE = 0.28, $p = 0.256$). When the index episode was entered in the model, however, significant main effects for episode ($B = 9.50$, SE = 3.55, $p = 0.007$) and time appeared ($B = 0.66$, SE = 0.29, $p = 0.024$), as well as a time versus episode interaction ($B = -1.55$, SE = 0.63, $p = 0.015$). Graphical inspection of the data demonstrated the interaction indicated: a significant increase of BDNF levels with time in those with a depressive episode ($r = 0.30$, $p = 0.019$), but a significant decrease in BDNF levels with time in those with a manic or mixed episode ($r = -0.47$, $p = 0.029$) (Fig. 1). The other predictors (treatment response, illness stage and sex) were not associated with change in BDNF levels (Table 2).

**Table 1**
Demographic and clinical characteristics of the sample at baseline according to index episode.

| | Depression ($n = 17$) | Manic/mixed ($n = 8$) |
|---|---|---|
| Female sex | 77% | 50% |
| Age | 36 (30–50) | 42 (29–55) |
| Years of education | 13 (12–17) | 14 (10–16) |
| Bipolar I disorder | 59% | 100% |
| Baseline CGI | 4 (3–5) | 4.5 (3–5) |
| Baseline HAM-D | 22 (18–26) | 22 (10–27) |
| Baseline YMRS | 0 (0–3) | 16 (13–20) |
| Number of previous episodes | 15 (6–20) | 14 (5–22) |

Shown as median (interquartile range).

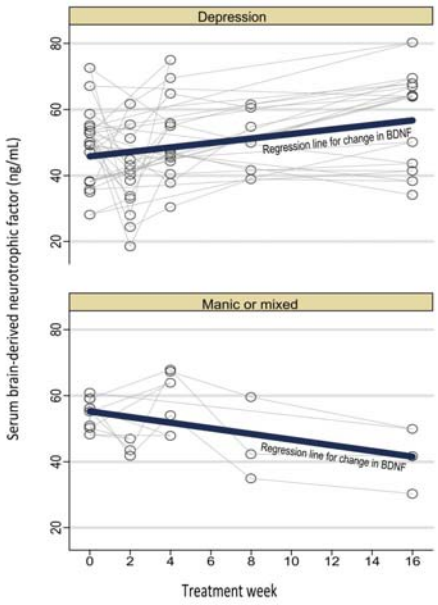


**Fig. 1.** Change in peripheral brain-derived neurotrophic factor levels in patients with depressive or manic/mixed index episode during the 16 week follow-up.

## 4. Discussion

As far as we know this is the first study to report prospective changes in peripheral BDNF levels in individuals who were initially drug-free in a manic, mixed or depressive episode and received treatment. While we initially expected that treatment would be associated with raising levels, the study revealed that mood episode polarity was a significant effect modifier. BDNF levels increased in patients with depression but decreased in patients in manic or mixed episode.

Why polarity of the index episode would have an influence on serum BDNF level variations after initial treatment is a question to unravel. These differences could be due to a number of factors. One possibility for explaining continuing low BDNF levels in mania is the prominent toxicity of the manic episode. In our study, although both groups presented with similar rates in the HAM-D score at baseline, the manic symptomatology seem to prompt BDNF levels variations. Albeit the biological evidence is limited, mania may be associated with greater systemic dysfunction (Kapczinski et al., 2011), including DNA damage (Andreazza et al., 2007) and oxidative stress (Andreazza et al., 2008). A clinical parallel to these findings would be the protracted cognitive and functional deficits often observed in patients who have undergone manic episodes (Conus et al., 2006; Grande et al., 2012; Sole et al., 2012). The number of manic episodes has been studied to be a predictor of poor cognitive performance, suggesting that the recurrence of mania may have a long-term neuropsychological impact (López-Jaramillo et al., 2010). Moreover, our findings in BD patients with depression are in accordance with results in longitudinal follow-up conducted in patients diagnosed with unipolar depressive disorder in a depressive episode (Yoshimura et al., 2007).

**Table 2**
Fixed effects and interactions with time of selected predictors of change in brain-derived neurotrophic factor (BDNF) levels.

| | Fixed effect | Time interaction |
|---|---|---|
| Index episode | $Z = 2.68$, $p = 0.007$ | $Z = 2.44$, $p = 0.015$ |
| Sex | $Z = 0.45$, $p = 0.656$ | $Z = 1.29$, $p = 0.197$ |
| Illness stage | $Z = 0.23$, $p = 0.0819$ | $Z = 0.96$, $p = 0.338$ |
| Treatment response | $Z = 0.87$, $p = 0.386$ | $Z = 0.30$, $p = 0.766$ |

Illness stage defined as less than 10 or ten or more previous episodes.

This study evidences some shortcomings. Firstly, as a preliminary study, the sample size is limited. As such, interesting associations between predictors of BDNF change were restricted and we employed rather simple models. Secondly, the open-label nature of the trial means we cannot separate the effect of use of quetiapine from the effect of illness course. Whether variations in peripheral BDNF levels were directly related to clinical improvement or treatment with quetiapine is difficult to discern. It is important to remark that the response rate was lower than in large previous clinical trials (Chiesa et al., 2012). In addition, a dose effect could have influenced on the different outcome as well, since higher quetiapine doses may be used for manic or mixed episodes (Vieta, 2005). Notwithstanding the fact that quetiapine has shown efficacy in mania and depression, this study was not designed as an efficacy trial. Thus, quetiapine was specifically selected as monotherapy because of preclinical suggestions of an effect in BDNF and neuroplasticity (McIntyre et al., 2007). Lastly, the BDNF levels were assessed peripherally. To what extent peripheral BDNF levels represent effective central nervous system levels versus other sources of the protein remains to be elucidated. However, preclinical studies have reported positive correlations between serum and cortical BDNF levels (Karege et al., 2002), as well as the influence of peripheral BDNF on brain behavioral and cellular functions (Schmidt and Duman, 2010). Moreover, serum BDNF levels have been related to neuronal integrity in spectrophotometry in healthy subjects (Lang et al., 2007).

The current study offers a suggestion on how BDNF levels may vary during pharmacological treatment. Our findings support the notion that pathways related to BDNF and neuroplasticity are associated with the pathophysiology of mood disorders. Larger controlled studies are warranted to elucidate relationships between peripheral biomarkers and bipolar disorder course.

### Role of the funding sources

This study was sponsored by an unrestricted study grant from AstraZeneca Brazil.

### Contributors

Flávio Kapczinski, Keila Mendes Cereser, Marcia Kauer-Sant'Anna, Benicio Frey designed the study and wrote the protocol. All the authors managed the literature searches and analyses. Pedro VS Magalhaes and Iria Grande undertook the statistical analysis, and wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

### Author disclosures

Prof. F. Kapczinski has received grant/research support from Astra-Zeneca, Eli Lilly, the Janssen-Cilag, Servier, CNPq, CAPES, NARSAD and the Stanley Medical Research Institute; has been a member of the speakers' boards for Astra-Zeneca, Eli Lilly, Janssen and Servier; and has served as a consultant for Servier.

Dr. Frey has received grant/research support from CIHR, Father Sean O'Sullivan Research Centre, Stanley Medical Research Foundation, Hamilton Health Sciences Foundation, Eli Lilly, Wyeth, and Bristol-Myers Squibb; has received speaking fees from AstraZeneca and Bristol-Myers Squibb; and has received travel support from Pfizer.

Prof Kauer-Sant'Anna has received research grants from CNPq-INCT-TM, CNPq Universal, CAPES, SMRI, NARSAD, Astra-Zeneca, and Eli Lilly.

Prof. E. Vieta has received research grants, and served as consultant, advisor or speaker for the following companies: AstraZeneca, Bristol-Myers Squibb, Eli Lilly, Forest Research Institute, Gedeon Richter, GlaxoSmithKline, Janssen-Cilag, Jazz, Lundbeck, Merck Sharp & Dohme, Novartis, Otsuka, Pfizer Inc, Sanofi-Aventis, Servier, Takeda, and UBC and research funding from the Spanish Ministry of Health, the Spanish Ministry of Science and Education, the Stanley Medical Research Institute and the 7th Framework Program of the European Union.

All other authors declare that they have no conflicts of interest.

## Acknowledgments

Dr. I. Grande has received a research grant from Hospital Clínic de Barcelona (HCB). Dr. Magalhães is supported by a post-doctoral scholarship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (Brazil).

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# 8. Discussió

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## 8.1. Resultats més rellevants

En aquesta tesi doctoral per compendi d'articles hem aportat evidència sobre un tema de vigent actualitat i altament debatut en el camp de la psiquiatria i, en concret, en el TB, com és l'ús de l'estadificació i la utilitat dels biomarcadors.

En l'***estudi 1***, realitzem una proposta d'estadificació amb uns barems, per tant dades numèriques, que ens permeten diferenciar grups de pacients des d'una perspectiva terapèutica i pronòstica diferencial. Mentre que els pacients en l'estadi definit com a "*primerenc*" presentaven menor nombre d'episodis, millor funcionament en la vida diària, major edat a l'inici del TB i major temps d'evolució de la malaltia, els pacients compresos en l'estadi "*tardà*" mostraven resultats diametralment oposats en aquestes variables. A més a més, aquesta classificació s'ha vist relacionada amb un biomarcador lligat a la inflamació, la IL-6.

En el TB, no només la IL- 6 s'ha establert com un potencial objectiu en l'estudi dels biomarcadors. El BDNF, llargament estudiat en els mecanismes de memòria a llarg termini a nivell hipotalàmic, també sembla jugar un paper en les malalties neuropsiquiàtriques. En els ***estudis 2 i 3*** d'aquesta tesi doctoral, el BDNF ha estat avaluat de forma longitudinal en una mostra de pacients amb TB que presentaren un episodi afectiu agut. S'ha vist que els nivells sèrics de BDNF variaven al llarg del tractament farmacològic en funció de la resposta al tractament, de ser portador de l'al·lel Met en el polimorfisme BDNF Met66Val, del tipus de tractament farmacològic administrat i de la polaritat de l'episodi inicial.

### 8.2. Estadificació

Des del nostre punt de vista, abastar la complexitat d'un trastorn psiquiàtric, com el TB i el procés neuroprogressiu que comporta en tan sols una variable pot arribar a no recollir tots els trets i particularitats de la malaltia. Les trajectòries de la malaltia varien enormement: existeixen pacients amb una òptima recuperació tot i un reguitzell de descompensacions clíniques a diferència de pacients que amb un episodi ja mostren afectació de per vida. El mateix s'evidencia amb la cronicitat, ja que aquesta no és definitòria d'una evolució més tòrpida. Per aquest motiu, en l'***estudi 1*** es va preferir recollir informació sobre diversos ítems clínics analitzats en la literatura sobre el model d'estadificació en el TB com el funcionament en la vida diària, el número d'episodis, l'edat a l'inici de la malaltia i el temps d'evolució de la malaltia per tal de definir millor una proposta d'estadificació basada en criteris clínics.

En base al funcionament, recentment Reinares i col.[64] han proposat una classificació d'estadificació diferenciant els pacients amb alt rendiment dels de baix rendiment. A partir d'una anàlisi latent de classe que delimità aquests dos grups de funcionament, es definiren com a predictors de funcionament a) *variables relacionades amb la gravetat clínica* com el nivell de símptomes depressius residuals i la densitat d'episodis, és a dir, la quantitat d'episodis en un any i, b) *variables neuropsicològiques* com la intel·ligència verbal estimada i el control inhibidor. Durant la recent elaboració de les recomanacions del grup de treball de la Societat Internacional de Trastorns Bipolars sobre un model d'estadificació en el TB s'ha debatut sobre el perill de caure en circularitats tautològiques en l'ús de variables com un baix rendiment cognitiu així com l'evolució tòrpida de la malaltia.

L'equip australià dirigit per Michael Berk ha treballat força al voltant del model d'estadificació basant-se en una classificació segons el número d'episodis. A partir de les dades de 12 assajos clínics controlats, randomitzats finançats per Eli Lilly&Co. per avaluar l'efectivitat d'olanzapina en episodis maníacs, depressius i en tractament de manteniment[52], Berk i col. dividiren la mostra en aquells pacients que havien presentat entre 1-5 episodis, entre 6-10 i més de 10. Les taxes de milloria en la majoria de mesures de resposta en mania eren majors en el grup entre 1-5 episodis podent arribar al doble comparat amb la resta de grups. Quant als estudis de depressió, les taxes de resposta eren significativament majors en el mateix grup, tanmateix només per a dues mesures d'avaluació. Pel que fa al tractament de manteniment, la probabilitat de recaiguda a mania o depressió es va reduir en un 40-60% en aquells pacients que presentaren 1-5 episodis o 6-10 episodis comparat amb aquell grup de pacients que havien patit més de 10 episodis, mostrant una diferència significativa només en les recaigudes maníaques en el grup de 1-5 episodis comparat amb el que havien patit més de 10 episodis. Seguint el mateix patró, Magalhaes i col.[65] van analitzar les dades obtingudes en l'estudi obert, prospectiu, multicèntric *Systematic Treatment Enhancement Program for Bipolar Disorder* (STEP-BD) seleccionant aquells pacients en un episodi agut depressiu que participaven en un assaig clínic amb tractament antidepressiu adjuvant[66]. Els pacients amb múltiples episodis presentaven, de forma consistent, pitjors resultats tant transversalment en forma de major discapacitat i menors dies d'estabilitat clínica, com prospectivament, en l'avaluació de símptomes depressius, maníacs, de qualitat de vida i de funcionament. Tanmateix, no s'evidenciava que la resposta antidepressiva variés en funció del nombre d'episodis afectius soferts. Per contra, en l'estudi clàssic de Swann, sí s'evidencià diferència en la resposta al liti en funció

del número d'episodis[51]. Rosa i col.[67] també van avaluar la funcionalitat a partir del número d'episodis evidenciant una major recuperació al llarg d'un any en termes generals en aquells pacients que havien presentat un únic episodi comparat amb aquells pacients amb un mínim de dos episodis afectius en la seva història clínica.

L'edat precoç a l'inici de la malaltia és una variable que s'ha associat *per se* a una major gravetat i pitjor funcionament diferenciant–la de les repercussions que té, de forma independent, la cronicitat de la malaltia[68, 69]. Per aquest fet, s'ha proposat l'edat precoç d'inici de la malaltia bipolar com un especificador de curs[70]. En l'estudi de Perlis i col.[68], els 1000 primers pacients consecutius de l'estudi STEP-BD foren classificats en inici *molt precoç*, abans dels 13 anys; *precoç*, entre els 13 i els 18; i inici *a l'edat adulta*, a partir dels 18. L'inici precoç es va associar a una major taxa de trastorns comòrbids d'ansietat i de substàncies tòxiques, major nombre de recurrències, períodes més curts d'eutímia, major probabilitat d'intents suïcides, de violència i major probabilitat de trobar-se en episodi afectiu agut a l'inici de l'estudi.

Considerant el temps de malaltia, Kauer-Sant'Anna i col.[37] van utilitzar aquest criteri per indagar en les bases biològiques del TB determinant nivells sèrics de factors inflamatoris i de BDNF en pacients que es trobaven en els primers tres anys després del primer episodi maníac comparat amb aquells pacients en què havien transcorregut, com a mínim, 10 anys des del diagnòstic de la malaltia. En aquest estudi es va observar que la cronicitat de la malaltia involucrava una menor capacitat neuroplàstica i una menor protecció vers la inflamació. La mateixa classificació de pacients en funció del temps d'evolució de la malaltia va ser utilitzada per Andreazza i col.[44] descrivint un disbalanç prooxidant-antioxidant al llarg dels anys.

### 8.3. Biomarcadors

En les diferents branques de la medicina, els biomarcadors han esdevingut de gran utilitat tant pel diagnòstic com pel pronòstic de les malalties. En els darrers anys s'estan realitzant avenços en aquesta vessant de la psiquiatria, i en concret del TB. El coneixement actual de la neurobiologia del TB s'ha desplaçat des del focus inicial del dèficit monoaminèrgic a la vigent evidència de canvis intracel·lulars que comporten repercussions sistèmiques a nivell neuroplàstic, oxidatiu i inflamatori. Aquest model, comprensible, és al mateix temps, capaç d'explicar particularitats clíniques del TB com el progressiu escurçament de l'interval interepisòdic entre cada recurrència i la reducció en la probabilitat de resposta al tractament al llarg de la progressió de la malaltia. Dades emergents en aquest terreny mostren que els biomarcadors varien al llarg de la malaltia en paral·lel a canvis neuroestructurals i neurocognitius. La comprensió dels mecanismes a nivell cel·lular faciliten així un fonament sobre possibles dianes terapèutiques i el desenvolupament de nous grups farmacològics.

L'IL-6 es va identificar en l'***estudi 1*** com un biomarcador viable en la caracterització de la progressió de la malaltia. Els nivells perifèrics d'IL-6 incrementaven al llarg de l'espectre del TB des dels familiars de primer grau de pacients diagnosticats de TB passant pels pacients en un estadi primerenc fins als pacients en un estadi més avançat. En el model d'estadificació basat en la cronicitat de la malaltia, Kauer-Sant'Anna i col.[37] també van determinar diferències en la IL-6 entre els estadis primerenc i tardà al igual que entre els estadi primerenc i tardà comparat amb el grup control. Considerant el paper de l'IL-6 en la neuroprogressió del TB, s'ha suggerit el potencial terapèutic de l'antagonisme de

l'IL-6[71]. Considerant l'autoimmunitat, estudis a nivell bàsic han mostrat el paper crucial de IL-6 en la inducció de la inflamació autoimmune crònica induint la diferenciació dels limfòcits T d'ajuda específics I[71].

Pel que fa a l'estrès oxidatiu, no vam trobar diferències al llarg del model d'estadificació proposat. Andreazza i col., utilitzant la variable del temps de la malaltia, van trobar alteracions en les reaccions d'oxidació-reducció de l'òxid nítric i la nitració de la tirosina[44]. En els pacients amb TB, tant en un estadi primerenc com tardà, es determinà un increment dels nivells de nitrotirosina-3 comparat amb el grup control. En el cas de l'activitat de la glutatió reductasa i la glutatió S-transferasa, aquestes es van trobar incrementades en aquells pacients en l'estadi tardà de la malaltia. D'altra banda, en un estudi recent basat en població general, només es va determinar dany oxidatiu proteic en aquells adults diagnosticats de TB comparat amb controls sans[72].

Quant a la darrera via suggerida en la neuroprogressió en el TB[1], no vam trobar canvis en els factors neurotròfics al llarg dels diferents estadis en l'***estudi 1***. Al contrari, Kauer-Sant'Anna i col. van descriure diferències en els nivells perifèrics de BDNF entre aquells pacients en l'estadi tardà comparat amb aquells en l'estadi primerenc i el grup control[37]. Tanmateix en l'***estudi 2*** i ***3*** sí que van evidenciar el possible potencial del biomarcador BDNF en la situació d'estat, en aquest cas d'episodi afectiu. En l'***estudi 2,*** tot i no evidenciar-se canvis en els nivells perifèrics de BDNF al llarg del seguiment a 16 setmanes, es va demostrar que tant el polimorfisme BDNF Val66Met com presentar resposta clínica al tractament influïen en la trajectòria dels nivells de BDNF. En concret, els portadors de Met presentaven nivells sèrics de BDNF menors al homozigots per Val a l'inici de l'estudi.

El polimorfisme BDNF Val66Met juga un paper fonamental en l'expressió del BDNF i, en conseqüència, en la neuroplasticitat cerebral. En un estudi recent, Matsuo i col.[73] estudiaren les funcions neurocognitives i el polimorfisme BDNF Val66Met en pacients amb TB comparat amb voluntaris sans. Els pacients amb TB mostraren regions cerebrals de menor volum que els controls sans. A més a més, aquells portadors de l'al·lel Met en ambdós grups presentaren volums cerebrals menors comparat amb els homozigots per Val. En la mateixa línea, Rybakowski i col.[74] avaluaren l'execució neurocognitiva en pacients amb TB comparada amb pacients diagnosticats d'esquizofrènia i subjectes sans. El polimorfisme BDNF Val66Met determinà que, només en els pacients amb TB, el genotip BDNF Val66Val s'associava a una millor execució en les tasques neuropsicològiques comparat amb els pacients amb TB amb el genotip BDNF Val66Met. Aquests resultats es poden explicar pel possible efecte del polimorfisme BDNF Val66Met en la secreció dependent d'activitat de BDNF que podria tenir pes, particularment, en un episodi afectiu més que en l'eutímia[75]. La disregulació en la secreció de BDNF per part del polimorfisme BDNF Val66Met pot explicar també la davallada inicial dels nivells sèrics de BDNF en el grup de portadors de l'al·lel Met en l'***estudi 2***. En definitiva, la secreció dependent d'activitat sembla ser crucial en la resposta clínica dels pacients amb TB a diferència dels resultats obtinguts en una recent meta-anàlisi en depressió unipolar[76].

Estudis pilots longitudinals previs sobre els nivells de BDNF en el TB descriuen un increment dels nivells de BDNF després que els pacients hagin rebut tractament farmacològic. Tramontina i col.[75] valoraren els nivells sèrics de BDNF en 10 pacients maníacs ingressats abans de l'admissió i un cop transcorregut una mitjana de 52 dies del tractament. En l'estudi dut a terme per Palomino i col.[77]

s'estimaren els nivells plasmàtics de BDNF en 14 pacients amb TB amb un primer episodi psicòtic. En ambdós estudis, es detectaren nivells de BDNF més baixos comparat amb els dels controls a l'inici de l'estudi, és a dir, durant l'episodi agut, sense mostrar diferències en els nivells de BDNF entre pacients i controls després del tractament agut i després de 6 mesos, respectivament. Aquests resultats són congruents amb els descrits en la literatura sobre depressió unipolar on, un increment dels nivells sèrics de BDNF segueix, de forma habitual, al tractament antidepressiu[78]. En els nostres ***estudi 2*** i ***3***, no vam trobar diferències inicials en els nivells sèrics de BDNF entre pacients i controls, com s'ha descrit en l'estudi de Rybakoswki i col.[79].

Tot i els avenços aconseguits en el camp dels biomarcadors en el TB, la seva possible utilitat com a predictor de milloria clínica i de resposta al tractament encara roman sense aclarir. En l'***estudi 3***, es va trobar una interacció entre temps i polaritat de l'episodi. La millora clínica des de la depressió es va associar a nivells incrementals de BDNF mentre que la millora clínica des de la mania o episodi mixt es va relacionar amb nivells decrementals de BDNF. Una possible explicació dels nivells baixos de BDNF en la mania és l'elevada toxicitat determinada en aquests episodis. Tot i que l'evidència a nivell biològic és limitada, la mania està associada a major afectació sistèmica[80], dany del DNA[81] i dany secundari a l'estrès oxidatiu[82]. El paral·lelisme clínic en aquestes troballes són els extensos dèficits, tant cognitius com funcionals, observats en pacients que han patit episodis maníacs[25, 83, 84]. El nombre d'episodis maníacs s'ha considerat com un predictor de baix rendiment cognitiu, suggerint que els episodis recurrents de mania repercuteixen a nivell neuropsicològic[85]. D'altra banda, els resultants obtinguts en la polaritat depressiva es troben en concordança amb els resultats descrits en el seguiment longitudinal

de pacients diagnosticats de trastorn depressiu unipolar durant un episodi depressiu agut[86]. En l'*estudi 2*, el fet d'assolir, tant resposta al tractament com remissió clínica, determinaren un increment en els nivells sèrics de BDNF, de forma independent a la polaritat de l'episodi índex. Resultats similars en funció de la resposta clínica s'han obtingut en mostres de pacients amb el diagnòstic de trastorn depressiu major. En el seguiment a 12 setmanes realitzat per Kurita i col.[87] es determinà un increment dels nivells plasmàtics de BDNF en aquells pacients amb un síndrome depressiu que assolien remissió a diferència d'aquells pacients no responedors que mostraren una disminució dels nivells plasmàtics de BDNF durant el monitoratge. Així mateix, en l'estudi transversal de Molendijk i col.[88] es comparà l'estreta relació entre resposta clínica i nivells sèrics de BDNF en pacients amb trastorn depressiu major. Els nivells sèrics de BDNF van ser menors en aquells pacients amb un episodi depressiu agut, amb independència de rebre tractament antidepressiu, comparat amb els controls sans. Tanmateix, els nivells van ser majors en aquells pacients en episodi depressiu que rebien tractament comparat amb aquells que no en rebien. Convé ressaltar que, la diferència en els nivells de BDNF trobada entre els pacients en episodi depressiu agut i els controls esdevenia imperceptible després de 6 mesos d'haver assolit la remissió sostinguda completa. Aquesta troballa pot indicar un efecte biològic perllongat de la simptomatologia afectiva sobre els nivells de BDNF.

### *8.4. Limitacions i punts forts de l'estudi*

Els estudis que conformen aquesta tesi doctoral tenen una sèrie de limitacions que cal esmentar.

En l'*estudi 1* no es van excloure els pacients amb simptomatologia subsindròmica. Tanmateix s'ha de tenir en compte que al llarg de la neuroprogressió de la malaltia i, sobretot, en els estadis tardans, la remissió clínica és difícilment assolible[89, 90]. Per tant, excloure aquests pacients hauria alterat la representativitat de la mostra en els estadis més tardans. En el mateix estudi, es va escollir un grup control positiu, incloent familiars de primer grau de pacients diagnosticats de TB sense malalties comòrbides. Vam preferir escollir aquest grup per tenir l'oportunitat de fer una millor descripció de les dimensions espectrals del TB. Així mateix, es podria argumentar que els resultats sobre la IL-6 són el resultat de la comparació múltiple. Tanmateix l'anàlisi de regressió logística mostrà resultats consistents en la comparació entre pacients i controls. A més a més, la IL-6 es va seleccionar prèviament com un biomarcador candidat essencial dels canvis inflamatoris en el model d'estadificació.

Pel que fa a l'*estudi 2* i *3*, un primer inconvenient podria estar relacionat amb la hipòtesi de la neuroprogressió, ja que hi ha dades que suggereixen canvis a nivell biològic en funció de cada estadi de la malaltia. Per tant, els nivells de BDNF podrien variar de forma significativa en determinats estadis[27, 64]. Aquesta situació pot haver donat lloc a un efecte sostre en aquests assajos clínics, veient que els nivells de BDNF no eren diferents entre els controls sans i els pacients a nivell basal. En segon punt, la naturalesa oberta de l'estudi fa difícil separar el possible efecte de l'ús de la quetiapina amb el curs de la pròpia malaltia. És a dir, és difícil de discernir si les variacions en els nivells de BDNF perifèric es troben directament relacionades amb la millora clínica o el tractament. Tanmateix, l'alta afinitat de la quetiapina pels receptors de serotonina 2A i el subseqüent increment de BDNF pot ser una possible explicació dels nivells més elevats de BDNF d'aquest grup[88, 91].

Altres estratègies terapèutiques com els estabilitzadors de l'ànim, els antidepressius i els antipsicòtics atípics s'han vist vinculades amb els vies on el BDNF juga un paper. Tot i que en els estudis es va avaluar el tractament amb quetiapina d'alliberació perllongada, amb liti i amb antidepressius, les altres estratègies farmacològiques van ser difícils d'avaluar a causa de la reduïda mostra sota aquests tractaments. Amb tot i això, el fet que els pacients es trobessin lliure de medicació a l'inici pal·lià l'efecte crònic de medicació prèvia, fet que reforça els resultats descrits.

Respecte a l'***estudi 3***, tot i haver seleccionat el tractament amb quetiapina, ja que es té evidència preclínica de l'efecte d'aquest fàrmac sobre els nivells de BDNF i la neuroplasticitat[92], un efecte de dosi pot possiblement haver influït en el resultat diferencial entre polaritat de l'episodi inicial ja que dosis majors a 300 mg/dia de quetiapina solen ser requerides en episodis maníacs o mixtos[93].

Finalment, en tots els ***tres estudis*** les determinacions dels biomarcadors es van realitzar perifèricament, en sèrum sanguini. Malgrat existir en estudis preclínics evidència de la correlació entre certs biomarcadors, com el BDNF, entre els nivells corticals i els sèrics i l'evidència que el BDNF travessa la barrera hemato-encefàlica[94], la presumpció d'aquestes troballes a l'escala humana s'ha de confirmar. Lang i col. van trobar relació entre els nivells de BDNF i la integritat neuronal en l'espectrofotometria en controls sans[95].

Malgrat les limitacions metodològiques dels tres estudis, cal destacar que els tres estudis d'aquest compendi tracten temes de vigent actualitat en el camp del TB. Referent al desenvolupament d'una classificació en el TB, un constructe com l'estadificació permetrà millorar la comprensió del debut, progressió i evolució de les malalties psiquiàtriques identificant possible fases amb particularitats

específiques. D'aquesta forma, es facilitarà la predicció del pronòstic de forma individual i la confecció d'intervencions, tant farmacològiques com psicoterapèutiques, específiques per a la situació individual de cada pacient. Per exemple, els pacients en un estadi més tardà potser no es beneficiaran tant de tractaments de primera línia com poden ser el liti, la psicoeducació familiar o la teràpia cognitivo-conductual comparat amb aquells pacients en un estadi primerenc[51, 53, 54, 96]. En sentit contrari, en estudis recents se suggereix que per a un grup de pacients amb TB en un estadi més tardà, teràpies enfocades en la rehabilitació funcional poden estar més indicades[97]. Aquesta classificació permetrà, així mateix, seleccionar grups més homogenis amb finalitat investigadora i poder determinar, segons el mètode científic, les intervencions eficaces en funció de l'edat i de l'estadi de la malaltia aconseguint minimitzar els efectes de la pròpia malaltia i de les estratègies terapèutiques sobre la trajectòria vivencial de cada individu. Per altra banda, els coneixements sobre els mecanismes biològics vinculats a la patofisiologia del TB i la seva evolució pot marcar un abans i un després en la farmacopea. En aquesta tesi doctoral hem mostrat resultats en aquest camp d'una banda, relacionant una proposta de classificació d'estadificació amb un biomarcador inflamatori i, de l'altra, aprofundint en els mecanismes biològics vinculats amb la resposta clínica. Les vies associades al BDNF i la neuroplasticitat estan relacionades amb la patofisiologia del trastorns afectius. Trobar un biomarcador perifèric que pugui predir la resposta al tractament permetrà, encara més, una personalització del tractament.

### 8.5. Línies de futura recerca

Els resultats obtinguts en aquesta tesi doctoral suggereixen nous reptes d'investigació de cara al futur. L'estudi descrit sobre models d'estadificació en el TB és de caire transversal. Per tant, considerant la complexitat de la patofiosiologia del TB, seria captivador confirmar els resultats amb estudis de cohorts al llarg del temps avaluant la reproductibilitat i el valor pronòstic d'aquesta classificació. A més a més, valorar la integració de nous biomarcadors de les vies neurotròfiques, d'estrès oxidatiu, i inflamatòries així com, possibles interaccions donades per polimorfismes d'aquestes molècules seria profitós. En aquesta mateixa línea, la possibilitat d'incorporar aspectes sobre neuroimatge per obtenir correlats neuroanatòmics tant de la neuroprogressió de la malaltia com de la resposta clínica al tractament aportaria evidència encara més consistent sobre les hipòtesis contemplades en aquest treball de tesi doctoral. Tot plegat, permetrà conèixer i comprendre millor les bases biològiques del TB i orientar futures investigacions relacionades amb nous tractaments tant farmacològics com psicològics.

# 9. Conclusions

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1. Les variables clíniques i funcionals que poden ser d'ajuda a l'hora de determinar un model d'estadificació basat en un estadi *primerenc* i un altre *tardà* en el TB són:
   a. La gravetat de discapacitat funcional avaluada a partir de l'escala FAST
   b. El número d'episodis
   c. L'edat a l'inici de la malaltia bipolar
   d. El temps d'evolució del TB

2. El biomarcador inflamatori IL-6 s'ha determinat estar relacionat amb un model d'estadificació en el TB mostrant un progressiu increment al llarg dels diferents estadis de la classificació des de familiars de primer grau de pacients amb TB no afectes de cap trastorn psiquiàtric, pacients que es troben en fases primerenques de la malaltia fins a pacients en fases tardanes.

3. Les neurotrofines i els reactants d'estrès oxidatiu semblen no assenyalar diferències remarcables entre els diferents estadis proposats en el model d'estadificació del TB.

4. Els nivells sèrics de BDNF varien al llarg del tractament farmacològic en funció de la presència de resposta al tractament en pacients amb TB que es troben en un episodi afectiu agut.

5. El polimorfisme BDNF Val66Met pot predir variacions dels nivells de BDNF durant el tractament en pacients amb TB que es troben en un episodi afectiu agut.

6. La polaritat de l'episodi sembla influir en la trajectòria dels nivells sèrics de BDNF al llarg del tractament en pacients amb TB que es troben en un episodi afectiu agut.

# 10. Bibliografia

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