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Brain-Derived Neurotrophic Factor (BDNF) and IL-1β in Pediatric and Adolescent' Depressive Disorders: a look towards precision psychiatry

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[I]f I had to live my life again, I would have made a rule to read some poetry and listen to some music at least once every week; for perhaps the part of my brain now atrophied would thus have been kept active through use. The loss of these tastes is a loss of happiness, and may possibly be injurious to the intellect, and more probably to the moral character by enfeebling the emotional part of our nature.

(Darwin, 1958, p. 138f)

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Abstract

Introduction Depression is one of the main and most debilitating mood disorders, as for personal functioning and social costs (Masi and Brovedani, 2011). It is estimated that depression affects more than 15% of the population lifetime (Kessler et al., 2003), with percentages in pediatric age that reach about 1-2% in prepuberal age and 4-6% in adolescence (Kessler et al., 2001). Although the early-onset depression usually tends to improve over time and resolve in a part of the cases, the functional impact of the disease is more difficult to recover, with consequences that tend to take a chronic trend and finally predispose to a high rate of recurrence of disease, a high risk of psychopathology in the future, as well as increase risk of suicide attempts and completed suicides (Masi and Brovedani, 2011). Studies on neuroanatomy, post-mortem brain observations and in vivo neuroimaging suggest that the impairment of neuroplasticity in specific neuronal networks may be involved in the pathogenesis of mood disorders (Biggio, 2011). In particular, recent studies have emphasized the role of the neurotrophic factor called Brain Derived Neurotrophic Factor (BDNF) whose depletion is associated with a reduction in neurogenesis and in the volume of important brain areas including the hippocampus, the prefrontal cortex and the amygdala (Biggio, 2011). Other researches found reduced BDNF concentrations in the blood of depressed patients, which increased after undergoing treatment with psychopharmacological therapy (Brunoni et al., 2008; Sen et al., 2008; Bocchio-Chiavetto et al., 2010; Molendijk et al. al., 2014). Scientific studies also found that the neuroinflammation process could contribute to the origin of some psychiatric conditions, especially depression (Dantzer et al. 2008; Miller et al.2009; Kim et al. 2014; Rosenblat et al. 2014). Depressed patients often present an increase levels of proinflammatory cytokines (like IL-1β) in blood and cerebrospinal fluid (Miller et al, 2009; Kim et al. 2014), so many authors have hypothesized their role as early clinical biomarker of pathology.

Objective The aim of the present study is to measure plasma levels of BDNF and IL-1β in subjects affected by depression in the developmental age compared to healthy peers. Secondary aims are: a) the evaluation in the depressed subjects of the trend of plasma levels before and after psychopharmacological treatment (at 3, 6 and 12 months); b) the analysis of the relationship between plasma levels of BDNF and IL-1β and clinical psychopatological features of depression. The last objective is to verify the usefulness of BDNF and IL-1β dosages as peripheral biomarkers of disease or of unresponsiveness to treatment, finally to improve diagnostic characterization of depressed patients and to target the treatment in pediatric age.

Methods Thirteen subjects aged 11-17,9 years, diagnosed with depressive disorder

(according to DSM-IV-TR; APA, 2002), as well as matched healthy subjects (n =12), were evaluated by trained raters and provided blood for BDNF and IL-1β measurements.

The diagnostic evaluation was conducted through free talks and the administration of a panel of structured (YSR-CBCL, CDI, MASC, CGI, C-GAS, SadPerson) and projective tests. The same panel was adminstrated to healthy subjects to explore subthereshold dimensions. Depressed subjects were treated with pharmacological therapy (Selective Serotonin Reuptake Inhibitors, mood stabilizers, SGAs) and psychotherapy.

The plasma levels of BDNF and IL-1β were measured according to definite timing (basal T0, before the start of therapy / psychotherapy, after 6 and 12 months). For depressed patients, an additional measure after 3 months of therapy was performed.

Analysis of BDNF andIL-1β levels were performed using specific ELISA kits (BDNF Emax ImmunoAssay System, Promega, Madison, U.S.A and Antigenix America, Huntington Station, NY, USA)

Results The depressed subjects, compared to the healthy peers, differ in a statistically significant way for a constant familiarity for psychiatric pathology, in particular in the maternal line, a history of events experienced as traumatic, a constant presence of conflicts within the family, scholastic and relational problems, sleep disorders and alchol/drugs use. Compared to heathy subjects at intake, plasma BDNF concentrations are lower, while IL-1β are higher in depressed patients, even if they do not reach a statistical significance. Regarding psychodiagnostic assessment, comparing test scores between depressed and non-depressed subjects at T0, there are significant differences in CBCL, YSR, C-GAS, CGI-S, CDI ($p < 0.05$) and SadPerson ($p < 0.001$) scores. No significant differences were found between the gropus in the MASC scores. Statistically significant correlations were found between BDNF levels at T0 of depressed subjects and problems of attention ($r = -.695$, $p = .026$) and school skills ($r = .878$, $p = .004$). At T6, BDNF inversely correlates to affective disorders ($r = -.975$, $p = .005$) reported at YSR T12. In depressed group, considering the presence of suicidal attempts, there are statistical significative differences for anxious-depressed ($Z = -2.158$, $p = .031$) and anxiety problems ($Z = -2.669$, $p = .008$) at YSR. The same analysis performed considering the personal history of trauma, highlighted statistical significative differences for rule break ($Z = -2.384$, $p = .017$) and conduct disorders ($Z = -2.037$, $p = .042$).

Conclusions Depressed pediatric patients present lower levels of BDNF and higher levels of IL-1β compared to healthy peers at the moment of diagnosis, although without a statistical significant difference. At intake BDNF appears to correlate to academic competences, while after 6 months of therapy higher BDNF levels orient to a better prognosis as for depression symptomatology. The evolution from suicidal ideation to SA appear to be linked to the perception of limited social support (reduced resilience) in adolescents with reduced levels of

BDNF.

Depressed children with a history of trauma present more externalizing symptoms, while the dimension of anxiety appears to be somehow protective from suicidal attempts.

The study supports the bio-psycho-social model of DD, highlighting the importance of a balance between neurogenesis, neuroinflammation and cerebral plasticity in the delicate period of developmental age. BDNF seems to be a symptoms or disease biomarker more than a severity index, so the integration of different instruments of evaluations, both clinical and biological (multimodal methods), as well as the integration of different voices (multi-informant approach) seems to represent the "best practice" for pediatric clinical assessment of mental health in line with precision psychiatry.

Further studies are needed to explore the role of BDNF and cytokines in the response of pediatric population to SSRIs, to identify biomarkers for predicting early treatment response, to produce new drug targets, to find preventive strategies and individually target therapy.

Abstract

Introduzione La depressione è uno tra i principali e maggiormente debilitanti disturbi dell'umore, sia in termini di funzionamento personale che di costi sociali (Masi and Brovedani, 2011). Si stima che la depressione colpisca almeno una volta nella vita più del 15% della popolazione (Kessler et al., 2003), con percentuali in età pediatrica che raggiungono circa l'1-2% in epoca prepuberale e il 4-6% nell'adolescenza (Kessler et al., 2001). Nonostante la depressione ad esordio precoce solitamente tenda a migliorare nel tempo e a risolversi in una parte dei casi, l'impatto funzionale della malattia risulta più difficile da recuperare, con conseguenze che tendono ad assumere un andamento cronico e che infine predispongono ad un alto tasso di ricorrenza di malattia, un alto rischio di psicopatologia futura, tentativi di suicidio e suicidi completati (Masi et Brovedani,2011).

Studi di neuroanatomia, osservazioni condotte sul cervello post-mortem e ricerche di neuroimaging in vivo suggeriscono che la compromissione della neuroplasticità a livello di specifiche reti neuronali possa essere coinvolta nella patogenesi dei disturbi dell'umore (Biggio, 2011). In particolare, studi recenti hanno sottolineato il ruolo di un fattore neurotrofico denominato Brain Derived Neurotrophic Factor (BDNF) la cui deplezione è associata a riduzione della neurogenesi e del volume di importanti aree cerebrali tra cui l'ippocampo, la corteccia prefontale e l'amigdala (Biggio, 2011). Altre ricerche hanno riscontrato concentrazioni ridotte di BDNF nel sangue di pazienti depressi e un successivo incremento a seguito di trattamento con terapia psicofarmacologica (Brunoni et al., 2008; Sen et al., 2008; Bocchio-Chiavetto et al., 2010; Molendijk et al., 2014). Altri studi scientifici evidenziano che anche processi di neuroinfiammazione possono contribuire all'origine di alcune patologie psichiatriche ed in particolar modo della depressione (Dantzer et al. 2008; Miller et al.2009; Kim et al. 2014; Rosenblat et al. 2014). I pazienti depressi infatti presentano spesso aumenti dei livelli di citochine proinfiammatorie (come l'IL-1β) nel sangue e nel liquor (Miller et al, 2009; Kim et al. 2014), pertanto molti autori ipotizzano un loro ruolo come biomarker precoci di malattia.

Scopo dello studio Lo scopo dello studio è la misurazione dei livelli plasmatici di BDNF e di IL-1βin soggetti affetti da depressione in età evolutiva rispetto ad un gruppo di soggetti sani. Obiettivi secondari sono: a) la valutazione nei soggetti depressi dell'andamento dei livelli plasmatici prima e dopo trattamento psicofarmacologico (a 3, 6 e 12 mesi); b) la valutazione della relazione tra livelli plasmatici di BDNF e IL-1β e le caratteristiche cliniche psicopatologiche della depressione. Obiettivo finale è verificare l'utilità del dosaggio del BDNF e dell'IL-1β, quali possibili biomarkers periferici di malattia o di mancata risposta alla terapia, per migliorare la caratterizzazione dei pazienti depressi e finalizzare il trattamento in

età pediatrica.

Materiali e metodi Tredici soggetti di età compresa tra 11 e 17,9 anni, con diagnosi di disturbo depressivo (secondo il DSM-IV-TR, APA, 2002), e un parallelo gruppo di soggetti sani appaiati per sesso ed età (n = 12), sono stati valutati da personale specializzato. Sono stati prelevati inoltre campioni ematici per la misurazione di BDNF e IL-1β. La diagnosi è stata condotta attraverso colloqui liberi e la somministrazione di test strutturati (YSR-CBCL, CDI, MASC, CGI, C-GAS, SadPerson) e proiettivi. Gli stessi test sono stati somministrati anche ai soggetti sani per esplorare la presenza di eventuali dimensioni depressive sottosoglia. I soggetti depressi sono stati trattati con terapia farmacologica (inibitori selettivi della ricaptazione della serotonina, stabilizzatori dell'umore, antipsicotici atipici) e psicoterapia. I livelli plasmatici di BDNF e IL-1β sono stati misurati secondo tempi definiti (T0 basale, prima dell'inizio della terapia/ psicoterapia, dopo 6 e 12 mesi). Per i pazienti depressi, è stata eseguito un ulteriore dosaggio dopo 3 mesi di terapia. L'analisi dei livelli di BDNF e IL-1β è stata eseguita utilizzando specifici kit ELISA (BDNF Emax ImmunoAssay System, Promega, Madison, U.S.A and Antigenix America, Huntington Station, NY, USA)

Risultati I soggetti depressi, rispetto ai coetanei sani, differiscono in modo statisticamente significativo per una costante familiarità per la patologia psichiatrica, in particolare in linea materna, una anamnesi positiva per traumi, una costante presenza di conflitti all'interno della famiglia, problemi scolastici e relazionali, disturbi del sonno e uso di alcol o sostanze. Rispetto ai soggetti sani, al momento del reclutamento, le concentrazioni plasmatiche di BDNF nei pazienti depressi risultano più basse, mentre quelle di IL-1β più alte, tuttavia i risultati non raggiungono una significatività statistica.

Per quanto riguarda la valutazione psicodiagnostica, confrontando i punteggi dei test tra soggetti depressi e non depressi a T0, vi sono differenze significative nei punteggi delle CBCL, YSR, C-GAS, CGI-S, CDI ($p < 0,05$) e SadPerson ($p < 0,001$). Nessuna differenza significativa è stata trovata tra i gruppi nei punteggi MASC. Nei soggetti con depressione è presente una correlazione statisticamente significativa tra i livelli di BDNF al T0 e problemi di attenzione ($r = -.695$, $p = 0,026$) e abilità scolastiche ($r = .878$, $p = 0,004$). A T6, i livelli di BDNF sono inversamente correlati a disturbi affettivi ($r = -.975$, $p = 0,005$) riportati al test YSR a 12 mesi. Nel gruppo di soggetti depressi, considerando la presenza di tentativi di suicidio, esistono differenze statisticamente significative per problemi ansioso/depressivi ($Z = -2,158$, $p = 0,031$) e di ansia ($Z = -2,669$, $p = 0,008$) riportati al YSR. La stessa analisi eseguita considerando l'anamnesi positiva per eventi traumatici, ha evidenziato differenze statisticamente significative per lo scarso rispetto delle regole ($Z = -2.384$, $p = .017$) e i disturbi del comportamento ($Z = -2.037$, $p = .042$).

Conclusioni I pazienti pediatrici depressi presentano livelli più bassi di BDNF e livelli più

alti di IL-1β rispetto ai coetanei sani al momento della diagnosi, sebbene il dato non raggiunga una differenza statisticamente significativa. Al momento del reclutamento, il livello di BDNF appare correlare con le competenze accademiche, mentre dopo 6 mesi di terapia livelli di BDNF più elevati orientano verso una prognosi migliore relativamente alla sintomatologia depressiva. L'evoluzione dall'ideazione suicidaria al tentato suicidio sembra essere collegata alla percezione di un limitato supporto sociale (ridotta resilienza) negli adolescenti con livelli ridotti di BDNF.I bambini depressi con una storia di trauma presentano sintomi più esternalizzanti, mentre la dimensione dell'ansia sembra essere in qualche modo protettiva rispetto al tentativo di suicidio.

Lo studio supporta il modello bio-psico-sociale dei DD, evidenziando l'importanza di un equilibrio tra neurogenesi, neuroinfiammazione e plasticità cerebrale nel delicato periodo dell'età evolutiva. Il BDNF sembra essere un biomarcatore di sintomi e di patologia, piuttosto che un indice di gravità, pertanto l'integrazione di diversi strumenti di valutazione, sia clinici che biologici (metodi multimodali), così come l'integrazione di diverse voci (approccio multi-informant) sembrano rappresentare la "migliore pratica" per la valutazione clinica pediatrica della salute mentale in linea con la psichiatria di precisione.

Ulteriori studi si rendono necessari al fine di esplorare il ruolo del BDNF e delle citochine nella risposta terapeutica agli SSRI nella popolazione pediatrica, per identificare biomarkers predittori precoci di risposta al trattamento, per la produzione di nuovi bersagli farmacologici, per trovare strategie preventive e mirare ad una terapia individualizzata nella depressione.

1. Introduction

Psychiatric diseases presenting in developmental age have obtained a growing attention in the last years all over the world. Estimates report a prevalence of mental diseases in children and adolescents of 7-10% (Avenevoli et al., 2015; GBD 2015 Mortality and Causes of Death Collaborators, 2016). In particular, 75% of mental disorders occurs within 25 years of age and most of them originate in adolescence with prodromes that can begin many years before the clinical manifestation of the disorder. In most neuropsychiatric disorders, early and timely treatment can change the natural course of the disease or prevent numerous sequelae, avoiding a chronic and disabling clinical trajectory. In addition to a timely diagnosis, therefore, a multidisciplinary management over time is required, as well as complex and coordinated interventions involving the family and the various life contexts, in close collaboration with other institutions and with the territorial services (Vicari and Vitiello, 2015).

In the present study, attention will be focused on mood disorders that represent the more frequent reasons for requesting a psychiatric evaluation and for access to child neuropsychiatric services. Among the mood disorders, those with depressive polarity and anxiety traits are more frequent and they are often complicated by deliberate self-harm behaviors and suicidal attempts (Pompili et al., 2012; Hawton et al., 2012).

The etiopathogenesis of DepressiveDisorders (DD) is not yet clear and numerous theories have been debated over the years. It is likely that identifying a single etiology for depression will not be achieved. Rather, many factors, risk or trigger factors, have been found to contribute to the onset of the disease. In recent decades, neurobiological theories concerning neurotrophins have gained particular interest: in particular, the Brain-Derived Neurotrophic Factor (BDNF) is one of the neurotrophic factors with greater scientific documentation, for its implications on brain development, in the physiological functioning of the Central Nervous System (CNS) and in the pathology (Huang and Reichardt, 2001; Duman, 2002a). Specifically, in addition to its functions for neuronal development and survival, BDNF plays a fundamental role for synaptic plasticity, especially in the regions of the hippocampus and prefrontal cortex, that are areas involved in affective regulation, in the genesis of anxiety and in cognitive functions (Berton and Nestler, 2006; Ressler and Mayberg, 2007; Dwivedi, 2009; Bathina and Das, 2015). Another recent interesting theory suggests a role of inflammatory process in the development or persistence of DD. Several studies have highlithed that the levels of proinflammatory cytokines (a family of proteins that mediate immune responses to injury, infection and other stress) are elevated in both serum and cerebral spinal fluids in patients with major DD (Dahl et al., 2014;Belem da Silva et al., 2017). Therefore, studying pro-inflammatory states may clarify whether inflammation could represents an early

biomarker for the pathophysiology of such emotional disorders.

Currently the diagnosis of DD is exclusively based on clinical evaluation, since no laboratory tests that could confirm the specialistic observations are available. Therefore the identification of peripheral biomarkers on blood or urine could help to formulate more specific diagnosis of these pathologies. More effective diagnostic assessment would allow to perform a better characterization of patients, improve the monitoring of the clinical course, as well as develop effective individualized therapies, according to the "evidence based" guidelines and "precision medicine" directions.

1.1 Depression: definition, epidemiology and classification

Every person presents physiological mood oscillations that normally allow to adapt our reactions to the conditions of the surrounding environment. When there is an alteration of these physiological mechanisms of adaptation with the inability of the individual to maintain his/her normal daily function, the subject suffer of a mood disorder (Cassano and Tundo, 2006). Mood disorders are highly prevalent, heterogeneous and recurrent neuropsychiatric conditions characterised by a broad range of symptoms (Zunzsais, 2013).

The Depressive Disorders (DD) in particular are all characterized by a persistent and pervasive alteration of mood (sadness), with irritability, loss of interest or pleasure in normally enjoyable activities and interactions (anhedonia). These aspects are also associated with other symptoms such as changes in appetite and sleep, energy loss, difficulties in memory and concentration, feelings of inadequacy and guilt, recurrent thoughts of death, with a serious impact on social and scholastic functioning (Masi et al., 1998). A fundamental characteristic for diagnosis of DD is the presence of a change of mood in a negative sense, associated with alterations of the cognitive and somatic functions (Fig.1). The alteration of cognitive functioning determines a reduction in the ability to concentrate and in attention skills, with the onset of thoughts of worthlessness and death until to thoughts of suicide. Somatic alteration, on the other hand, involves a reduction in physical activity, a slower motor activity, modifications of appetite and sleep rhythms (Vicari and Vitiello, 2015).

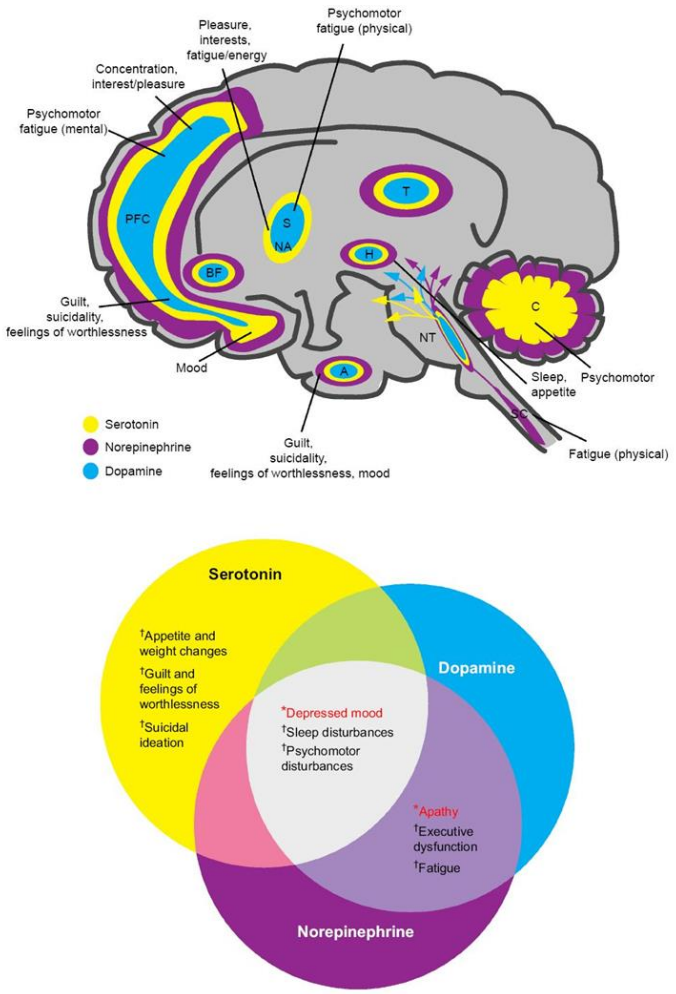


Fig. 1 Neurotransmitters and their hypothetically malfunctioning brain circuits in regions associated with the diagnostic symptoms for depression. (From Lum and Stahl, 2012).
A, amygdala; BF, basal forebrain; C, cerebellum; H, hypothalamus; NA, nucleus accumbens; NT, neurotransmitter centers; PFC, prefrontal cortex; S, striatum; SC, spinal cord; T, thalamus.

The World Health Organization (WHO) recognized DD as an emerging public health problem, estimating it will be the second leading cause of disease burden by 2020, after ischaemic heart diseases, and therefore promoting a series of interventions to reduce the impact of the disease and overcome the gap between the possibility of effective treatments and the number of subjects who use them (30-50%), finally to early identify subjects with DD and granting them access to appropriate and effective treatment (Avenevoli et al., 2015).

The WHO estimates that 121 million people suffer from psychiatric disorders worldwide; of these 10-20% are children or adolescents. The estimated prevalence of DD is one person in six (1/6), with a probability of relapse ranging from 35% to 65% (WHO, 2004). DD represent the third cause of disability in the world and the first cause in Europe, affecting strongly the Quality of Life of the entire family. In 2002, unipolar depression constituted the 4.5% of world morbidity and the 7.6% of European morbidity (WHO, 2004).

The diagnosis of DD is clinical and it is based on the observation of precise signs and

symptoms, which must be severe enough to significantly compromise the adaptation and functioning of the subject affected in all areas of life.

The DD diagnostic criteria are listed in the main nosographic systems of classification: DSM-IV-TR (APA, 2002), DSM-5 (APA 2013) and ICD 10 (WHO 2004). DD are classified on the basis of severity, chronicity, presence of episodes of mania and cyclicity. In the international classification of Diseases, ICD 10 (WHO 2004), that is currently used in medical records, the DD belong to the Affective Syndromes (codes F30-F39) and the depressive episodes are subdivided according to the severity and the presence of "biological" symptoms. According to the DSM-5, the patient must experience functional impairment related to the change of mood that cannot better be explained by another condition, for example, medical disorder or substance abuse. In the ICD-10 it is not formally required a functional impairment of the individual, but it is described as a frequent accompaniment of depression (WHO, 2010). Comparing to the previous version DSM.IV-TR (APA, 2002), in DSM-5 (APA, 2013), authors introduce some novelties supported by more recent evidenced-based studies. In particular Unipolar Depressive Disorders (UDD) come to constitute an autonomous category, a bridge between the Anxiety Disorders and the Bipolar Disorders (BD), from which they were separated (with BD collocated between DD and Psychotic Disorders). The category of DD includes Disruptive Mood Dysregulation Disorder- DMDD (new diagnostic condition, diagnosed under 18 years), Major Depressive Disorder-MMD (which includes Major Depressive Episode), Persistent Depressive Disorder- PDD (which includes previous diagnoses of Dysthymia and Chronic Major Depression), Premenstrual Dysphoric Disorder (new entity), Depressive Disorder induced by substances/drugs, Depressive Disorder due to another medical condition, Depressive Disorder with other specification and Depressive Disorder without any specification.

In order to avoid overdiagnosing and treatment of BD in children, the newly introduced entity DMDD refers to children with severe persistent irritability and frequent episodes of temper outbursts occurring in youth at least aged 6 and with onset prior to age 10. These suffering children in adolescence and adult age present more frequently UDD or anxiety disorders, rather than BD.

The MDD represents the classical condition in this group of disorders, and also in DSM-5 the diagnostic criteria presented in the DSM-IV-TR are globally maintained (Appendix 1). The only notable change is the elimination of the mutual exclusivity between bereavement and depression.MDD is characterized by distinct episodes of at least 2 weeks of persistent changes in affective, cognitive andneurovegetative functions, and inter-episodic remissions. PDD (dysthymia), a more chronic form of depression, can be diagnosed when mood alterations last at least 2 years in adults or 1 year in children (Appendix 2).

The DSM-5 also includes a section entitled: "Conditions that require further studies", in which the new diagnostic categories of Suicidal Behavior Disorder and Nonsuicidal Self-Injury are presented without coding (Appendixes 3 and 4).

1.2 DD in developmental age

Epidemiological studies of the last 20 years shown an increase in the frequency of psychopathological disorders in developmental age, in particular in the prevalence of depression throughout lifecourse. Each incoming generation comparing to the previous one seems to be at a greater risk of developing a DD in an earlier age (Birmaher et al., 1996; AACAP 1998; Park and Goodyer, 2000). In Sweden, between 1997 and 2007, the number of adolescents between 16 and 19 years, who requested an evaluation and an admission to the specific services for diseases related to anxiety and depression has increased by 400 % (The Swedish National Board of Health and Welfare, 2009). In an Italian pediatric reality (Padua) mood disorders account for the 46% of emergency admissions to Psychiatric Unit (Zilli et al., 2017).

Over the years, the nature of depression as a real disorder in children and adolescents has been frequently debated. Literature about DD in the developmental age before 1970 is very small. In 1971 the Union of European Child Psychiatrists officially declared that depression can also manifest in infancy and adolescence and in the last 40 years many authors have contributed to a better definition of these disorders in childhood (SINPIA, 2007). Therefore only since the 1980's the academic articles on these topics are starting to become increasingly rich and abundant (Lanzi et al., 1994).

Currently, it is recognized that DD in childhood have a consistency and stability of disease similar to those of adults, although with some significant differences. In particular, in children, mood is more often dysphoric, symptoms of presentation are more often behavioral and somatization is very frequent (Lanzi et al., 1994). In adolescence, the characteristics of affective and impulsive liability linked to the specific developmental phase determine a higher risk of aggressive behaviours directed to oneself or to other person (Thapar et al., 2012). Furthermore, DD are often in comorbidity with other psychiatric disorders (40% to 90%). Comorbidity can be determined by sharing the same etiology or may be the cause as well as the consequence of depression (Angold and Costello, 1993; Angold et al., 1999). Anxiety, for example, is a frequent precursor of depression and share the same genetic diathesis (Middeldorp et al., 2005). ADHD and depression are often comorbid, but in this situation it is necessary to consider that symptoms of loss of attention that worsen significantly during mood changes should be depressive symptoms. Comorbidity between depression, behavioral disorders and substance abuse can be related to family risk factors such as abuse, exposure to

violence and substance abuse by parents (Harrington, 1990).

The Oppositional Defiant Disorder (ODD) can predispose to depression because of difficulties with the regulation of emotions and a tendency to negate affectivity (Burke, 2012). Depressive disorders can therefore have a serious impact on the personal, social and scholastic functioning of the child or teenager with the risk of chronicity, of predisposition to other psychiatric conditions, substance abuse and obesity (Keenan-Miller et al., 2007; Fletcher, 2008).

Some studies report the following prevalences related to DD in childhood: 0.3-1% in preschool age, 0.4-2.5% in school age, 4-6% in adolescence (WHO, 2004). According to more recent studies, the dramatic prevalence of depression, in puberty, in the female population (1:1= M:F ratio in prepubescent age; 1:2 in adolescents) could be related to the increase in estradiol and testosterone levels. Precocious puberty could increases the risk of depression in females, as we can observe in the USA and now in Europe (Blom et al., 2016).

Studies on neurodevelopment have shown that the remodeling of prefrontal and limbic structures on CNS implicated in social attributions, regulation of emotions and reward responses that typically happen in adolescence, could contribute to an increase in vulnerability to developing depression. Additionally, these structural modifications colud be associated with an increase in sleep-wake rhythm dysregulation and the risk of developing alcohol and substances abuse (Casey et al., 2011). Neuroimaging studies in depressed adolescents reveal an hyperactivation of the amygdala and of the anterior cingulate cortex which leads to hypersensitivity to stress and to an anomalous connectivity between the areas involved in processing and regulation of emotions (Bricolo et al., 2010).

The risk of recurrent depression after an initial episode ranges from 30% to 70% in the first two years, and it increases in young patients with chronic depression, psychiatric comorbidities and intrafamilial conflicts (Masi and Brovedani, 2011).

Evidences show that the clinical course of depression that begins before puberty is different from an onset after puberty. Children with apre-pubertal onset of depression have an increased risk of developing other mental disorders such as BD in adulthood; while boys who develop depression in late adolescence are at greater risk for developing recurrent depression or other mental disorders (Richardson and Katzenellenbogen, 2005). The risk of an evolution in a BD in subjects with early onset of depression is estimated at around 10-20% with a high risk in patients presenting hypomania in response to treatment, psychotic symptoms, hypersomnia and BD in family history. Suicidal behavior is a common sequela in early onset depression and the mortality rate in young depressed patients due to suicide is 10 times higher than in non-depressed ones (Brent and Maalouf, 2015).

Due to the absence of clearly recognized "causal" factors, researchers paid much attention to

possible "predisposing" and "triggering" factors, also defined risk factors (Vicari and Vitiello, 2015). Over 50% of DD in developmental age seem to be preceded by a negative event of strong emotional impact or by the combination of several stressful events (Fonagy, 2001; Nomura et al., 2002; Purper-Ouakil et al., 2002). In Beardslee et al (1996), on 139 adolescents, a single risk factor (maternal depression, non-affective psychiatric disorders of the parents or in the child him/herself) can increase the risk of developing an affective disorder from 7% to 18%, while the presence of 3 risk factors increase the risk up to 50%.

Risk factors can be summarized in individual factors (internal factors of the subject) and environmental factors (external factors). In the first group there are the biological factors, in particular the biochemical ones such as the reduction of serotonin, the decrease in catecholamines (dopamine-noradarenaline), the endocrine dysfunction, the abnormal response of the growth hormone-GH and so on (Vicari and Vitiello, 2015). A family history positive for psychiatric disorders is another individual factor: 61% of children with parents suffering of MDD will develop a psychiatric disorder during childhood and adolescence. These children present 4 times the risk of developing a mood disorder compared to their peers without affected parents. Sons of depressed mothers have suicidal thoughts and behaviors more frequently than their peers (Celi, 2015). Some characteristics of the child/adolescent may contribute to the development of depression such as high anxiety, low self-esteem, high self-criticism, cognitive distortions, poor academic performance and poor social skills (Richardson and Katzenellenbogen, 2005).

Among the external risk factors, there are factors linked to family and social context in which the subject lives: family conflicts, a low socioeconomic status, an early death of a parent, sexual abuse, physical abuse, neglect. Other environmental risk factors are negative life events such as divorce or separation of parents or loss of friends (Richardson and Katzenellenbogen, 2005).

Studies on environmental factors involved in neurodevelopment and pathophysiology of increasing DD (Blom et al., 2016) highlitghted that accelerated pace of life in modern age coincided with the increase in the prevalence of mental health problems related to chronic stress in developmental age. Stress can limit the ability of parents to bond with their children and to support their emotional development: an inconsistent, unreliable or detached attachment with parents during childhood can interfere with a safe and stable psychic development.These factors could reduce the child's ability to cope with stressful life events, thus increasing the risk of depression (Malik et al., 2014).

Modern ages are also charachterized by the massive spreading of media and technology: the use of media in adolescence has been associated with an increased risk of depressive symptoms. The messages transmitted by these thecnologies can favor a depressive

symptomatology as children/adolescents are exposed to highly idealized and simplified characters and situations that lead the subject to confront with unrealistic images (Bailin et al., 2014). Bullying and cyberbullying are even more frequent, leading to an increasing in susceptibility to peer victimization, to the social rejection and finally to depressive symptoms (Aboujaoude et al., 2015). Some other studies state that the increase in depressive pathology among young people can also be related to climate changes and pollution (Shaftel, 2015).
In recent years, many authors have also focused their attention on recognition of protective factors, in particular on resilience.
Resilience is the ability of the individual to adapt to stressful life events in a safe and flexible way and to modulate the expression or inhibition of his/her affections in accordance with the demands of the surrounding environment in order to preserve and improve the balance of the person (Vicari and Vitiello, 2015).

In developmental age, the definition, the diagnosis, the classification systems and the treatment of DD are still problematic, since the pathology is characterized by a variable clinical expression in the different age, symptoms are often difficult to describe for a child and clinicians tend not to use uniform diagnostic tools. Therefore depression, when it occurs in children and adolescents, is often underestimated or unrecognized and, to aggravate the situation, there is a tendency to consider the disease similar to that of an adult (SINPIA, 2007). For such critical issues, in May 2007, the Italian Society of Child and Adolescent Neuropsychiatry (SINPIA) published the Guidelines for Depressive Disorders in developmental age, underlining the peculiarities of the pathology in these delicate ages (SINPIA, 2007).
As for adulthood, also in pediatric settings clinicians use the diagnostic criteria of ICD-10 and DSM, with some specifications, especially for earlier age groups. Already in the DSM-IV-TR (APA, 2002) there appeared some specific notes for the clinical diagnosis in the developmental age, maintained in DSM-5. In particular, irritability was recognized as a peculiar manifestation of mood deflection in children and adolescents, the inability to reach normal body weight in the child was emphasized as an indication of psychopathological suffering and, in the dysthymic disorder (now Persistent Depressive Disorder), the time threshold for diagnosis was reduced to 1 year (instead of 2 as in adults). These criteria, however, are generally more easily applicable from 3 to 5 years old, while there are greater difficulty under 3years of age.
In DSM-5 (APA, 2013) a new diagnostic specific entity for the developmental age has been introduced, the "Disruptive Mood Dysregulation Disorder- DMDD" (Appendix 5). It refers to a condition characterized by the presence of frequent episodes of temper outbursts that are disproportionate with respect to the triggering event, very intense and associated to a chronic

alteration of mood in a dysphoric sense. According to literature, patients in the developmental age affected by this disorder are prone to develop DD in adulthood. The prevalence of this disorder is estimated to be 2-5%, with higher rates in high school males (APA, 2013).

In the developmental age, DD present a variable phenomenology in relation to the development phase in which the disorder begins and it is possible to identify different clinical situations (Tab.1). The DD is thus characterized by the presence of depressive feelings associated with specific depressive symptoms that vary according to chronological age and the socio-cultural context (SINPIA, 2007).

Between 2001 and 2002, the Task Force on the Research Diagnostic Criteria Infancy and Preschool, set up by the American Academy for Child and Adolescent Psychiatry, proposed to extend the diagnostic criteria of the DSM-IV to the age group of 0-5 years by elaborating the criteria called Research Diagnostic Criteria-Preschool Age (RDC-PA, 2003) consisting of some DSM-IV criteria changes for 13 psychiatric conditions occurring in pre-school age. These criteria were revised in 2016 and adapted to the new DSM-5 (Appendix 6).

The smaller the child is, the more he/she expresses DD through his/her body with a typically psychosomatic symptomatology such as anorexia, poor growth, sleep disorders, motor slowdown or less frequently diarrheal episodes, dermatological problems, respiratory diseases and so on. Usually, however, the most represented depressive feeling is irritability (SINPIA, 2007) (Tab.1).

In children between 3 and 5 years, the depressive symptomatology can be recognized in its most typical expression during a separation or a loss. The child presents retirement, a deflection of mood with withdrawal from activities that previously gave him/her pleasure, listlessness and sometimes regressive behaviours. He/she can present moments of deep calm alternate with agitation and feelings of anger. Thoughts and fantasies usually go in the same way with emotional states, so it is often present the theme of death expressed through play and drawings. Even in this age group problems of sleep, feeding and sphincter control can occur. Children with DD are worried children who require continuous reassurance and who often fail to express their needs; they demonstrate an important sensitivity to the separation from parental figures, in particular a regressive relationship of considerable dependence is established with the mother (Lanzi et al., 1994) (Table 1). With the years passing (6-11 years) the subject becomes more and more able to express his/her psychic suffering, manifesting feelings of self-underevaluation and fear of not being loved. To these sentiments, the children respond with opposition, protest, impulsiveness and aggressiveness. There could be a fall in academic performance due to an attention lability, difficulties in concentration and easy fatigue. The children live moments of failure that leads them to sense of guilt and relational difficulties. Some children suffer from headaches or other somatic disorders (Lanzi et al.,

1994)(Table 1). In adolescence, DD are more similar to those of adulthood, but there are anyway some crucial points to keep in mind. The diagnostic complexity in this age group is due to the observation that some descriptions of depressive symptoms are similar to the physiological adolescent suffering. Adolescents with or without depression, present an incomplete development of the frontal cortex and of the frontolimbic connections that are involved in the regulation of emotions, in the control of impulses, of the capacities of planning and reflection; therefore, adolescents are more exposed to the risk of a depressive pathology (Lanzi et al., 1994).

In adolescence there could be a state of boredom or of depressed mood with episodes of crying, emotional instability and moments of excitement. Death ideas are frequent (until 60% in Stoep et al., 2009) and they can be associated with sleep and feeding disorders. In severe cases, there is also a psychomotor deceleration with thoughts focused on issues of inadequacy, inability, guilt, ideas of death up to the idea of self harm and suicide attempts. Relationships with others are reduced and behavioral disturbances may occur (Table 1) (Lanzi et al., 1994).

Tab. 1: Clinical expressiveness of depression in relation to age (adapted from SINPIA, 2007)

0-3 years
He/she is not interested in games
He/she is restless from the motor point of view
He/she complains/cries too much / is irritable
He/she moves little and / or slowly
He/she hits themselves
He/she uses their eyes very little to communicate
He/she smiles/ laughs very little
He/she has difficulty in reaching body weight adequate for age
He/she has psychomotor delay or regression
He/she are not very reactive
He/she does not use vocalizations/ babbling/ acquired language
He/she has little/no facial expressions
He/she has sleep problems
He/she has problems with eating
He/she is not interested in being with other children
He/she is aggressive
He/she has difficulties in new situations, he/she is frightened by new challenges
He/she has difficulties in separating from his/her parents
He/she has somatic problems (vomiting, asthma, dermatitis, alopecia, etc ...)
He/she is not very curious, he/she explore very little

3-5 years
He/sheis not easily involved in games/activities
He/she appears sad most of the time
He/she is irritable (temper outbursts, etc.)

He/she manifests provocative, defiant, oppositional, disobedient behaviours
He/she is always on the move
He/she is not interested / not entertained during games or other pleasant activities for their age (eg sports, videogames, etc)
He/she complains / cries often and without reason
He/she cannot pay attention
He/she is capricious, grouchy, grumpy, sullen
He/she moves little and / or slowly
He/she hits / gets hurt
He/she plays fiction games with sad/ scary/ death contents
He/she reproaches themselves, he/she apologizes for minor infringements
He/she appears tired, with little energy,he/she gets tired easily
He/she has a psychomotor regression (loss of motor functions, language, control sphincters, etc)
He/she smiles / laughs little
He/she has difficulties in reaching body weight adequate for age
He/she behaves like a smaller child
He/she has little/no facial expressions
He/she plays in a monotonous and repetitive way
He/she is too scared / anxious / angry
He/she is shy, closed, embarrassed
He/she speaks little
He/she has sleep problems (difficulties in falling asleep, awakening, nightmares, etc.).
He/she has problems with eating (eats a lot or little, coprophagia, pica, etc).
He/she is not interested in socializing with peers,he/she prefers to be alone or with adults
He/she is aggressive
He/she feels inadequate, incapable
He/she is worried
He/she has behavioral problems
He/she surrenders to difficulties
He/she is easily frustrated by reproaches or failures
He/she has difficulty in new situations, he/she is frightened by new challenges
He/she feels rejected by others, alone, unloved
He/she has difficulties in separating from parents (dependent on parents, separation anxiety, etc.)
He/she needs reassurance and gratification to improve its performance.
He/she is not very curious, he/she explores little

6-11 years
He/she is not easilyinvolved in games/activities
He/she appears sad most of the time
He/she is irritable (temper outbursts, etc)
He/she manifests provocative, defiant, oppositional, disobedient behaviours
He/she is always on the move
He/she is not interested/does not get excited during games or other pleasant activities for their age, previously pleasant
He/she complains/cries often and without reason
He/she cannot pay attention
He/she is capricious, grouchy, grumpy, sullen
He/she moves little and/or slowly

He/she hits/gets hurt
He/she makes fiction games, drawings, dreams with sad/scary/death contents
He/she reproaches him/herself
He/she has problems in following the rules
He/she is not very self confident
He/she does not react
He/she appears tired, with little energy, gets easily tired
He/she has excessive guilt feelings
He/she smiles/ laughs little
He/she has difficulties in reaching body weight adequate for age
He/she behaves like a smaller child
He/she plays in a monotonous and repetitive way
He/she is always scared/ anxious/ angry
He/she speaks little
He/she has sleep problems
He/she has fears of loss or abandonment
He/she is shy, closed, embarrassed
He/she has problems with eating
Somatic complaints (vomiting, asthma, dermatitis, allergies, abdominal pain, headache, alopecia)
He/she is not interested in socializing with peers, he prefers to be alone or with adults
He/she is aggressive
He/she has scholastic difficulties (loss of performance, failures, withdrawal, etc.)
He/she feels inadequate, incapable, inferior to others
He/she is worried
He/she has behavioral problems
He/she surrenders to difficulties
He/she is easily frustrated by reproaches or failures
He/she has difficulties in new situations, he/she is frightened by new challenges
He/she feels rejected by others, alone, unloved
He/she has difficulties in separating from parents (dependent on parents, separation anxiety, etc.)
He/she needs reassurance and gratification to improve its performance
He/she feels inadequate and incapable
He/she has difficulties in concentrating
He/she reports thoughts of death, suicidal ideas, he/she has made suicide attempts

12-18 years
He/she is not easily involved in games/activities
He/she appears sad most of the time
He/she is irritable (temper outbursts, etc)
He/she is not interested/does not get excited during games or other pleasant activities for their age, previously pleasant
He/she has problems in following the rules
He/she complains/cries often and without reason
He/she can not pay attention
He/she moves little and/or slowly
He/she hits/gets hurt
He/she makes drawings, reads books with sad/ scary/ death contents
He/she has delay in the acquisition or loss of higher cognitive functions

He/she reproaches him/herself
He/she is not very self confident
He/she does not react
He/she appears tired, with little energy, gets tired easily
He/she has excessive guilt
He/she smiles/ laughs little
He/she is irritable
He or she is too scared / anxious / angry
Speaks little
He or she has trouble sleeping
He/she is shy, closed, embarrassed
He/she has problems with feeding
He/she has abdominal pain, headache, widespread pain, delayed puberty, neurovegetative disorders
He/she is not interested in being with friends,prefers to be alone
He/she is aggressive
He/she has scholastic difficulties (loss of performance, failures, withdrawal, etc.)
He/she feels inadequate, incapable, inferior to others

As in adult age, pediatric depression presents different variants, included in the DSM-5 (2013) as "specifiers": DD with anxiety, with mixed features, with melancholic features, with atypical features, with psychotic features, with catatonia, with a seasonal trend.

Mixed features associated to depression are recognized to be significative risk factors for an evolution to BD (APA, 2013). Melancholic characteristics, uncommon in pediatric age, are a loss of reactivity to pleasant experiences, a particular mood quality (despair, discouragement, "empty mood"), the presence of early awakenings with worse mood in the morning, loss of body weight.

In 1994, the DSM-IV introduced the criteria for "atypical features", characterized by significant mood reactivity (mood brightness in response to actual or potential positive events) and two or more of the following symptoms: significant weight gain, increase in appetite (craving for carbohydrates), hypersomnia, leaden paralysis, and a long-standing pattern of interpersonal rejection sensitivity that results in significant social or occupational impairment (de Rénoche and Condini, 2004). Atypical depressive features show association with worse course and less treatment response in unipolar depression (Quitkin et al. 1993; Stewart et al. 1993; Akiskal and Benazzi 2005). The Columbia group pointed out that patients suffering of atypical depression, when compared to patients with melancholic depression, had a significantly earlier onset of the illness, much more chronic course of the illness, and less frequently family members with a recurrent and severe depressive illness, but more often family members who were chronically depressed (Łojko and Rybakowski, 2017; Stewart et al; 1993).

Psychotic features in pediatric age can be represented by auditory hallucinations, which often do not disturb the child, while delirium is more typical in adolescence. These symptoms can be congruent or not with the mood.

To understand deeply DD during the developmental age, it is important to keep in mind some concepts about cerebral maturity during the course of life. The ideal condition for a healthy growth of a child is to have an adequate genetic patrimony at birth and to live in an environment in which both the physical aspects and the educational rules are able to give adequate stimuli to every age of life. The CNS starts to develop from the first week after conception, from the cellular layer called ectoderm. At the end of the first 8 weeks, the human embryo presents rough skeleton of almost all the organs of the body and the brain is the fastest growing organ representing half of the total size of the embryo (Bricolo et al., 2010). The weight of the brain varies in different stages of life and can therefore be considered an indicator of the processes that lead to full brain maturation. The brain's shape and connections

depend mainly on the genetic dispositions that direct the production of every cellular protein. Genes therefore represent intrinsic factors of central importance. Brain development is also influenced by extrinsic factors such as maternal nourishment or neurotrophic factors, important elements that can regulate cell death or growth. Furthermore the everyday experience and the set of knowledge acquired through the stimuli provided by the external environment lead to the development of new brain connections and reinforces those already existing (Bricolo et al., 2010).

The child's CNS grows according to two main rules: "Top-Down" and "Bottom-Up". This means that the individual develops according to the interaction between genetically pre-established potentialities and stimuli coming from the environment. Top-Down mechanism is generally the internal genetic drive, while for Bottom-Up mechanism we mean the stimulation coming from the external environment (Taylor et al., 2010). It is necessary to consider this interaction to fully understand the phenomena that occurs during growth in the developmental age. The hormone/brain relationship stimulates the need for strong emotions and sensations, while the brain areas responsible for judgment are still immature: that is why teenagers have more difficulties in making mature decisions and understanding the consequences of their actions. This leads them to be more vulnerable to risk situations, such as, for example, consuming drugs or having transgressive behaviours. The brain has the ability to change continuously during life and it is dependent on the experience that guide CNS adaptation to a constantly changing environment. This cerebral capacity of continuous adaptation is defined as "neuroplasticity" and depends on the characteristics of each neurons, that modify their ability to communicate with each other through synapses and neurotransmission. Neuroplasticity is therefore a physiological mechanism that allows brain maturation in the child, but also occurs in adulthood during learning and memorization, or after trauma and subsequent rehabilitation. Understanding the mechanisms involved in mature brain neuroplasticity is relevant for understanding normal and pathological plasticity (Bricolo et al., 2010).

The ability of a child and an adolescent to pursue goals is based on mechanisms that we could indicate with the term "driver" and "controller". By "driver" we mean the motivation that is at the basis of, for example, thirst, hunger, sleep and sex. For "controller" we mean the function that allows one to decide if, where, when and how to achieve that need. The “controller” has the function of filtering the needs according to the variables of place, time and modalities. An adolescent's brain is partially developed and strongly linked to emotions. The limbic system that mediates emotions and impulses develops in fact precociously, and it is located in the deep structures of the brain. The prefrontal and frontal cortex, which are

linked to rationality, cognition, social functions and language, mature later, around at 20-25 years. Just these regions modulate the decisions taken on impulse under the thrust of emotions (Bricolo et al., 2010). The prevalence of risk behaviours during adolescence could therefore be easily explained by the immaturity of some brain regions compared to others, as for example, the low control of the frontal cortical regions on primary impulses. Some authors (Casey et al., 2008) state that the only immature function of the prefrontal cortex is not sufficient in explaining adolescent behaviour because in this case children should be remarkably similar to adolescents or even worse, given the immaturity of their prefrontal cortex and cognitive abilities. According to this model, the subject is more influenced by the functionally mature limbic system during adolescence (ie, imbalance of limbic control compared to prefrontal areas), compared to children, in which both systems (ie, limbic and prefrontal) are under development; and compared to adults, who have fully matured systems. This perspective therefore focuses on an early maturation of the limbic system compared to the prefrontal areas with immature top-down control (Casey et al., 2008) (Fig.2).

At the beginning of adolescence, there is a new period of intense synaptogenesis (the first period of synaptogenesis is between the 3rd and 6th month of intrauterine life), that is the proliferation of new synapses, which ends only with the death of the individual. In this period of life, there is a progressive increase in gray matter, which reaches a peak in density. Then a period of stasis occurs (Bricolo et al., 2010). Synaptogenesis is a process of formation and maturation of the neuronal synapses necessary for the high specificity of cellular connections. In adolescence, however, there is also a new period of synaptic pruning (the first period of synaptic pruning is in the last months of gestation). The gray matter therefore increases in density and reaches the plateau with an "inverted U" shape pattern: the maximum neuronal density of the gray matter in the frontal cortex occurs at around 12-13 years of life. Subsequently, due to the phenomenon of "synaptic pruning", there is a reduction in the total cortical volume due to the elimination of the less used neuronal connections, and by the consolidation of the most heavily used networks (Fig. 3)(Casey et al., 2008). The "use it or loose it" rule helps to understand the "drive" and "controller" model more accurately and to interpret it in the different stages of life. This rule requires that the most used neuronal connections are structured and strengthened, while the poorly used connections tend to be less structured. In other words, the environment is fundamental to assure stimuli that maintains a balance between drive and controller. Therefore, the educational system in which a child/adolescent is living should favour the full development of control abilities, ie provide stimuli that inhibit behaviours aimed exclusively to satisfying the impulses (drives); finally to obtain a full structuring of the controller in the prefrontal cortex (Giorgio et al., 2010). Supporting this hypothesis, some studies conducted in the 2000's investigated the activation

of brain areas in adults and adolescents. The two groups were evaluated for the same task, showing different results attributable to the different brain functioning of adults and adolescents. Specifically, adolescents activate the orbitofrontal cortex (OFC), the ventrolateral frontal cortex (VLPFC), the dorsolateral prefrontal cortex (DLPFC) and the cingulate cortex (CC) (Bricolo et al., 2010). Therefore adolescence represents a risk period, since the limbic regions, areas of the gratification system, mature before the frontal regions responsible for control (Fig. 2) (Casey et al., 2008).

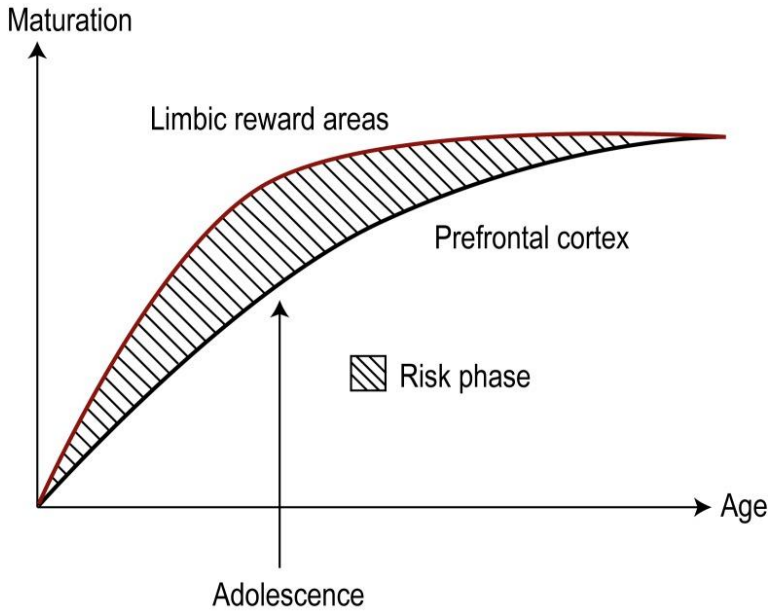


Fig. 2: The risk period in adolescence. Nonlinear maturation processes of subcortical and prefrontal brain areas lead to an imbalance of neural networks in adolescence. The period of risk is defined by the dotted area: the limbic regions, responsible for the gratification system, mature before the frontal regions assigned to control (Adapted from Casey et al., 2008).

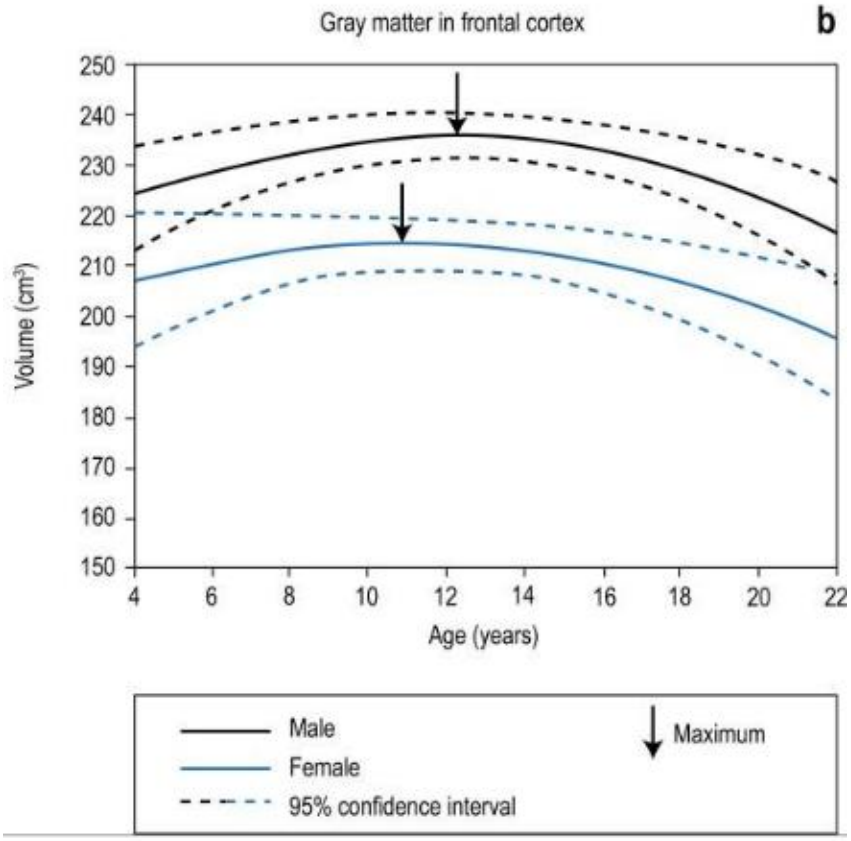


Fig. 3: Development of the cerebral gray matter. The density peak is reached at around 12-13 years of life (as indicated by the arrow), then volume decreases at around 20 years of age (inverted U-shape trajectory) (Adapted from Giedd et al 1999).

1.3 Differential Diagnosis

When we approach to DD, the most important differential diagnosis is between unipolar and BD. In developmental age, the presence of manic symptoms do not mean that the patient certainly suffer of BD, since only the 25% of youth with these kind of symptoms meet criteria for BD (Findling et al., 2010). Another important entity to differentiate is the psychotic onset, for the presence of psychotic symptoms or paranoid contents. Alteration in concentration are not only depressive symptoms, but they could be core symptoms of ADHD. In case of irritability, a Conduct disorder or an Oppositional Defiant Disorder should be excluded (Brent and Maalouf, 2015).

1.4 Self-injurious behaviors and suicide

Suicidal ideation is a form of thought characterized by meditations focused on self-injurious actions or suicide. It includes the will to kill oneself by making a plan about the place, the day, the way and also thinking about the possible impact of the suicide on the close surrounding people.

Self-injurious behaviours are any form of intentional poisoning and self-inflicted injury (cuts,

substance overdoses, self-locking, running into traffic) without distinction regarding the underlying intentionality to commit or not suicide (completed suicides and suicide attempts). Suicide consists in death as a direct result of self-injurious behavior (Vicari and Vitiello, 2015).

Suicidality behaviours ("self-harm behaviors" that include suicidal ideation, self-injurious behaviours with suicidal intent or suicide attempts-SA) refer to situations of "absolute urgency" as they require immediate and non-deferrable interventions for the potential danger of life or of serious damage to health of the patient (Vicari and Vitiello, 2015).

1.4.1 Self-injurious behaviours

Self-injurious behaviours (including SA) affect about 13% of the population during lifetime, with annual rates of 10% and an increasing trend since the 1960s (Evans et al., 2005). In adolescence it is estimated a lifetime prevalence of 28-45% (Nock, 2010; Brunner et al., 2014), with Italian rates of 20% (Brunner et al., 2014) and an increasing trend since 2006, observed comparing the estimated rates of the CASE (Madge et al., 2008) and SEYLE studies (Carli et al., 2013).

Considering these behaviours from a classification point of view, scientific literature present two tendencies. The first unify in a continuum the self-injurious behaviours with SA, stating that in the same patient can coexist at the same time behaviours with or without suicide intentionality and that the clinical evaluation is useful to distinguish what the subject feels and intends to communicate. The second orientation tends to differentiate non-suicidal self-injurious (NSSI) behaviours from SA, since the clinical populations seem to have different underlying motivations, severity and cognitive characteristics, they use different methods and there is also a different neurobiology (Nock, 2010). In DSM-5 (APA, 2013), the distinction between these two entities was introduced in the third section entitled "Conditions requiring further study" (Appendixes 3 and 4), however, since suicidal ideation is a dimensional phenomenon, a clear categorical distinction is often not possible in clinical practice.

Self-injurious behaviours are generally more common in poor socio-economic classes and among females between 12 and 15 years old. In this range of age there is a male: female ratio of 1:5-6 (Hawton et al., 2003; Olfson et al., 2005). Access to services is estimated to be 1 in 8 cases, reflecting the enormous spread of these behaviours in the population and the reduced access to treatment (Hawton et al., 2002; Madge et al., 2008). The reasons for the increase of the phenomenon in recent years are not clear, however, the availability of medical drugs, the inappropriate consumption of drugs and alcohol, adolescent stress, and an interpersonal hypersensitivity and to some other external factors seem to contribute. Other factors are the increased incidence of depressive symptoms, the precociousness of initiation of sexual activity, contagion or social transmission and bullying (including cyberbullying) (Fig.4)

(Hawton et al., 2012a).

Among the most common methods of self-harm there are self-cutting, (Hawton et al., 2002; Madge et al., 2008), more frequent among females, get superficial burns with cigarette butts or lighters, followed by inappropriate self-medication with excessive medical drugs assumption and by risk behaviours (indirect self-harm). In most cases, the purpose is to reduce negative emotions such as tension, anxiety, self-reproach and/ or to resolve an interpersonal difficulty. Subjects often refers only an immediate feeling of relief. The adolescent often does not seem to be fully aware of the reasons that led him/her to act; he/she does not seem to be able to describe the reasons and can present a greater tolerance to pain. All these factors lead to the "normalization" of such behaviours. Subjects often learn this behaviour through someone else's suggestions or observations. When this type of behaviour occurs frequently, a sense of need and craving can be developed with behaviors similar to those of addiction. In self-cutting the lesion is procured with a knife, a needle, razor blades or other sharp objects. The most affected areas are usually the thighs and the forearms. The wounds inflicted can gradually become more numerous and deep. Scientific studies have hypothesized a particular neurobiological vulnerability related to the puberty phase of girls who experience self-cutting (Hawton et al., 2012b). The repetition of NSSI behaviours is common in adolescents, particularly in those who use self-cutting (more than 55% in Madge et al., 2008). Predictive factors of repetition of self-harm behaviours include mood disorders, a history of sexual abuse, exposure to self-harm and sexual orientation problems (Hawton et al., 2012). Although there is not a declared intentionality of suicide, the task of the clinician, as well as to perform a careful objective examination, is not to underestimate these behaviours. Self-harm behaviors are part of the clinical psychopathological manifestations and are decisive for the therapeutic choices, as predictive of suicide (more than a previous history positive for SA, as stated in Ougrin, 2012) or of a tendency to act or predictive of a possible escalation potentially fueled by activating drugs. The task of the specialist is also the characterization of the behaviours as occasional or repeated episodes (≥4/ year), habitual and non habitual patterns, and analyzing the context or phase in which they occur (Ougrin, 2012).

In recent years, the identification of predictive thoughts or characteristics of suicidal risk in the population of self-harm patients (such as impulsivity) has attracted increasing interest, but the theme still needs further studies. Literature agrees that the risk of suicide is more frequent in male adolescents, in those with underlying psychiatric disorders, in those who experience repetitive self-injuries and among self-cutters (Hawton et al., 2012).

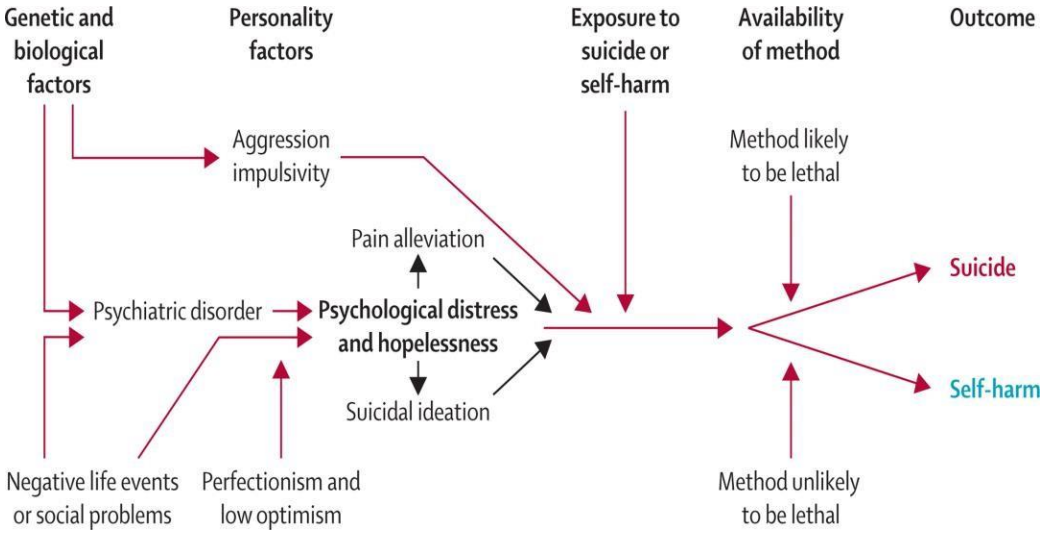


Fig. 4: Risk factors for self-harm and suicide in adolescence (Adapted from Brent and Weersing, 2008).

1.4.2 Suicide

According to the WHO, suicide is the second leading cause of death in the 15-24 age range group, after road accidents (GBD, 2016). In the US, from 2005 to 2011, it was found to be the second leading cause of death among children between 12 and 17 years (Perou et al., 2013). In Italy up to 2002, data reported a suicide rate of 2.3 per 100,000 per year in adolescents (12-19 years) with a higher suicide rate among males (Pompili et al., 2009). From 1971 to 2008 all causes of death among children and adolescents showed a significant decrease in frequency with the exception of suicide (Pompili et al., 2012) (Fig.5). The means used to suicide vary depending on the availability of lethal means in the various countries (ingestion of substances, firearms, hanging, defenestration, asphyxia and so on) and on sex (Pompili et al., 2012). According to data presented to the SINPIA Congress (2004), in Italy the poisoning from drugs or other substances is at the first place (90%), then cutting veins, throwing themselves down, hanging and suffocation. For the SA the most used means is poisoning with medical drugs.

According to Vicari and Vitiello (2015), three profiles of adolescents who come to suicide can be recognized. The first is the youth who faces a suicidal crisis: despite a discrete global functioning, he/she suddenly findhim/herself in a critical period, generally following a stressful event such as the loss of a relative, or internal factors such as loss of self-esteem, self-confidence etc. The second profile is the model of suicidal young recognized as "the solitary", usually a white male. In this case, the sufference begins early in adolescence while the suicidal action happen close to adulthood. This subject is usually isolated with very few relationships and he/she is affected by mood disorders, schizoid traits or even borderline personality disorders. The third profile is that of girls of mixed ethnicity who have illegal,

dangerous, violent and aggressive behaviours. They are generally rebels and abuse of drugs and alcohol. The difficulties emerge at the beginning of adolescence with the arrival of depressive symptoms to which they cannot cope and react spontaneously with aggressive behaviours. When the act is no longer enough to contain the anguish, a profound crisis takes place that puts the adolescent at risk of suicide. These patients in many cases have a borderline personality (Vicari and Vitiello, 2015).

Suicide is usually the result of a long chain of events in which the opportunity to intervene effectively has failed. Suicide is often the result of a combination of genetic, biological, psychological, social, cultural and environmental factors associated with the crisis of protective factors that vary depending on the moment, the situation and the individual. The subject is in a "perturbed state" whereby suicide is the only solution for put an end to a psychological pain that has become unbearable. Suicide is associated with psychiatric disorders such as mood disorders, DD and BD, eating disorders, psychotic onset and substance dependence/ abuse (Vicari and Vitiello, 2015). As for mood disorders, it is notable that most teenager affected by depression do not act suicide, but a significant number of suicidal adolescents have an underlying or concomitant affective disorder (Condini et al., 2005). The main cause of a SA or a complete suicide is a previous attempt. For every SA, the risk that another SA occurs over the next two years increase by over 30% (Malone et al., 1995).

Among the risk factors there are also dysfunctional family factors such as violent domestic environments, poor parental assistance, frequent quarrels, divorces and stressful life events such as early death of a parent, school failures and pressure from the peer group (Vicari and Vitiello, 2015) (Table 2).

In this context, there is a central debate on the relationship between antidepressant drugs and completed suicides (Masi et al., 2013). The use of antidepressants in the developmental age has been increasing during the 1990's, but in recent years their use has been decreasing, due to the fear that they may favour suicidal thoughts or behaviours during treatment (Olfson et al., 2006). The risk of suicide during the antidepressant treatment is probably weak, but constantly reported in the various studies and metanalysis, and therefore requires a great preliminary attention to the chose of treatments (in particular an explicit exploration of suicidal ideation or previous SAs in the subject and/or in family members). This risk is significantly greater in younger people than in adults, and could involve suicidal ideation and self-injurious behaviors, while there are unclear data about the relationship between antidepressants and complete suicides. A predictor of risk is the persistence of depression and therefore the goal of the therapeutic strategy should always be to reduce depressive symptoms. There is a risk that the fear of suicidal events during antidepressive therapy may

lead not to treat pharmacologically subjects with more severe depressions, which are instead those at greater suicidal risk and with greater efficacy of response to drug therapy (Masi et al.,, 2013). In these cases, all possible suicide risk factors must be carefully evaluated, starting from previous SAs (the most important predictor), to clinical factors (BD, borderline personality disorder), the use of substances, but also obviously environmental, family and social factors (Masi et al, 2013).

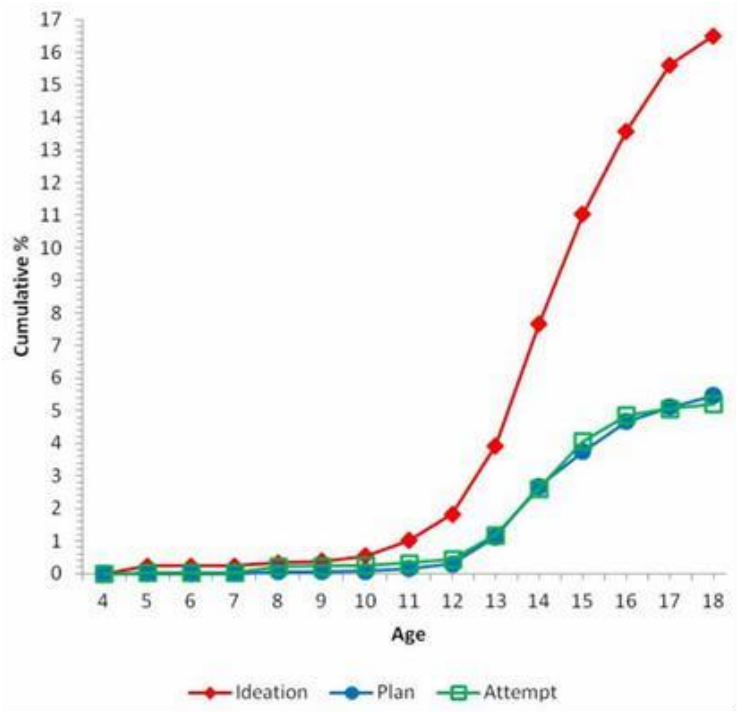


Fig. 5: Self-harm and age (Adapted from Moran et al., 2012). Increasing prevalence in adolescence (12-17 years)

Tab. 2: Risk factors for SAs. Adapted from Cepeda, 2010.

RISK Factors
History of impulsivity
History of a previous suicide attempt
Trigger events
Presence of depression, guilt, despair or anger
Presence of cognitive deficits (poor problem-solving strategies)
Presence of maladaptive coping strategies (social isolation, drug abuse)
Interpersonal difficulties with boyfriends or parents
Family conflicts (family violence, physical violence or sexual abuse)
Personal losses (losses, divorces, transfers)
Rejection of peers
Physical illnesses
Contact with a person who has committed suicide or who is planning it
Emulation or imitation of suicidal behaviour

In clinical practice, the main risk factors can be summarized in the acronym SADPERSON (Patterson et al., 1983):

- **S**ex: Males are more at risk of committing suicide than females. Males complete suicide about 4 times more frequently than the females, although the attempts are more frequent among females.
- **A**ge. The age at greater risk for suicide varies over time, thus it is useful to consult current statistical data.
- **D**epression. The suicide rate among clinically depressed subjects is about 20 times greater than that of the general population. Loss of hope is an aspect of depression closely linked to suicide. These two aspects, depression and despair are the strongest predictors of desire for premature death.
- Anamnestic **P**recedents. About 80% of completed suicides were preceded by a first attempt.
- **E**thanol/substances abuse.
- **R**ational thinking loss (psychosis). Psychosis ('I hear a voice in my head ordering me to kill myself') increases the risk. Some estimates suggest that 20-40% of schizophrenics make a suicide attempt sooner or later, and the risk is much higher in the early stages of the disease.
- **S**ocial support lacking. For children/adolescents it can be the break with the "first love", which they can live very seriously even if others, such as for example parents, can consider it a trivial event.
- **O**rganized plan
- **N**egligence of parents, presence of significant stressors, history of suicide in the family. Carelessness, abuse, trauma.
- **S**chool problems (including cyberbulling) and sickness.

1.5 Pathogenesis of Depressive Disorders

Affective and anxiety disorders are among the most common childhood psychiatric disorders (Ipser et al. 2009). Despite the high percentage of these debilitating and complex disorders, their pathogenesis is incompletely understood (Amitai, 2016).

In literature, etiopathogenesis of depression is attributed to different causes and processes that are of psycologic, biologic and social nature. The main attitude of researchers is that of considering DD as a complex illness with a multifactorial aetiology, according to a biopsychosocial model. It is likely that alterations in several interacting systems underlie its pathogenesis.

To understanding depression as a boundary event between mind, brain and body, multiple

causal factors must be considered and weighed. These factors concern the patient's psychological structure, his genetic and physiological constitution, and finally the family and social environment inthe first years of life.

Sigmund Freud and his followers (1917) were among the first to propose an entirely psychological explanation of the genesis of depression. According to his theory, depression is the subjective reactions of mourning for the loss of the object of love, associated to the anger, which is first directed against the object (an internal object) and therefore against oneself. This hypothesis was subsequently modified and supplemented by other psychoanalytic theoreticians, in particular by supporters of object relations theory, who underline how the inner life of a person is profoundly influenced by the early relationships between the child and the parental figures (Klein, 1934; Kohut, 1971; Kernberg, 1976). More recently, the English psychoanalyst John Bowlby (1953) proposed the famous theory of attachment), synthesizing methods of psychoanalysis and cognitive psychology. Empirical studies on mother-child relationships, on separation and loss of the mother, initially conducted on primates and later replicated by Mary Ainsworth (1978) on very young children, permitted to define some models of early caregiving that, if insufficient on a practical or emotional level, can predispose the child to catastrophic experiences of abandonment or failure in adult life. Finally, it is necessary to mention the models developed by Aaron T. Beck and his school (1987) who interpret depression as the result of a precise style of thought of the individual, consisting of a stably negative image of oneself, one’s own future and his/her own environment (neuroticism). From this theory derives the cognitive-behavioural therapy (CBT) that currently, according to most international guidelines, is the first-choice psychological treatment for DD. CBT aims to correct the cognitive distortions of the subject, to improve coping strategies and finally to enhance the subject's ability to experience pleasure and gratification in everyday activities (Beck et al., 1987).

In the biological field, n the same years when the first psychotropic drugs were discovered, various theories have been changed according to favorable or contrary evidences. The monoaminergic hypothesis suggest the role of dysfunctions in the regulation of noradrenergic, serotoninergic and dopaminergic neurotransmitter systems. The onset and maintenance of the psychic and physical symptoms of depression were mainly attributed to a decrease in the cerebral serotonin rate, and this led to the production of many antidepressant drugs aimed to enhance serotoninergic transmission. This hypothesis has undergone several revisions in recent years, with a substantial shift of attention from the anomalies of the monoaminergic neurotransmitters to the alterations of the receptors. In a study conducted with the Single Photon Emission Computerized Tomography (SPECT), authors demonstrated a reduction of the binding to serotonin transporter in depressed patients compared to healthy ones (Malison

et al.,1997) . With respect to dopamine, neuroimaging have shown a lower binding and a reduction in transporter density. However, since the brain is composed of a complex neural network, in which groups of cells and functional areas are in close connection through numerous neurotransmitters, the most widely accepted hypothesis is that the aetiology of depressive disorders does not depend so much on the dysregulation of a single neurotransmitter system but rather on an imbalance of several morphologically and functionally related systems (Castren, 2005). In this view, studies has investigated that the role of other neurotransmitters and neuroactive molecules. For example, glutamate, an aminoacid that mediates excitatory neurotransmission and plays an important role in learning and memory, appears to be involved through mutations or polymorphisms of the gene encoding the NMDA (N-methyl-d-aspartate) receptor, especially in the hippocampus (Schiffer et al., 2002).

Another line of research postulates the presence of alterations at the level of the molecules that are responsible for the transduction of signals from the receptor to the cell (second messengers). Most serotonin receptors are associated with the G protein, which modulates the activation of the second messenger cAMP (cyclic adenosine monophosphate), which in turn causes cellular activation. The involvement of this protein in mood disorders has been evidenced by several pharmacological researches on animals. Antidepressants, lithium and lamotrigine regulate different α subunits of G protein, and their gene expression, in different brain areas.

In general, neuropeptides are recognized to have a key role in the neurobiology of depression (Malison et al, 1997).

Furthermore, it was suggested that the endocrine system may have a role in the development of DD. In different subtypes of depression, it was possible to identify a different neurochemical substrate of action of CRH (Corticotropin Releasing Hormone) and of the cortisol-related inhibition. In depression with melancholia, an increase in central secretion of CRH is observed and therefore an increase in circulating cortisol; on the contrary in the atypical depression there is a reduction in the activity of the CRH and, consequently, a reduction in the levels of cortisol. The two different endocrine situations seem therefore to be related to distinct clinical pictures: one (melancholia) characterized by slowing / psychomotor agitation, insomnia and loss of appetite, the other (atypical) by asthenia, hypersomnia and hyperphagia (Murphy, 1991; Heuser et al., 1994; Mackin et al., 2006).

Another neuropeptide probably involved in the etiopathogenesis of depression is substance P, which is a neurobiological mediator of pain response. It is involved in the reaction to stress and is produced in areas of the CNS that are responsible for the regulation of affectivity. The antagonist action of substance P is recently used, with some positive effects, in the treatment

of depression.

Finally, the use of the thyroid hormone (which has the effect of stimulating the general metabolism and the psychophysiological activation), as an additional treatment to the antidepressant in resistant forms, has now become common practice, confirming the involvement of this endocrine axis in depressive pathology (Murphy, 1991; Heuser et al., 1994; Mackin et al., 2006).

Another important theory suggested by researches is that of the synaptic plasticity induced by the activity of neuronal networks. Depression, as well as other diseases of the CNS, would be due to morpho-functional alterations of the networks of some neurons that are formed during brain development through interaction with the environment. These neurons perform the function of processing cognitive information and emotion.

The synthesis and the release of the neurotransmitters and of the molecules of transport of the signal would depend on the quality and the quantity of the interaction with the environment. Particular experiences in the developmental age could lead the construction of the neuronal networks of the adolescent first and then of the adult in a dysfunctional sense.The action of antidepressants on neurotransmitters and membrane transporters, would stimulate, with the continuation of treatment, the general plasticity of the entire network, triggering a process of structural "self-repair". Unfortunately the limit of this hypothesis is that it is mainly supported by indirect evidences, since the exiguity of experimental data. Some evidences derive from studies on children with trauma (or severe deprivation) in early stages of psychological and neurological development, and subsequently followed up at different times, after trauma and after removal from the traumatic environment (Chugani et al. , 2001).

Neuroimaging studies in depressed patients show a reduction in volume of the prefrontal cortex and the hippocampus, probably linked to a reduction in complexity and neuronal connectivity: in some cases a reversibility of this morphological picture was observed following antidepressive therapy (Gould et al., 2002).

In particular, it has been shown that brain infusion of the trophic factor BDNF produces an antidepressant effect in some experimental models of depression based on the analysis of the rats' behaviour placed in front of unresolvable tasks: this suggests also the involvement of nerve growth factors and therefore of an altered plasticity in depressive symptomatology. Under stress conditions, the BDNF gene is repressed and therefore its synthesis is reduced; as a result, the hippocampal neurons undergo atrophy and apoptosis. Neuroimaging studies in depressed patients show a reduction in the volume of brain structures related to the hippocampus, confirming the hypothesis that in the course of depression the hippocampal neurons are reduced in number, volume and functionality. It was finally observed that prolonged treatment with antidepressants may increase the expression of BDNF and its Trk-

β receptor in the structures of the limbic brain (Shirayama et al., 2002).

The frequent association between mood and immune disorders suggest also the possibility that inflammation could contribute to the origin of some psychiatric conditions, especially depression (Dantzer et al. 2008; Miller et al.2009; Kim et al. 2014; Rosenblat et al. 2014).

The inflammatory hypothesis, also known as the malaise or cytokine theory of depression (Maes et al. 2009; Miller et al. 2009; Ur et al. 1992) asserts the role of psycho-neuroimmunological dysfunctions with a hyperactivation of the immune system (Zunszain et al.,2013) during mood disorders. In this way, inflammation is considered the final process of an interaction between external and internal stresses that could determine the onset of the disease, like in somatic pathologies.

According to the biopsicosocial model, psychosocial stress can precipitate episodes of depression (Kendler et al. 1999) and it is known that episodes of stress, for example difficult caregiving and hostile marital relationships (Miller et al., 2008) are associated to inflammatory processes (Black 2003). Interestingly a stressful childhood could produce neuroendocrine and immunological abnormalities that are thought to condition the development of a proinflammatory phenotype in adulthood (Chida et al. 2007; Elenkov 2008; Zunszain et al., 2013).

Evidences in scientific studies in adult population highlithed that MDD patients present often an altered peripheral immune system that is an impaired cellular immunity, an increase in the levels of proinflammatory cytokines in the blood and cerebrospinal fluid, an increase in blood concentrations of acute phase proteins, chemokines and adhesion molecules (Miller et al, 2009; Kim et al. 2014). Cytokines influence the metabolism of neurotransmitters, the neuroendocrine function and finally the CNS activity (Dantzer et al. 2008; Schiepers et al. 2005). These molecules can lead to changes in neuronal apoptosis, oxidative stress and metabolic derangement, as well as to impairing processes of synaptic plasticity and neurogenesis (Hayley et al., 2005; Leonard and Maes, 2012; Maes et al., 2009; McAfoose and Baune, 2009; Song and Wang, 2011).

Administration of high levels of proinflammatory cytokines has been shown to cause changes in behaviour, such as low mood, fatigue, anxiety, sleep disturbances, anhedonia and cognitive dysfunction, all of which similar to what it is observed in depressive disorders (Capuron and Miller 2004; Pollak and Yirmiya, 2002).

Studies suggest that only some groups of depressed patients presents increased inflammatory molecules, in particular who presents resistance to treatment, a history of childhood maltreatment, and obesity (Danese et al., 2008; Shelton and Miller, 2010; Lanquillon et al., 2000).

In literature, many serum inflammatory molecules were measured in adults with anxiety and

depression to understand wich one could be a useful clinical biomarker for depression. Some of the most frequently studied are cytokines produced by innate immune cells, including interleukin-1 (IL-1), IL-6 and tumour necrosis factor-alpha (TNF-a) and the acute-phase C-reactive protein (CRP) (Dowlati et al,2010; Howren et al., 2009).

Little is know about the association between inflammatory processes and neuropsychiatric disorders in children and adolescents (Belem da Silva et al., 2017). Even though the literature supports a pro-inflammatory state across internalizing disorders in adult samples (Dowlati et al., 2010; Hoge et al., 2009; Howren et al., 2009), there are fewer studies in pediatric age (Mitchell and Goldstein, 2014). Therefore, studying pro-inflammatory states in youngsters may clarify whether inflammation represents an early biomarker for the pathophysiology of emotional disorders.

The monoaminergic theory, the involvement of the HPAA, the role of the neurotrophic factors and the neuroinflammation appear finally to be thightly connected faces of the same complex process in pathogenesis of depression.

Thus, in a basal situation of genetic vulnerability, unfavourable environmental conditions could affect gene expression with structural and functional alterations of the systems involved in affective regulation. These alterations could subsequently be self-regenerating, finally manifesting themselves as a DD.

1.6 Neurotrophins

During the early 50's a fortuitous discovery opened a new and fundamental field of investigation for biology. Studies conducted by Rita Levi Montalcini and collaborators on mice's nervous system and on tumor cells called Sarcoma 180, led to the discovery that these tumor cells synthesize and release a protein that plays an essential role in the differentiation and function of two species of sensitive and sympathetic cells (Levi-Montalcini, Hamburger, 1953; Cohen et al., 1954). This molecule is called the "Nerve Growth Factor" (NGF) and it is considered the prototype of the entire neurotrophin family (NT). Subsequently, several studies were undertaken to isolate other proteins structurally and functionally related to NGF. In the early '80s Barde and colleagues succeeded in isolating from the pig brain a protein factor with a high sequence homology with NGF, called "Brain-Derived Neurotrophic Factor" (BDNF) (Barde et al., 1982; Leibrock et al., 1989). Subsequently, researchers isolated many other components of the NTs related to mammals, including neurotrophin 3 (NT-3) (Ernfors et al., 1990) and neurotrophin 4/5 (NT-4/5) (Barde, 1990). These genes encode pre-pro-neurotrophins. Mature proteins weigh about 13 kDa and are present in solution as non-covalently bound homodimers. They have basic isoelectric points, an unusual feature for

secreted proteins that permit to limit their range of action. The promoter is characterized by a conformation of disulfide bridges known as "cysteine knot", later identified also in other secreted proteins, such as PDGF (Platelet-Derived Growth Factor) and TGF-βs (Transforming Growth Factor- βs) (McDonald and Hendrickson, 1993). With the exception of NT-4/5, neurotrophin sequences are highly conserved in mammals. The family of neurotrophins plays an important role in the survival, differentiation and functioning of different neuronal populations, both belonging to the central and peripheral nervous system. For this reason the NT's are at the center of numerous studies in the field of neuroscience, aimed not only at understanding their physiological role but also to find their involvement in the pathogenesis of certain diseases of the nervous system, characterized by the death of specific neuronal populations. Among these the most studied are neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, and more recently numerous psychiatric diseases, such as depression.

1.7 BDNF

The Brain Derived Neurotrophic Factor (BDNF) was the second neurotrophin to be characterized (Barde et al., 1982), about thirty years after the Nerve Growth Factor (NGF). Thanks to this brilliant discovery, in the fifties, Rita Levi-Montalcini won the Nobel Prize for medicine (Levi-Montalcini and Hamburger, 1953).

In humans, the BDNF gene is located in chromosome 11p14 and consists of several exons 5' and one 3' exon (exon IX) that encodes the mature BDNF protein (Fig. 6).

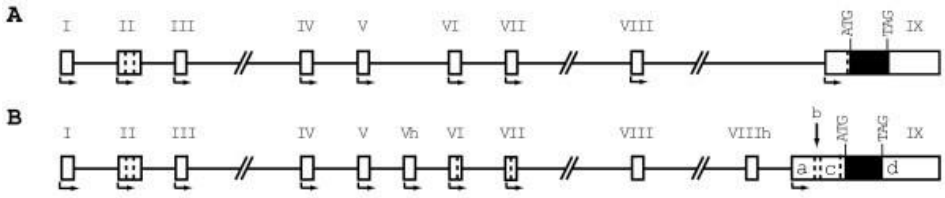


Fig. 6: Schematic representation of the BDNF gene in mouse (A) and in man (B).
In mouse, gene is composed of 8 non-coding exons (I-VIII) (white) that are joined by splicing to a common coding sequence, indicated with "IX" (in black) (the sites of the beginning of the transcription are indicated with the arrows). The dotted lines indicate alternative splicing sites. Although the human gene is very similar, it differs for the additional exons Vh and VIIIh and for a more complex alternative splicing pattern involving the exon IX (Adapted from Koppel I. et al., 2009).

From this gene, which includes 9 different promoters, 22 different mRNA isoforms are transcribed. The different isoforms are expressed in specific ways and times at the cerebral, pulmonary or cardiac levels. Analyzing the gene, the Val66Met polymorphism (replacement of a valine in methionine in codon 66, exon IX) is particularly interesting, since it is

considered to play a role in the regulation of intracellular trafficking and in the release of mature BDNF. It seems to be correlated to the hippocampal volume, to learning and episodic memory in humans (Egan et al., 2003). The Val66Met polymorphism does not appear to be related to the serum protein levels in the adult (Shen et al., 2014), however, the literature recognizes a role in the genesis and expression of mood disorders, both in children and in adulthood (Kaufman et al., 2006; Hilt et al., 2007). The Met allele, in mice (Chen et al., 2006) as in humans (Egan et al., 2003; Pezawas et al., 2004; Bueller et al., 2006), was demonstrated to be related to an increase in anxious behaviours and MDD (Verhagen et al., 2010), being a marker of interpersonal hypersensitivity.

The BDNF protein, the most widespread neurotrophin in the CNS, shares about 50% of the aminoacids with the other neurotrophins (NGF, NT-3 and NT-4/5) and, like the other neurotrophins, consists of a homodimer. The transcript includes a promoter region and a signal peptide (pro-region) with a glycosylation site. Initially a precursor peptide is synthesized, pre-proBDNF, at the level of the rough endoplasmic reticulum, then the promoter peptide is cleaved by a specific serin protease (convertase) and then the proBDNF (truncated form of about 32KDa) is converted into the mature form (13 -14KDa) by extracellular metalloproteases (Fig. 7) (Yoshida et al., 2012a). In the past, it was believed that only the mature secreted form was biologically active, whereas proBDNF was thought to be an inactive intracellular precursor. Evidences have shown that the two forms are both active: the proBDNF peptide has different structural and biological characteristics with respect to the mature BDNF, binding with different affinity to the receptors (p57NTR for the proBDNF, with activation of apoptotic pathways, and TrkB for the mature BDNF, with stimulation to the formation of dendritic spines) (Lu, 2003).

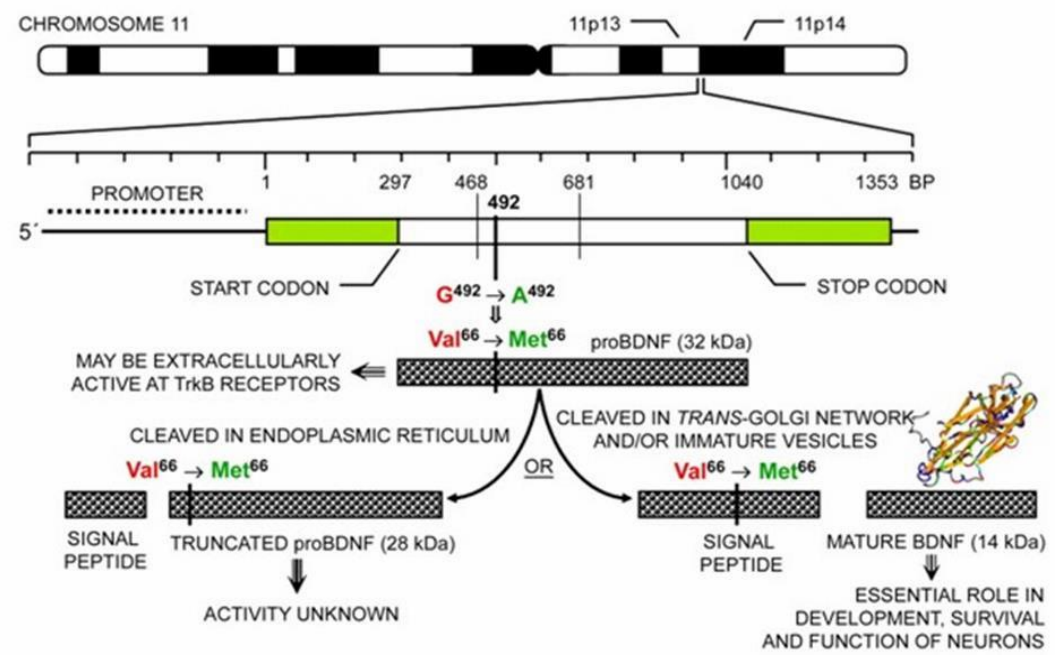


Fig. 7: BDNF synthesis (Adpted from Murer et al., 2001).

All neurotrophins share the same three-dimensional primary structure consisting of two pairs of antiparallel beta strands and disulfated bonds between six cisterna residues in highly conserved positions. They also have common biochemical characteristics such as molecular weight (13.2-15.9 kDa) and isoelectric point (pH 9-10). The NTs bind to two different types of transmembrane receptors: the Trk (Tropomyosin Receptor Kinase) and the receptors of the neurotrophins p75. The mature form of BDNF binds the p75NTR receptor with low affinity and the receptor tyrosine kinase B (TrkB) with high affinity (Huang and Reichardt, 2003; Lu, 2003). When a neurotrophin binds to a membrane receptor of the tyrosine kinase family (Trk), this binding with the ligand determines the receptor dimerization and its consequent activation. This process results in an autophosphorylation of receptor's tyrosine residues that become binding sites for intracellular proteins. The activation of these target proteins (e.g, phofolipase C, PLC, gamma1, phosphatidylinositol-kinase, PI3-K) in turn determines the activation of a cascade of intracellular signals such as the Ras-MAP kinase cascade (ERK, Protein Kinase activated by Mitogens) and CREB protein (cAMP Responsive Element Binding protein), responsible for the expression of some genes in the hippocampus (including the BDNF) (Patapoutian et Reychardt, 2001) (Fig.8).

The CREB protein induces a phosphorylation resulting in inactivation of the pro-apoptotic protein BAD (cell death factor), while the self-phosphorylation of the CREB itself induces an upregulation of the Bcl-2 protein that promotes survival and neuronal plasticity (Duman, 2002b).

The TrkB receptor (tyrosin kinase type 2, NTRK2) which binds BDNF with high affinity is present in a full-length or in a truncated form; the latter one seems to play a role in modulating

the full-length form. For example, following severe trauma, there is a greater production of the truncated form that inhibits the naive form, modulating the vulnerability of astrocytes and the sequestration of BDNF within them (Binder et Scharfman, 2004). All the neurotrophins also bind to p75 receptors, linked in turn to the TNFR receptor family for Tumor Necrosis Factors. This binding, which determines the specificity of the molecules, activates various intracellular cascades, including that of NF-kB, which mediates programmed cellular death or apoptosis (Binder et Scharfman, 2004).

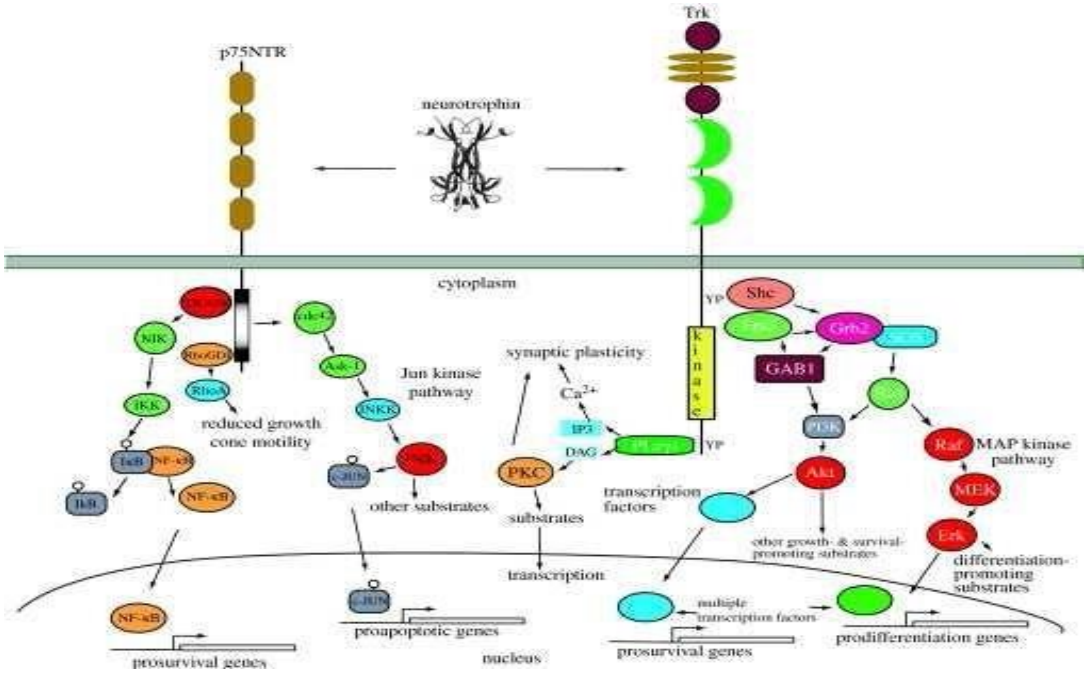


Fig. 8 Representation of the intracellular pathways mediated by Trk and p75 receptors (Reichardt, 2006).

The p75 receptor regulates three main routes:

• activation of NF-kB which determines the transcription of several genes, including many promoters of cell survival;

• activation of the Rho pathway that controls the growth of the axon's growth cone;

• activation of the pro-apoptotic signal mediated by p75 which requires the presence of sortilin.

Trk receptors control three main ways:

• Ras activation that involves the activation of MAP kinases that promote neuronal differentiation including axon augmentation;

• activation of PI3K through Ras or Gab1 that promotes the survival and growth of neurons;

• activation of the PLC-γ that causes the activation of the Ca2 + dependent protein kinase (PKC) that promotes synaptic plasticity

BDNF plays a central role in synaptic plasticity. The protein is involved in the promotion of cell growth and survival of many neurons, including dorsal root ganglion cells, cortical and hippocampal neurons and specific sensory peripheral neurons (eg. neurons of the vestibular and petrous ganglia). Unlike the NGF, BDNF does not appear to affect sympathetic or motor neurons (Binder and Scharfman, 2004). Neurotrophin seems to play a role in brain maturation, in the formation of correct axonal pathways and in dendritic morphology. Among the actions of BDNF there are the ability to contribute to the stabilization and maturation of pre-existing synapses and the ability to generate new synaptic contacts (Gomez-Palacio-Schjetnan and Escobar, 2013).

The protein seems to be able to re-activate cortical plasticity under appropriate environmental guidance, similar to what happens in the developing brain, modulating neural networks (Castre'n et Rantamaki, 2010) and protecting the CNS from excitotoxic, hypoxic insults and hypoglycaemia.

Studies on homozygous knock-out mice showed death after three weeks of life, while on guinea pigs with heterozygous gene, it was observed a reduced survival of immature neurons in the dentate gyrus with development of aggressive behaviours, hyperphagia and obesity, reduced seizure threshold and spatial difficulties, associated with the dysfunction of the 5-HT system (Lyons et al., 1999; Sairanen et al., 2005).

At the synaptic level, BDNF seems to have a protective role maintaining the morpho-functional integrity. It can strengthen the excitatory glutamatergic synapses and weaken the GABAergic inhibitory synapses. In a study on adult rats, Kang and Schuman (1995) showed that exposure of hippocampal sections to the neurotrophin resulted in a long-term potentiation (LTP) of afferences at the level of the pyramidal cells of the tissue. At the present time it is unclear whether this potentiation is due to a presynaptic action (eg increased release of vesicles containing glutamate) or to postsynaptic mechanisms (eg through the phosphorylation of the NMDA receptors) (Binder and Scharfman, 2004).

BDNF leads to a weakening of GABAergic synapses, through different pathways (modulation of the phosphorylation of GABAA receptor, down-regulation of ionic transporters) (Binder and Scharfman, 2004) (Fig. 9).

In addition to glutamate, BDNF also interacts with the main neurotransmission systems considered implicated in the genesis of psychiatric disorders and in particular with serotonin (Szapacs et al., 2004).

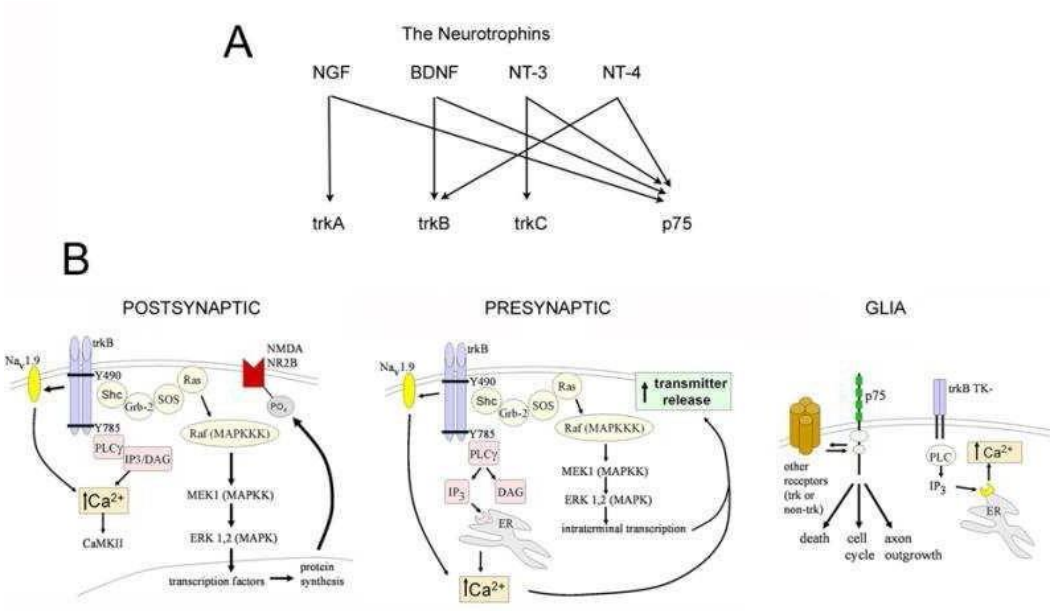


Fig. 9: Neurotrophic receptors and BDNF targets (Binder et al., 1999).

(A) Neurotrophins family includes NGF, BDNF and neurotrophins 3 and 4. NGF is a specific ligand of TrkA and TrkC (NT-3 is its specific ligand), whereas TrkB have two potential ligands, BDNF and NT-4. All neurotrophins bind p75. BDNF is considered the major ligand of TrkB in the hippocampus.

(B) A schematic illustration of the targets of the TrkB translation pathway for the presynaptic, postsynaptic and glial processes.

Approximately 10 years ago, Rosenfeld and colleagues detected the presence of BDNF at the blood level (Rosenfeld et al., 1995). BDNF levels are about 10 times higher in serum than in plasma, maybe because platelets release high amounts of BDNF when activated. In fact, platelets are not able to produce BDNF but they capture it from the plasma through a mechanism not yet known (Fujimura et al., 2002). Furthermore, the role of BDNF in platelets is also unknown: it probably has a specific function in tissue traumas, nerve lesions and hemorrhages. When activated platelets release BDNF, it would probably play a role in inflammation and in proliferation of cells and smooth muscle. Plasmatic BDNF is likely to be produced by endothelium, smooth muscle cells, activated macrophages and lymphocytes. Authors suggested conflicting views about the passage of BDNF across the blood-brain barrier (Pan et al., 1998) so it is not clear how central neurons and glia could actually influence the peripheral blood concentration of BDNF. Other peripheral growth factors such as VEGF and IGF can reach the brain (Pan et al., 1998) similarly to BDNF, influencing neurogenesis and CNS function. In rats, peripheral BDNF levels have been shown to undergo changes during maturation of the CNS similar to what is observed in developmental processes. Futhermore there is a positive correlation between BDNF blood and cortical levels (Teixeira et al., 2010).

In a study of Pan et al. (1998), the authors hypothesized a bi-directional BDNF passage from the brain to the plasma, through a saturable transporter, but further studies are needed to support the existence of an active neurotrophin transporter (Sen et al., 2008). It appears that circulating BDNF may have functional effects on the brain (Sen et al., 2008; Schmidt and Duman, 2010; Klein et al., 2011), resulting in an antidepressive response (Duman, 2004).
Therefore, due to the difficulties in studying brain BDNF levels, which would require invasive methods, the researches aimed at the evaluation of the peripheric BDNF activity from blood or saliva samples. Salivary, plasmatic, and, to a lesser extent, serum BDNF concentrations would appear to increase to a maximum level after birth, and then decrease with age in the adult population (Lommatzsch et al., 2005). The levels also appear to have circadian variations, with a peak in the first part of the day (Piccinni et al., 2008a; Tirassa et al., 2012) and increase with exercise (which also has benefits on the mood) (Pareja-Galeano et al., 2013). There seem to be a relationship between BDNF and body weight/ food intake: BDNF protein increases energy expenditure and reduces appetite (Pelleymounter et al., 1995).
The BDNF seems to have a series of regulatory functions on energy balance and neuronal plasticity, which could also explain the seasonality of DD (and suicides), as shown by some studies on the adult population (Molendijk et al., 2012). These researches measured a peak in blood concentration of the neurotrophic factor at the end of the summer and a reduction at the end of the winter, probably related to the number of hours of sunshine in the 7-8 weeks prior to the sample collection.
The literature also argues that there is an interaction between expression and activation of BDNF and glucocorticoid cascade. BDNF modulates the response to stress through the regulation of the activation of the hypothalamus-pituitary-adrenal axis (HHHA), with increased cortisol (Carbone and Handa, 2013). Early stress (eg separation from the mother) could lead to alterations in mediators and finally to persistent hippocampal atrophy even in adulthood. BDNF is also recognized to be the mediator of the effect of some steroid hormones on neuronogenesis (eg estrogen, testosterone, DHEA) (Pluchino et al., 2013).
Since the discovery of BDNF involvement in the modulation of brain activity-dependent synaptic plasticity, and in light of its greater concentration at the level of the hippocampus, numerous studies have been developed to define the role of the factor in brain processes. The neurotrophin, ubiquitous in the CNS, seems to be implicated in all the physiological and pathological mechanisms in which there are abnormalities of the trophic support (eg neurodegenerative diseases) or of excitability processes (eg epilepsy and central sensitization to pain) suggesting common pathophysiological mechanisms and justifying some comorbidities (Sen et al., 2008):

- **Learning and memory**: studies on rats and humans have shown that the deficiency

of BDNF in the hippocampus leads to a reduction in learning, visuo-spatial (Linnarsson et al., 1997) and episodic (Egan et al., 2003) memory. These cognitive difficulties are also present in depression (in both these situation there are underlying hippocampal changes).

- **Epilepsy**: recent in vivo and in vitro findings highlighted the role of BDNF in modulating the electrophysiological changes that underlie epileptogenesis (Hong et al., 2014). Epileptic seizures appear to increase BDNF levels in the hippocampus in animal models (Nibuya et al., 1995). The inhibition of BDNF or its binding to the receptor inhibits epileptogenesis. Conversely, an over expression of the factor leads to spontaneous crisis (Binder, 2004). The hippocampus and limbic structures appear to be determinant for the pro-epilectogenic effect of BDNF (Binder et al., 1999a-1999b). It should also be remembered that some psychotropic drugs, which have an effect on BDNF, result to favor epileptic seizures.
- **Acute neurological disorders**: BDNF levels appear to vary in primary headaches (Fischer et al., 2012, Blandini et al., 2006) and in multiple sclerosis.
- **Neurodegenerative disorders**: a reduced expression of BDNF is found in patients with Alzheimer's disease, Parkinson's, Huntington's disease (we can recall the role of neurotrophins in memory processes), producing an increasing scientific interest for a possible therapeutic role of the molecule (Michalski and Fahnestock, 2003; Ventriglia et al., 2013)
- **Pain**: BDNF plays an important role in the neuro modulation of pain, also being synthesized by the neurons of the dorsal horns. Its expression determines hyperalgesia and nociceptive hypersensitization, up to allodynia, while a block of BDNF inhibits such central sensitization (Pezet et al., 2002; Pezetand McMahon, 2006).
- **Obesity and Metabolic Syndrome**: altered levels of BDNF can cause type 2 diabetes, obesity and metabolic syndrome (Hristova, 2013).
- **Cardiovascular disorders**: altered levels are found in patients after stroke or in person affected by atherosclerosis (Ejiri et al., 2005).
- **Rheumatological disorders:** neurotrophin appears to be elevated in patients with acute rheumatoid arthritis, with good response to immunomodulatory therapy (Forsgren et al., 2011).
- **Particularly traumatic or stressful situations** can reduce the expression of this neurotrophin in the rat hippocampus (Ueyama et al., 1997) and at the peripheral level in humans as occurs in adult patients suffering from post-traumatic stress disorder- PTSD (Dell'Osso et al., 2009). In these patients, reduced levels of BDNF would once again contribute to the loss of dendritic spines and increased neuronal vulnerability, with the possibility of hesitating in psychosis (van Winker et al., 2013).
- **Other neuropsychiatric diseases**: BDNF levels appear to be reduced in adult

populations (Hashimoto et al., 2004) affected by eating disorders of anorexic type (Nakazato et al., 2003-2009; Day et al., 2009; Brandys et al., 2011), autism spectrum disorders (Hashimoto et al., 2006; Nishimura et al., 2007; Connolly et al., 2006), schizophrenia (Iannittelli et al., 2007; Green et al., 2011; Koeva; et al., 2014), first psychotic episode (Sotiropoulou et al., 2013), obsessive compulsive disorder (Dos Santo et al., 2011; Maina et al., 2010; Fontenelle et al., 2012), anxiety disorders (Suliman et al., 2013), BD (Tsai, 2004). On the contrary, conflicting data appear in pediatric studies related to ADHD (Scassellati et al., 2014). Low levels of BDNF would also be related to particular personality traits, the so-called neuroticism, which reflects a chronic tendency to experience negative emotions (Lang et al., 2004; Sotiropoulou et al., 2013). Neuroticism characterizes a personality profile with psychopathological vulnerability.

- **Suicide:** Dwivedi et al. (2003) found a significant reduction of BDNF and its mRNA in the prefrontal cortex and in hippocampus of suicide victims in comparison to healthy subjects, as well as a reduction in full-length form of the TrkB receptor. Similar results with reduced BDNF in the prefrontal cortex (but not in the hippocampus) are found by Pandey et al. (2008) on a sample of post-mortem adolescents (Dwivedi et al., 2009; Lee et Kim, 2009).
- **Other factors**: environmental stimulation, neonatal maternal care, electroconvulsive therapies, CNS active drugs and psychotherapies (Palma and Brugnoli, 2007) can modulate BDNF gene expression.

1.8 BDNF, depression and treatment

Recently researches focused their attention on the processes of neuronal plasticity and in particular on the delicate balance between biological processes such as cellular necrosis, apoptosis and neuronal regeneration also in the case of pharmacological treatment. According to neurotrophic theory, depression is the clinical manifestation of a protracted neuronal suffering that is biologically susteined by the depletion of synaptic connections followed by the reduction of neuronal survival up to the atrophy of the affected areas (Bathina et al., 2014). Studies in neuroanatomy, postmortem brain observations and neuroimaging suggest that the impairment of neuroplasticity in specific brain regions such as the hippocampus, striatal nucleus and prefrontal cortex may be involved in the pathogenesis of depression (Dwivedi et al., 2009). This hypothesis is supported by evidences such as the reduction of hippocampal volume in depressed patients, the correlation of this reduction with the duration of the disease and, conversely, the protective effect associated with the antidepressant treatment. The biological rationale of these neuroanatomical alterations is the evidence that in experimental models of depression, prolonged stressful events associated with high levels of corticosterone can determine a neuronal atrophy in the same brain areas that are morphologically and functionally altered in human pathology. In man, the action of physiological concentrations of cortisol is fundamental to guarantee the neuronal trophism, the plastic abilities of the neurons and the ability of the hippocampus to express neurogenesis. Despite this premise, the excess of cortisol for prolonged periods, in concomitance with the activation of the excitatory synapses, induces a serious insult to cell homeostasis that often facilitates the onset of depression recurrences. The evidence of reduced trophism and reduced neurogenesis in brain areas involved in the control of affective, emotional and cognitive functions suggests the need to understand if this phenomenon is only a consequence of the pathology not effectively treated (lack of well-timed or delayed treatment, inadequate drug's dosage) or if it is due to a pre-existing genetic basis (one can be born with a reduced volume of the hippocampus, cerebral cortex or amygdala), which makes the individual more vulnerable to stressful events and more in general to environmental insults. Both these possibilities can occur and constitute two equivalent causes of vulnerability. Experimental studies clearly demonstrated that antidepressant drugs are capable of stimulating not only trophism but also neurogenesis, that is, enhancing in the brain the differentiation and proliferation of new neurons with significant functional and learning properties (Fig. 10) (Biggio, 2011). These evidences demonstrate that a complete process of neurogenesis is a neessary phenomenon to promote the effective action of antidepressant drugs. This concept is also consistent with the evidence that the differentiation and maturation of newly synthesized neurons occurs in a few weeks, and can

therefore be temporally correlated with the emergence of the clinical response to drugs. The efficacy of antidepressant drugs is closely related to the synthesis of trophic factors and consequently to neuronal trophism and to the neurogenesis, phenomena regulated by complex molecular mechanisms. In this contest, it is of crucial importance to guarantee sufficiently constant plasmatic and cerebral concentrations of drug during a chronic treatment in order to favor an equally constant and lasting action of the BDNFin the target proteins (Biggio, 2011).

As previously reported, BDNF depletion is associated with a reduction in neurogenesis and in the volume of brain areas including the hippocampus, prefrontal cortex and amygdala (Piccinni and Veltri, 2010). Specifically, the Val66Met polymorphism carriers, who represent the 20-30% of the Caucasian population, have a significantly reduced hippocampus and sometimes presents alterations in the learning processes and memory. Recently agroup of American researchers developed the animal model of this polymorphism, initially described only in humans, with the generation of a line of transgenic mice carrying the aforementioned polymorphism. The most fascinating aspect of the animal model is the evidence that these mice have (as happens in humans) a hippocampus of reduced volume, little trophic neurons, few dendritic spines and they are not very sensitive to treatment with antidepressant drugs. All these observation confirm that the neuronal trophism and the molecular mechanisms involved in neuroplasticity are at the base of the efficacy of antidepressant drugs, of the adherence to therapy and of the same vulnerability to the emotional and affective disorders (Biggio, 2011).

The clinical observation that the therapeutic response to antidepressant drugs is evident only after prolonged treatment has led to the hypothesis thatit is necessary to produce modifications in structural and functional plasticity in order the response can occur (Duman et al., 1997; Nestler et al., 2002). In 1995, Duman et al.demonstrated for the first time that chronic, but not acute, treatment with different type of antidepressants (ATC, SSRI, IMAO, and electroconvulsive stimulation) can induce the expression of BDNF and its TrkB receptor in the rat hippocampus (Nibuya et al., 1995).

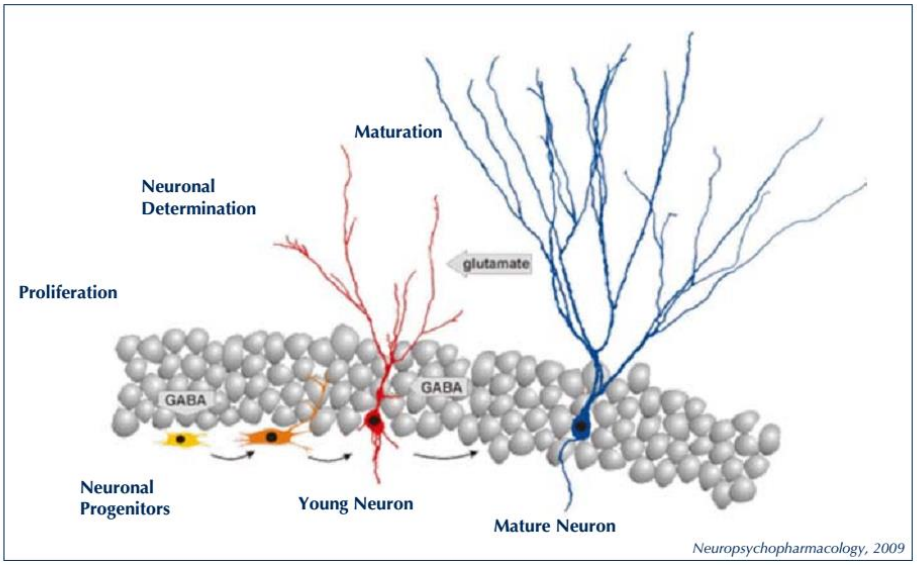


Fig. 10: Antidepressant drugs stimulate neurogenesis in the human brain (Adapted from Biggio 2011).

Scientific evidences on animal stress, confirm what has long been widely documented by the clinical experiences on therapeutic efficacy: only the chronic administration of antidepressants increases the levels of BDNF in the affected brain areas (Nibuya et al., 1995; Duman and Vaidya, 1998; Malberg et al., 2000), as well as electroconvulsive therapy (Nibuya et al., 1995; Duman and Vaidya, 1998). In the aforementioned studies, the therapeutic treatment could prevent or contrast BDNF reduction under stress conditions. The induction of BDNF by antidepressants is at least partly mediated by the transcription factor CREB; in fact the gene expression of BDNF is induced both in vivo and in vitro by CREB (Tao al 1998, Conti et al., 2002). Most of antidepressants are able to increase the expression of CREB in different brain areas, including the hippocampus, through the activation of protein kinase systems that positively modulate CREB activation, with consequent nuclear translocation and increase of BDNF synthesis, as well as of antiapoptotic molecules of the Bcl-2 family (Nibuya et al., 1996; Thome et al., 2000) (Fig.11).

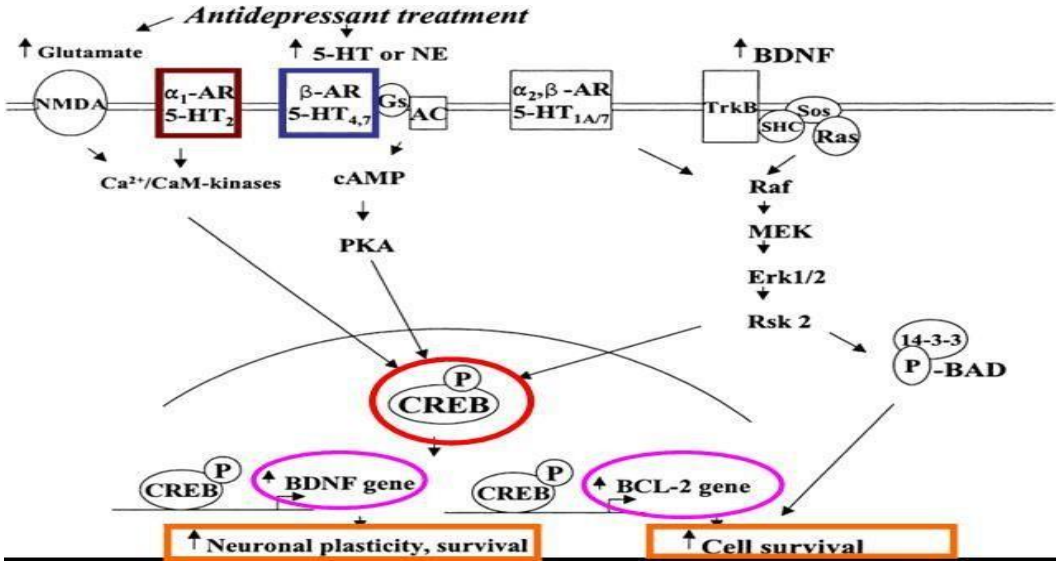


Fig. 11: Molecular action of antidepressant drugs and BDNF.
Antidepressant therapy increases synaptic concentrations of serotonin (5-HT) and noradrenaline (NE). From this derives the cascade activation of the intracellular transduction signal and therefore the activation of the processes that involve the CAMP-CREB system. Chronic administration of antidepressants determines the increased activity of CAMP, protein kinase (PKA) and CREB. The common genetic target of antidepressant therapy and of the CAMP-CREB cascade system is the synthesis of BDNF that participates in the cellular processes that finally underlie neuroplasticity and cellular survival (Adapted from Palma et al, 2007).

Other studies show how treatment with antidepressants can regulate complex phenomena such as neurogenesis and synaptic plasticity (D'Sa and Duman, 2002).

Long-term treatment with antidepressants produces a prolonged activation of the intracellular system of cAMP and CREB in rat hippocampus, resulting in the transcription of numerous genes (Nibuya et al., 1995; Thome et al., 2000; Blom et al., 2002). Some post-mortem studies have shown that in depressed patients there is a reduced concentration of CREB in the temporal cortex compared to the control group, while depressed patients during treatment have values comparable to the control group (Dowlatshahi et al., 1998). The induction of neurogenesis was observed after chronic, but not acute, administration of antidepressants of different classes (SSRI, ATC, IMAO) or following electroconvulsive stimulation (Malberg et al., 2000). This data seems to indicate that an increase in neurogenesis is a common target for different antidepressant treatments (Duman and Monteggia, 2006). In addition, treatment with antidepressant drugs blocks or reverses the inhibition of neurogenesis caused by stress (Fig.12) (Malberg and Duman, 2003). Santarelli et al. 2003 suggested that hippocampal neurogenesis is necessary for the antidepressive action: in vitro experiments demonstrate that activation of the cAMP pathway or incubation with BDNF increases neuronal differentiation and growth of axons of progenitor cells (Palmer et al., 1997). This finding suggests that upregulation of CREB and BDNF in response to antidepressants may actually increase both

cell differentiation and survival.

One of the most important issue in experimental neurobiology and clinical pharmacotherapy of affective and emotional disorders is therefore to understand when and for how long a DD should be treated in a timely and appropriate manner (appropriate and protracted dosage over time), in order to avoid that a delayed start or an early stop of therapy can severely impair neuronal homeostasis, particularly in brain areas such as the hippocampus, the amygdala, the cingulum cortex, whose morphology and function are altered in depression (Duman and Monteggia, 2006).

Studies shown that in depressed subjects that were not treated for a sufficient time, the number of recurrences at 6 months after the stop of therapy was marked higher comparing to patients treated for at least 2 years (Campbell et al., 2004). Furthermore, in patients with repeated relapses, the volume of the hippocampus was significantly reduced compared to what was observed in the same patients at the beginning of the therapy (Biggio, 2011). These results suggest that to protect the brain from the insult of the DD and to prevent possible relapses, timely treatment is required with adequate dosages, in order to avoid serious fluctuations in the concentrations of the drug. Furthermore treatment should be protracted beyond the remission of symptoms. Experimental and neurobiological evidences suggest that the time necessary to restore anadequate tropism to guarantee optimal functional neuron responses is much longer than those necessary to improve symptomatology. This conclusion implies that, to be effective in restoring and maintaining the neuronal trophism, especially in severe depression, pharmacological therapy:

a) must be sufficiently protracted over time;

b) must ensure constant plasma and brain concentrations throughout the treatment (fluctuations that must remain within a narrow range of values) (Biggio, 2011).

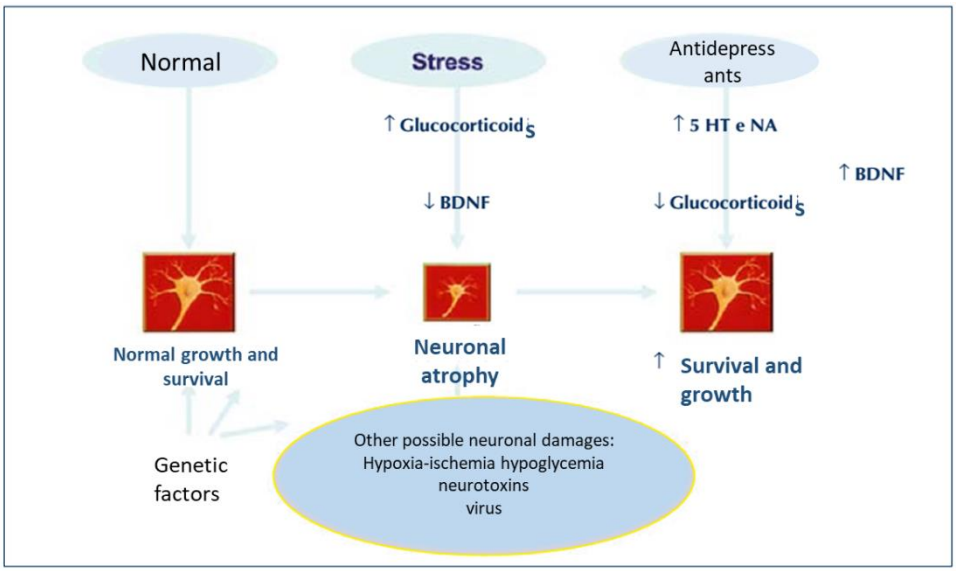


Fig. 12 Antidepressant drugs moderate the reduction of trophism, induced by chronic and severe stress, in neurons of different brain areas (Adapted from Biggio2011).

Expression of BDNF in the hippocampus, frontal cortex and other brain regions are reduced by stress. The reduction in BDNF expression is counterbalanced by treatment with antidepressant drugs so that the BDNF has an antidepressant effect.

1.9 Scientific literature

Searching in PubMed, the term "*BDNF*" corresponds to 21.363papers (1219 in 2018; 1704 in 2017; 1610 in 2016). Extending the research with "*Brain Derived Neurotrophic Factor*", there are 20.156papers (923 in 2018; 1494 in 2017; and 1442 in 2016). Limiting the researches to the developmental age (0-18), we respectively reach 1003 and 928 studies. These works are mainly reviews of literature and papers centered on the biological role of neurotrophins in brain plasticity. Considering the research for "*BDNF and depression*" and for "*Brain-Derived Neurotrophic Factor and depression*", in developmental age, and limiting it to the last10 years, we obtain 180 and 176 papers.

Of these, only 25 are clinical studies, and only 4 out of 25evaluated the protein dosage on peripheral blood (Table 3).

Karege et al. (2002) were the first to discover that the serum BDNF level in depressed subjects was significantly lower than in healthy controls. This reduction was directly correlated with the severity of depression. A few years later, the same group of researchers suggested that the alteration in plasma or serum BDNF levels was not due to changes in peripheral blood levels, but rather it was related to alterations in central release mechanisms (Karege et al., 2005). Since then, numerous studies have been conducted to identify the role of the neurotrophin in psychiatric pathology. Other studies examinated serum BDNF in depressed patients by comparing levels before and after antidepressant treatment. These studies found reduced levels of serum BDNF in depressed patients and a significant increase after treatment with

antidepressants (Gonul et al., 2005; Piccinni et al., 2008), stimulation of the vagus nerve, repetitive transcranial magnetic stimulation (Lang et al., 2006) or electroconvulsive therapy (Bocchio Chiavetto et al., 2006). In two metanalysis (Brunoni et al 2008, Sen et al., 2008), authors confirmed that the BDNF levels are lower in depressed patients than in healthy controls and that the BDNF levels became higher following an antidepressant treatment.

It is unclear if the low blood concentration of BDNF represents more a "trait marker" (Lang et al., 2004; Terraciano et al., 2011; Bus et al., 2012) or a "state marker" (Molendijk et al., 2011; Bus et al., 2015) of illness. A "trait marker" reflects the behavioral and biological characteristics that play a role in the pathophysiology of the psychiatric disorder before the onset (also causal factors), while a "state marker" reflects the condition of clinical manifestations in patients with already established pathology. Since this assumption, it is unclear whether the alterations of BDNF constitute a primary or secondary phenomenon of depression. Some authors (Lang et al., 2004; Terracciano et al., 2011) support the hypothesis of a genetic vulnerability for affective disorders by describing a negative correlation between BDNF and the "neuroticism", that is the tendency to an emotional lability with a anxious components associated with depression. In this hypothesis, the low level of BDNF is more a "trait marker" reflecting a tendency towards depression. Ihara et al. (2016) recently demonstrated that BDNF levels, at the time of diagnosis, do not differ between patients (major depressive episode, minor depressive disorder and minor depressive disorder with a history of major depression) and healthy subjects. In follow-up, serum levels of the neurotrophin decrease in subjects with a major depressive episode or minor depressive disorder with a history of major depression more then in those with minor depressive disorder, supporting the hypothesis that BDNF could be a "state marker" of disease.

As it is possible to see, studies are very heterogeneous for samples characteristics, methods, outcome measures (for example, some studies measured the protein in serum, others in plasma), units of measure, thus limiting the comparability of papers.

Studies in developmental age are still limited to date (Table 3). The most recent study is that of Tsuchimine et al. (2015) which, in contrast to the results of previous pediatric studies, does not show a significant difference in serum levels between the group of depressed patients and the healthy group. Pallavi et al. (2013) stated that the levels of BDNF among depressed adolescents (of both sexes) are significantly lower than in healthy controls. Also Sasaki et al. (2011) attested lower serum BDNF levels in depressed patients, but only in male subjects. In line with the previous ones, Pandey et al. (2010) confirmed that the BDNF protein expression is significantly reduced in the platelets of pediatric depressed subjects compared to non-depressed peers. Another notable study on the pediatric population is that of Dmitrzak-Weglarz et al., (2013) in Polish adolescents with anorexia nervosa in which low serum BDNF

levels have been reported.

Tab. 3Clinical studies on pediatric population

Author Year State	Type of study	N patients, average age	Method	Test Used	ELISA kit	BDNF level in depressed (M + SD)	Results
Tsuchimine2015 Japan	Transverse Case-control	24 vs 26 healthy (M: 16 yrs)	Serum protein level	DSRSC BDI	n.a.	n.a.	No significative differences of BDNF level between depressed and healthy
Pallavi 2013 India	Transverse Case-control	84 vs 64 healthy (M: 15 yrs)	BDNF, NDG, NT3, GDNF on serum	BDI STAI C	Promega	8.10±3.4ng/ml	BDNF reduced in depressed
Sasaki, 2011 Japan	Transverse Case-control	30 vs 22 healthy (M: 13 yrs)	Serum protein level	CDRS Mini KID	Promega	8.13±4.1ng/ml	Reduced levels in depressed. BDNF correlate to the lenght of the disease in males
Pandey 2010 United States	Transverse Case-control	14 vs 14 healthy (M: 14 yrs)	Expression and gene and protein on platelets and lymphocytes	CDRS YMRS	Promega	18.85 ± 7.4 ng/mg proteins	Reduced levels in depressed

DSRSC: Depression Self Rating Scale for Children. BDI: Beck's Depression Rating Scale. STAIC: State and Trait anxiety Inventory for Children. CDRS: Children Depression Rating Scale. Mini KID: Mini International Neuropsychiatric Interview for Children and Andolescents. YMRS: Young Mania Rating Scale.yrs: years. n.a. not available.

On the other hand, there are more studies on adult depressed populations without comorbidities. Since 2002 it is possible to identify about sixty papers, the main and most recent of which are described in Appendix 7. The use of different dosing methods in different papers does not allow complete comparability between the studies. Researches currently available in the literature about BDNF assay in humans, use the ELISA technique as a measuring tool, which until a short time ago did not allow the distinction between mature BDNF and proBDNF, since the specificity of the two forms being minimal. Yoshida et al. (2012b) affirmed that only the mature form is reduced in the depressed adults (we can remember that the proBDNF would seem to induce apoptosis), thus opening the way to further investigations. Results of all the clinical studies about BDNF dosage in depressed adults, although not selected and therefore affected by numerous other pathologies, in comorbidity or treated, are best analyzed in four brilliant metanalysis (Brunoni et al., 2008; Sen et al.,

2008; Bocchio-Chiavetto et al., 2010; Molendijk et al., 2014). In all of them is highlighted the reduction of peripheral protein levels in depressed compared to healthy groups. Fernandes et al. (2014) state that the reduced level of peripheral BDNF can be considered a biomarker of disease in the major psychiatric disorders. In this study, the authors suggest that the reduced level of BDNF may be related to the suppressive effect triggered by the stress due to acute event (the psychiatric pathology) (unipolar, bipolar depression, schizophrenia).

On the contrary, there are studies, albeit in the minority, that demonstrated higher levels of BDNF in depressed patients compared to healthy (Groves, 2007; Elfving et al., 2012; Kheirouri et al., 2016).

Some interesting studies on pregnant women are of particular interest (Lommatzsch et al., 2006; Gazal et al., 2012; Gao et al., 2016) showing reduced levels of neurotrophin in the peri-partum period, consisting in a possible substratum of susceptibility to development of depression in this delicate moment of life. Gao et al. (2016) showed that serum BDNF levels are lower in women with more severe forms of depression.

In recent years, many researchers concentrated in studying the relationship between stressful traumatic events and BDNF in patients suffering from Post-Traumatic Stress Disorders (PTSD). The results indicated that patients with PTSD have significantly lower levels of BDNF than healthy subjects and suggested that a reduction in BDNF levels may be implicated in PTSD neurobiology, but further longitudinal studies are needed to confirm this observation (Dell 'Osso et al., 2009).

1.10Proinflammatory cytochines

Proinflammatory cytokines are signaling polipeptidic molecules mainly produced by helper T cells (Th) and macrophages and involved in the upregulation of inflammatory reactions (Zhang and An, 2007).

According to a functional classification, this family include primary proinflammatory cytochine, as theinterleukin-1 (IL-1), IL-6 and tumor necrosis factor (TNF), and secondary molecules for specific immune regulation (like interferon gamma,IFN-γ). IL-1, IL-6 and TNF (inflammatory triad) play a role in initiating the inflammatory responseand in regulating the host defence against pathogens mediating the innate immune response. These cytochine are pleiotropic and present biological functional redundancy, even if they bind to different receptors. IL-1 and TNF play a role in the very first phase of the immune response, while IL-6 lead to the production of acute phase proteins in a second time. More in details, IL-1 and TNF cause in the local area the production of adhesive molecules, chemochines, growth factors and lipidic mediators like prostaglandine and NO. These local mediators amplify the

leukocyte recruitment and survival into the tissue. The presence of leucocytes at the local level amplifies in situ the mechanisms of innate immunity, and this activates specific immunity. The amplification of innate immunity is very important to guide the specific immunity to type I responses, characterized by the production of IFN-γ, or to type II responses characterized by IL-14 and IL-13 production. At the systemic level, IL-1 and TNF act through IL-6 on the liver. The liver's response to IL-6 is the production of 'acute phase proteins' that diffusely amplify the mechanisms of innate immunity and tissue remodeling. The cascade of inflammatory cytokines is subject to negative regulation circuits, which act both locally or at a systemic level. A first negative regulating action is due to anti-inflammatory cytokines (IL-10, TGF-β), which are mainly produced by the same cells that produce IL-1 and TNF, the monocyte-macrophages. A second negative regulating circuit is constituted by the Hypothalamic-Pituitary-Adrenal Axis (HPAA): IL-1 and TNF induce the production of releasing factors on the hypothalamus that cause the production of ACTH (hypophysis), which in turn stimulates the production of glucocorticoid hormones (adrenal). Glucocorticoid hormons finally tend to shut down the production of IL-1 and TNF.

Some inflammatory cytokines have also additional roles such as acting as growth factors (Fitzgerald et al, 2001) or they can influence neurogenesis (Hayley et al. 2005; Leonard and Maes 2012; Maes et al. 2009; McAfoose and Baune 2009; Song and Wang 2011). For example, TNF-α and IFN-γ can produce inhibition of neural progenitor cell proliferation (Ben-Hur et al. 2003). Furthermore, TNF-α induced neuronal cell death through blockade of the glutamate transporter activity, thereby potentiates glutamate neurotoxicity (Zou and Crews 2005).

1.11 IL-1 superfamily

In 1985 two distinct, but distantly related, complementary DNAs encoding proteins sharing human IL-1 activity were isolated from a macrophage cDNA library, thus defining two individual members of the IL-1 family: IL-1α and IL-1β (March et al, 1985).

IL-1 family is a group of 11 cytokines, which rapidly emerged as a key player in the regulation of inflammatory processes. IL-1α and IL-1β, that are the most studied members, are two agonist cytokines encoded by different genes on chromosome 2q14. They have similar biological properties and bind to the same receptors (IL-1R1), activating signals via MyD88 adaptor. Both of them include a beta trefoil fold, although from a structural point of view they share only the 20% of the sequence. (Fig. 13)

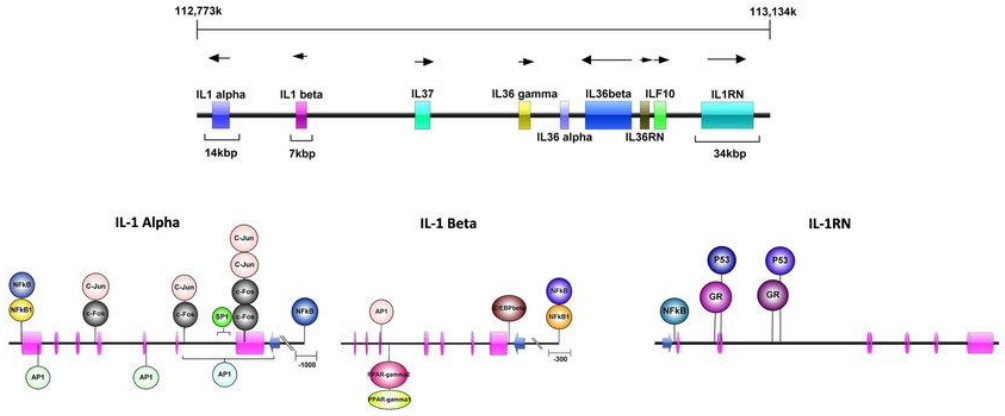


Fig.13 The interleukin-1 gene cluster. Representation of the human chromosome-2 locus containing the IL-1 gene cluster showing the relative size and positions of the known IL-1 genes within the IL-1 gene cluster (upper panel). Transcriptional direction is designated by black arrows, and the overall gene size for IL-1α, IL-1β and IL-1RN is indicated below each corresponding gene in kilobases (kb). Lower panel shows organization of IL-1α, IL-1β and IL-1RN genes, whereby exons are represented by pink bars and promoters are represented by solid blue arrows. Transcription factor binding sites including Activator protein-1 (AP-1), c-Jun, the proto-oncogene c-Fos, the tumor protein p53 (P53), Peroxisome Proliferator-Activated Receptor (PPAR), CCAAT/enhancer-binding protein (C/EBP) and Nuclear Factor kappa-light- chain-enhancer of activated B cells (NF-κB) are marked with closed circles. For distal promoter binding sites, the sequence upstream of the gene promoter is indicated using a black bar, and the DNA length is indicated in Kb. (fromKhazim et al, 2018)

IL-1α and IL-1β are synthesized by activated macrophages as propeptides of a molecular weght of about 33 kDa. Propeptides are proteolytically processed within the cell by 'caspase-1' or IL-1 converting enzyme (CASP1/ICE) to a mature active form of 17 kDa, which is secreted through unconventional mechanisms, since IL-1β does not have a signal sequence.

IL-1 precursors in fact do not have a clear signal peptide for processing and secretion and none of them is found in the Golgi; they belong to so-called leaderless secretory protein group.

IL-1α precursor form can bind to the receptor and can activate signal transduction, while IL-1β precursor require proteolytic cleavage by either intracellular ICE or extracellular neutrophilic proteases.

The ICE was the first identified enzyme of the family of caspases that play a crucial role in the regulation of apoptosis.

IL-1 is mainly produced by monocytes-macrophages after a variety of pro-inflammatory stimuli, but the microbial ligand of the Toll family receptors and of primary cytokines are particularly important. IL-1 is induced by IL-1 itself and by TNF, triggering an amplification of the inflammatory response.

IL-1α and IL-1β present some distinctions, mainly on immunity, inflammation and cancer.

The IL-1α precursor is constitutively present in epithelial layers of the entire gastrointestinal tract, lung, liver, kidney, endothelial cells, and astrocytes. Upon cell death by necrosis, as

occurs in ischemic diseases such as stroke and tumor necrosis, the IL-1α precursor is released. It is fully active and functions as an "alarmin" by rapidly initiating a cascade of inflammatory cytokines and chemokines, which accounts for early phases of sterile inflammation (Chen et al., 2007; Rider et al., 2011). Circulating IL-1α is rarely detected even in persons with severe infections but it is contained in apoptotic bodies released from endothelial cells (Berda-Haddad et al., 2011).

IL-1β is produced by hematopoietic cells such as blood monocytes, tissue macrophages, skin dendritic cells and brain microglia in response to Toll like receptors, activated complement components, other cytokines (such as TNF-α) and IL-1 itself (Dinarello, 2011). The IL-1β precursor is not active but is cleaved by caspase-1, releasing the active cytokine into the extracellular space. Although caspase-1 is abundant in hematopoietic cells, the proenzyme (procaspase-1) first requires cleavage by a multiprotein formation called inflammasome.

Elevated secretion of IL-1β causes autoinflammatory diseases.

Not all IL-1β-mediated inflammation is due to caspase-1 activity. Mice deficient in caspase-1 develop the same IL-1β-mediated disease as do wild-type mice (Dinarello, 2009, 2011). Extracellular cleavage of the inactive IL-1β precursor by neutrophil enzymes such a proteinase-3 and elastase generate active IL-1β because the cleavage site isclose to that of caspase-1 (Dinarello, 2011).

Another distinction between IL-1α and IL-1β can be found in carcinogenesis. Mice deficient in IL-1β develop fewer tumors compared IL-1α-deficient or wild-type mice (Krelin et al., 2007). IL-1β induces tumor angiogenesis and metastatic spread of tumors (Carmi et al., 2013). IL-1 is indeed an important component of the inflammatory microenvironment of tumors (Mantovani et al., 2008).

IL-1α is a component of the intrinsic pathway linking genetic events causing cancer (Ras mutation) and the orchestration of cancer-related inflammation (Salcedo et al., 2013). Moreover, IL-1 plays a critical role in inflammatory conditions which increase cancer incidence (extrinsic pathway) as revealed by carcinogenesis in the pancreas, skin and liver (Salcedo et al., 2013). Neutralizing antibodies to IL-1α have been used in clinical trial for patients with terminal colon cancer with encouraging results (Hong et al., 2011).

Recent results have highlighted differential induction and role of IL-1α and IL-1β in fat induced vascular responses and atherosclerosis (Freigang et al., 2013; Garlanda et al., 13)

Differences between IL-1α and IL-1β are mainly due to cell sources and release mechanisms: IL-1α localizes to the nucleus and functions as a component of transcription whereas IL-1β has never been observed in the nucleus. The IL-1α has a pro-piece that may act as an oncoprotein. The IL-1α precursor shuttles between the cytosol and nucleus with amazing rapidity (Cohen et al., 2010). Upon a signal to initiate apoptosis, cytosolic IL-1α moves to the

nucleus and remains tightly bound to chromatin. On the other hand, with a signal to undergo necrosis, for example due to hypoxia, IL-1α leaves the nucleus and resides in the cytosolic compartment (Cohen et al., 2010). With necrotic cell death, the IL-1α precursor is released (Rider et al., 2011) initiating neutrophilic inflammation (Rider et al., 2011), whereas in cells dying of apoptosis, chromatin-bound IL-1α is unavailable for initiating inflammation.

The IL-1 family includes also a natural receptor antagonist (IL-1ra), of which there are different isoform, produced by the same cells and induced by the same signals, even if anti-inflammatory cytokines induce the preferential production of IL-1ra. IL-1ra is an antagonist: it binds to the receptor, gives no biological response and it also prevents agonist molecules from interacting with the receptor.

IL-1α or IL-1β bind first to the first extracellular chain of IL-1RI, that recruits the IL-1 receptor accessory protein (IL-1RAcP), serving as a coreceptor and is necessary for signal transduction. IL-1ra interacts only with the IL-1RI chain, preventing the formation of an active receptor complex by agonist molecules.

After the formation of receptor heterodimeric complex (IL-1α or IL-1β, IL-1RI and IL-1RacP), two intracellular adaptor proteins are assembled by conserved cytosolic regions called Toll- and IL-1R-like (TIR) domains. They are called the myeloid differentiation primary response gene 88 (MYD88) and interleukin-1 receptor-activated protein kinase (IRAK) 4. Phosphorylation of IRAK4 is followed by phosphorylation of IRAK1, IRAK2 and tumor necrosis factor receptor-associated factor (TRAF) 6. TRAF6 is an ubiquitin E3 ligase, that in association with ubiquitin-conjugating enzyme (ubiquitin E2 ligase) complex attaches K63-linked polyubiquitin chains to some of IL-1signaling intermediates, for instance TGF-β-activated protein kinase (TAK-1). That facilitates the association of TAK-1 with TRAF6 and with MEKK3. These complex signaling pathways lead to activation of many transcription factors, such as NF-κB, AP-1, c-Jun N-terminal kinase (JNK) and p38 MAPK (Weber et al, 2010; Simi et al., 2007).

A second receptor, IL-1RII or “decoy receptor”, binds IL-1 but does not produce any signal transduction. This receptor is present in a free form or it is linked to membrane. This receptor also forms a complex with an agonist and the accessory chain (IL1RAcP), which does not transduce any signal. In this way, it subtracts the accessory chain that is essential for the formation of a transducing complex.

The complexity of this tightly controlled regulation underlines the importance of IL-1, which is potentially devastating for the body's integrity (Figure 14).

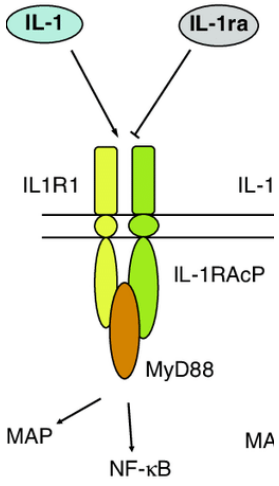


Fig.14 Schematic representation of receptors for IL-1. IL-1 receptor (IL1R1) forms a heterodimer with the IL-1 receptor accessory protein (IL-RAcP, aka IL1R3) upon binding of IL-1α or IL-1β, leading to intracellular recruitment of MyD88 and downstream signalling to NF-kB and p38MAP. Interleukin-1 receptor antagonist (IL1Ra, aka IL1F3) competes with IL-1 for binding to IL1R1. (Corthay and Haraldsen, 2017)

IL-1 cytokines are important mediators of the inflammatory response, and are involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis (Fig. 15).

IL-1 virtually affects all cells and organs and is a major pathogenic mediator of autoinflammatory, autoimmune, infectious and degenerative diseases (Dinarello, 2009, 2010; Dinarello et al., 2012; Gabay et al., 2010; Sims and Smith, 2010). It is sufficient a very limited number of receptors, perhaps one per cell, to activate the cellular response. Many of the effects of IL-1 are indirect, that is that the final effect depends on the induction of secondary mediators, like IL-6 and hemopoietic growth factors (GM-CSF and M-CSF).

IL-1 acts primarily on hematopoietic cells (hematopoietic precursors, T and B-lymphocytes), stimulating the proliferation and differentiation. It markedly prolongs the lifespan and stimulates the effector function of neutrophils and macrophages (Mantovani et al., 2011). In addition, IL-1 guides the differentiation and function of innate and adaptive lymphoid cells.

A second important target of IL-1 action is the Central Nervous System. IL-1 is a potent activator of host defense responses to infection and injury both in the periphery and the CNS (Rothwell and Luheschi, 2000). On the other hand, IL-1 can exacerbate damage in the CNS resulting from acute insults.

In a normal healthy brain the concentration of IL-1β is low but increases as a result of peripheral infection, surgery, brain injury, social isolation or during neurodegenerative diseases such as Alzheimer's disease (Rothwell and Luheshi, 2000; Tong et al., 2008). The increase in IL-1 after traumatic brain injury is followed by neuronal loss. Antibodies to IL-1

attenuate neuronal loss (Luet al., 2005) and in IL-1 deficient mice neuronal loss and infarct volumes are reduced (Boutin et al., 2001). The early primary source of IL-1 is microglia, but later the cytokine is also produced by astrocytes and its expression in oligodendrocytes and neurons has also been detected (Rothwell et al., 2000). There is a general consensus that after an insult, IL-1 has adverse effects in the brain in vivo, but observations on cultured neurons are less clear. It seems that IL-1β is neurotoxic only at high concentrations and after relatively long exposure (Araujo and Cotman, 1995). Several mechanism by which IL-1 may induce neuronal death have been proposed including the involvement of glia (Rothwell and Luheshi, 2000) and the regulation of NMDA receptor (Ma et al., 2000; Viviani et al., 2003).

During neuroinflammation, there are increased levels of TNF and IL-1 in the brain, and their presence may cause the breakdown of the blood-brain barrier (Hofman, 1986).

IL-1 is the classic endogenous pyrogen (Dinarello, 2009, 2010; Sims and Smith, 2010): in the hypothalamic centers, it induces the production of IL-6 and prostaglandins that are the mediators of the pyrogenic effect. Moreover, IL-1 induces anorexia and asthenia during the systemic inflammatory response.

IL-1 also leads to the activation of the HPAA. Glucocorticoid hormones finally have many effects, including the ability to shut down the inflammatory response by inhibiting the production of IL-1 itself and increasing the production of anti-inflammatory molecules such as the IL-1 decoy receptor. Thus, the activation of HPAA constitutes a negative feedback loop of the inflammatory activity. Cortisol downstream of the HPA axis has a regulatory function on innate immunity and inflammation.

IL-1β and TNF-α have been shown to inhibit long-term potentiation, that is the neuronal mechanism that underlie learning and memory (Cunningham et al. 1996), both of which are frequently affected in MDD. IL-1β also reduced hippocampal neurogenesis, both in animal (Goshen et al. 2008; Koo and Duman, 2008; Kuzumaki et al. 2010) and human in vitro models (Zunszain et al. 2012).

The detrimental effects of stress on neurogenesis have been reversed by hippocampal transplantation of neural progenitor cells that overexpress IL-1 receptor antagonist (Ben Menachem-Zidon et al. 2008), or by using IL-1 receptor knockout mice (Koo and Duman 2009). Concomitantly, this blockade of cytokine action has led to a decrease of depressive symptoms in these models.

In CNS, IL-1 induce the cyclooxygenase-2 (PTGS2/COX2) thatis found to contribute to inflammatory pain hypersensitivity.

Other main targets of IL-1 activity are vascular cells: the cytochine induces the production of chemokines and adhesion molecules in the vascular endothelium, amplifing the recruitment

of inflammatory cells at the local level. Furthermore, IL-1 induces the expression of enzymes that lead to the synthesis of prostacyclin (PGI2) and NO, molecules with vasodilatory activity. IL-1 can also modifies the anticoagulant properties of the vascular endothelium. In basal conditions, the vascular endothelium has anticoagulant properties, constituting a non-thrombogenic surface. IL-1 induces the production of procoagulant activity (tissue factor), which activates the coagulation cascade and inhibits the anticoagulant axis constituted by the C protein and by trombomodulin. Finally it the vascular endothelium, IL-1 activates a proinflammatory and prothrombotic genetic program.

The targets of IL-1 action are also cells of bone, cartilage, synovium and connective tissue. In these cells, IL-1 can induce the production of proteases and prostaglandins, that cause dissolution and damage of the tissue and phenomena such as bone remodeling observed in many pathological conditions. IL-1 induces the production of acute phase proteins at hepatic level, through IL-6.

IL-1 and TNF can inhibit directly cardiac contractility and promote protein catabolism of the skeletal muscle.

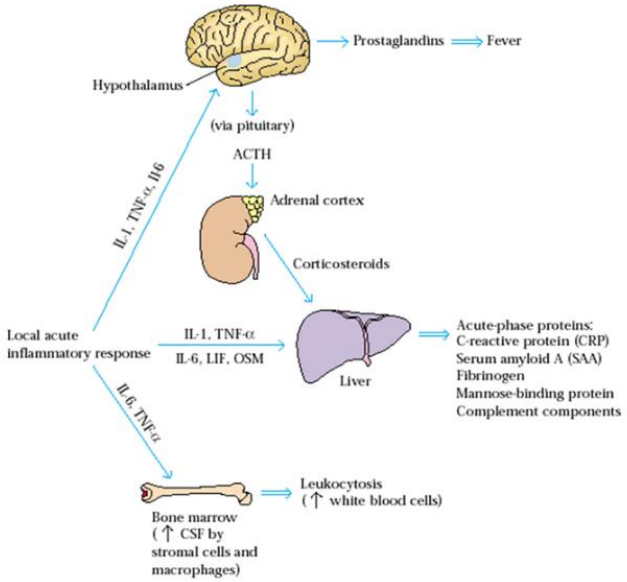


Fig.15 Organs and mediators involved in a systemic acute-phase response. IL-1, Il-6 and TNF α mediate acute phase effects. LIF: leukemia inihibitory factor; OSM: oncostatin M.

Recent meta-analyses reported positive associations of some immune molecules with depression, in both serum and plasma (Dowlati et al. 2010; Howren et al. 2009). It is important to specify that these increased levels are much lower than those found in autoimmune or infectious diseases.

Levels of IL-1β have been described as elevated in cerebrospinal fluid (CSF) of MDD patients

(Levine et al. 1999). Associations with changes in cellular immunity, including reduced natural killer cell activity and lymphocyte proliferation have also been reported (Blume et al. 2011; Zorrilla et al.2001). Additionally, postmortem gene analyses in the prefrontal cortex of MDD patients have suggested an upregulation of a variety of pro and anti-inflammatory cytokines (Shelton et al. 2011).

Bipolar patients, including children, present an increased monocyte phagocytic activity (McAdams and Leonard, 1993; Padmos et al., 2008) and increased levels of pro-inflammatory cytokines like IL-1β and IL-6 (Modabbernia et al., 2013). To discuss this topic more in detail, it is well known that mood episodes can be precipitated by exposure to stress, resulting in the release of glucocorticoid hormones, such as cortisol. In physiological conditions, cortisol exerts an inhibitory effect on cytokines production by immune cells. During chronic stress, this inhibitory effect is lost: continuous release of pro-inflammatory cytokines, particularly IL-1β, causes the activation of intracellular pathways (including mitogen-activated protein kinases (MAPK) pathways), inhibiting the glucocorticoid receptor response to cortisol. The suppression of glucocorticoid receptor by cytokines may also contribute to the persistent elevated levels of cortisol during chronic stress (Barbosa et al. 2014).

An abnormal activation of the HPAA in DD may suggest a defective neuroendocrine control over the immune system, partially explaining why DD patients present increased circulating levels of pro-inflammatory cytokines. As mood episodes may trigger stress responses, a cyclical abnormal activation of the HPAA may take place, leading to chronic increased levels of inflammatory markers.

Glucocorticoids regulate not only immunity response but also other functions such as metabolism. Glucocorticoid excess, secondary to HPAA activation, could leads to metabolic dysfunction and central obesity. Related medical comorbidities might also contribute to higher levels of stress perpetuating HPAA activation. Therefore, the increased pro-inflammatory status in these patients may be one of the consequences of the increased rates of chronic medical illness.

Some studies suggest that only some groups of depressed patients presents increased inflammatory molecules, in particular who presents resistance to treatment, a history of childhood maltreatment, and obesity (Danese et al., 2008; Shelton and Miller, 2010; Lanquillon et al., 2000).

Polymorphisms in IL-1 genes have been found to contribute to genetic susceptibility to some cancers (Xu et al., 2014) and Grave's disease (Liu et al., 2010).

The use of IL-1 antagonists has been linked to beneficial effects in patients with hereditary

autoinflammatory conditions associated with excessive IL-1 signaling, such as cryopyrinopathies and IL-1Ra deficiency. Successful treatment with IL-1 blockers has also been reported in other hereditary autoinflammatory diseases, as well as in nonhereditary inflammatory diseases, such as Schnizler syndrome, systemic-onset juvenile idiopathic arthritis and adult Still disease. Finally, some preliminary findings indicated that IL-1 targeting is efficacious in type 2 diabetes (Gabay et al., 2010).

The blockade of IL-1 activity (especially IL-1β) is a standard therapy for patients with autoimmune diseases or lymphomas. Anakinra (IL-1Ra) is an FDA-approved therapy for patients with rheumatoid arthritis (Anakinra 2014) because it reduces symptoms and slows joint destruction of this inflammatory disease. It has also been prescribed to patients with indolent or smoldering myeloma with a high risk of progression to multiple myeloma. In combination with other medication, IL-1Ra provides a significant increase in the number of years of progression-free disease in its recipients (Dinarello, 2011).

1.12 Literature on IL-1β and depression

Researching on PubMed the terms "*IL-1 AND depression*" it is possible to obtain 1122 studies (38 in 2018; 69 in 2017; 79 in 2016). Considering the limit of developmental age, these numbers dramatically reduced to 71 results overall. Of this, only 7 studies, measured IL-1β (Table 4).

Studies on adult population are limited too. The primary ones are presented in Appendix 8.

In most of them, authors confirm the presence of higher level of IL-1β in depressed patients comparing to healthy subjects. Studies also reported an increasing attention in evaluating depression in specific patients groups, in particulare in adults with heart diseases.

Tab. 4Clinical studies on pediatric populations

First author; year; State	Study's type	N patients, average age	Methods	Test used	Kit ELISA	Il-1 levels in patients M+ SD	Results
Spindola 2017 Brazil	Cross sectional Case-control	20 vs 61 (M 9.8 years)	Gene expression	DAWBA CBCL.	na	na	IL-1β reduced in MDD group
Miklowit 2016 USA	Cross sectional Case-control	18 BD vs 13 MDD vs 20 healthy peers (M:14.8)	IL-1β, IL-6, IL-8, TNF-α, and IL-10	K-SADS-PL YMRS CDRS-R	Performance High Sensitivity Human Cytokine, R& D Systems, Minneapolis, MN)	0.3 ± 0.1 pg/ml	Higher levels of IL-1β in BD e MDD, but no statistically significative

Amitai 2016, Israel	Longitudinal	41(M 14 yrs)	IL-1β before and after therapy	K-SADS-PL CGI CDRS-R BDI SCARED	Siemens Medical Solutions Diagnostics, Los Angeles, CA	0.62±0.34 pg/ml	Higher levels of IL-1β predict nonesponse to SSRI
Scola, 2016, Canada	Cross sectional Case-control	16 vs 13 healthy (M=15,5)	lipid peroxidation (LPH, 4-HNE, 8-ISO), protein carbonyl, and IL-1α-β, IL-6, IL-10, IFNγ, TNFα	WASH-U-KSADS WASI YMRS HDRS/ HAM-D	Luminex, USA	472.4±1273.4(pg/mL)	LPH reduction in depressed patient, no differences for IL-1β
Henje Blom 2012 Sweden	Cross sectional Case-control	42 girls vs 60 healthy (M=16.8 yrs)	IL-1β, IL-2, IL-6, IL-10, IFN-γ, TNF-α	Developme nt and Well-Being Assessment Emotional subscale of SDQ	Evidence Investi-gator TM®, Randox Laboratories Ltd	2.4±1.3	IL-1β higher in MDD patients
GAbbay, 2009 USA	Cross sectional Case-control	30 vs 15 healthy (M=16.6 yrs)	IL-1β, IL-4, IL-6, IFN-γ, TNF-α	K-SADS-PL CDRS BDI-II CGAS	n.a.	0.15 ±0.06	IL-1β increased in suicidal MDD patients but without statistical significance
Brambilla , 2004; Italy	Cross sectional Case-control	11 vs 11 healty (M=12.2)	IL-1β and TNF-α	KSADS PRSD RRSA	Medgenic Diagnostic (Italy)	29.2±24.2 pg/ml	No differences

DAWBA: Development and Well-Being Assessment. CBCL: Child Behavior Checklist.KSADSPL: Kiddie Schedule for Affective Disorders and Schizophrenia, Present and Lifetime Version. YMRS: Young Mania Rating Scale. CDRS-R: Children's Depression Rating Scale-Revised. CGI: Clinical Global Impressions Scale. BDI: Beck Depression Inventory. SCARED: Screen for Child Anxiety Related Emotional Disorders. WASH-U-KSADS: Washington University in St. Louis Kiddie-Schedule for Affective Disorders and Schizophrenia. WASI: Wechsler Abbreviated Scale of Intelligence.HDRS/HAM-D: Hamilton Depression Rating Scale. PRSD: Poznanski Rating Scale for Depression. RRSA: Reynold Rating Scale for Anxiety; SDQ: Strengths and Difficulties Questionnaire

1.13 BDNF and IL-1

Signaling by BDNF via the TrkB receptor is an important mechanism supporting neuronal survival and activity dependent plasticity, which is critical for cognitive function (Huang and Reichardt, 2003), both in development and in pathology (see above). Receptor trafficking, including endocytosis, sorting and transport, is a key process in BDNF signaling (Bronfman et al., 2007). BDNF binds to its receptor, TrkB, and signal transduction is mediated through the receptor tyrosine kinase domain of the TrkB receptor. The activated complex is propagated to both local and distal subcellular compartments in endosomal vesicles. These endosomes can carry complete signaling complexes capable of activating multiple intracellular cascades leading to plasticity and survival.

In literature, the central role of endosomal trafficking dysfunction was studied mainly in neurological diseases, like Alzheimer's disease, since the detection of a pathological enlargement of early endosomes in the brain (Pottier et al., 2012).

Conditions that may interfere with BDNF signaling may affect a variety of downstream neuronal functions and may contribute to neurodegenerative diseases.

IL-1β is a key component of the microglial-mediated immune response in the brain and it could have deleterious effects on cognition and synaptic plasticity at high or chronic levels of exposure (Goshen et al., 2008) and it is considered the most potent pro inflammatory cytokine in neurodegeneration. In the mouse hippocampus, sustained expression of IL-1β impairs contextual and spatial memory while sparing short term and nonhippocampal memory (Hein et al., 2010). Elevated IL-1 causes cognitive decline, especially on hippocampal dependent tasks (Rachal Pugh et al.,2001).

IL-1 administration to rodents has been popularly used as a model for studying the interaction between inflammation, brain functions, and memory deficits in neurodegenerative and psychiatric diseases (Song, 2002; Patel et al., 2006).

IL-1β suppresses long-term potentiation (LTP), the major cellular mechanism of learning and memory, and BDNF-induced Akt, and CREB activation (Tong et al., 2012; Smith et al., 2014; Prieto et al., 2016; Tong et al., 2008), while others have shown a link between acute neuroinflammation and disruption of specific neural circuit functions and cognitive impairment (Czerniawski andGuzowski, 2014).

Studies have also shown that blocking IL-1β signaling can restore cognitive function (Prieto et al., 2016;Ben Menachem-Zidon et al.,2008).

Neuroinflammation may be beneficial in promoting homeostasis and neuron survival, but it can also results in tissue injury through the over-action of inflammatory mediators. Evidence suggested that neuroinflammation may trigger neuroprotection or neurodegeneration through the neurotrophic system (Leibinger et al., 2009).

Song et al (2013) evidenced that acute intracerebroventricular IL-1 injection markedly up-regulated mRNA and protein expressions of NGF and BDNF, while 8 days repeated IL-1 injection down-regulated BDNF mRNA and protein expression without significantly changing in NGF expression and concentration in the hippocampus.

IL-1β suppresses BDNF signal transduction and gene regulation in low density cortical neuronal cultures (Tong et al., 2008). In Carlos et al (2017), the authors found that IL-1β can alter proper endosomal function and compromises BDNF/TrkB signal transduction and synaptic plasticity, placing the brain at risk for cognitive decline and neurodegeneration.

IL-1β alone did not affect the viability of neurons, but compromised their BDNF-induced survival suggest, therefore, a subtle regulatory mechanism that may contribute to increased

neuronal vulnerability resulting from inflammation in the brain (Tong et al., 08).
These results suggest that treatments that preserve neurotrophic signaling in brain may help to overcome aging-dependent memory decline and some neurodegenerative diseases.

1.14 Assessing depression: the problem of self-assessment

In order to minimize the subjectivity in clinical evaluation, some diagnostic interviews and rating scales are currently usedfor helping the diagnosis of DD. It is recommended to test children and/or their parents and teachers. Tools can be centered exclusively on symptomatology or on different dimensions of psychopathology. Although the usefulness of interviews and rating scales is recognized as evaluation tools in screening, diagnosis and follow-up, it is necessary to carefully evaluate the limits of these instruments. In fact, the concordance estimates of the information obtained from multiple sources are low, due to many variables (eg depressed parents have a much lower threshold for detecting depressive symptoms than non-depressed parents), resulting in greater sensitivity, but less specificity of the information obtained. Furthermore, the "attenuation effect" should be considered, so that repeated administration of the same instruments over time will result in a reduction in the frequency of diagnoses and in the severity of symptoms. Therefore, their use should be reserved for clinicians with some experience in the field, who have trained and are able to detect the limits of these tools. Trained rater should know how to "actively" contribute to the survey of information, data evaluation, interpreting and integrating the conflicting information, in the perspective of a global assessment of the cognitive-emotional skills of the subject.
Interviews can be unstructured, semi-structured and structured. Semi-structured interviews are more used in clinical practice.
One of the most used interviews is the Kiddie-Schedule for Affective Disorders and Schizophrenia (K-SADS; Puig-Antich and Chambers, 1978). This is a diagnostic interview, used mainly for the 6-18 age group, for the assessment of psychopathological disorders (present and lifetime) in children and adolescents according to the DSM III-R and DSM-IV criteria. It consists of: an unstructured introductory interview, a diagnostic screening interview, a checklist for the administration of diagnostic supplement, five diagnostic supplements with DSM criteria (mood disorders, psychotics disorders, anxiety disorders, attention deficit disorder and disruptive behaviour disorder, substance abuse), a general checklist on patient's clinical history and a scale for the global assessment of child’s level of current functioning. It is administered to children and their parents by psychologists or pediatric psychiatrists.
Other interviews are Diagnostic Interview for Children and Adolescent (DICA, Herjanic and

Reich, 1982), Diagnostic Interview Schedule for Children (DISC, Shaffer et al, 2000) and the Child and Adolescent Psychiatric Assessment (CAPA; Angold, 1989; Angold and Costello, 1995).

Assessment scales (rating scales) specific for depression represent another type of diagnostic tool. Rating scales are helpfull for diagnosis and they can be used as screening tools and in follow-up to monitor the clinical trend and the effectiveness of the treatment (Costello and Angold, 1988). The rating scales can be self-assessed or they can be completed by the parent, the teacher or the clinician, on the basis of observations and collections of information from some other person. The rating scales completed by the clinicians are semi-structured interviews focused on a limited area of the symptomatology, but usually they do not provide information on the duration and on differential diagnosis. The ratings scales for patients and those for parents and teachers are self-administered questionnaires, focused on current symptoms and behaviours. They are not sufficient to make a diagnosis. Obviously, an optimal procedure should be based, when possible, on self information and evaluations from other sources (parents, teacher, rater observations). They are particularly useful in screening, selecting subject needing a subsequent diagnostic investigation, in case of high scores.

The rating scales most used for the characherization of DD are (SINPIA, 2007):

- Children's Depression Inventory (CDI, Kovacs, 1992): a self-administered questionnaire that assesses depressive symptomatology in children and adolescents aged between 8 and 17 years. It consists of 27 items, evaluated on a scale from 0 to 2 points, which investigates a wide variety of symptoms related to mood, interpersonal relationships, self-esteem, the sense of ineffectiveness and anhedonia;
- Hamilton Depression Rating Scale (HDRS or HAM-D; Hamilton, 1960). The scale, completed by the rater on clinical observations, provides a simple way to quantitatively assess the severity of depressive symptoms, the patient's condition and to document changes.
- Children's Depression Rating Scale-Revised (CDRS-R, Poznanski and Mokros, 1979), completed by the rater. Based on the Hamilton scale, it evaluates the severity of depressive disorders in the age group 6-12, but it is also used in adolescents. The most recent version (Poznanski and Mokros, 1999) evaluates cognitive, somatic, affective and psychomotor symptoms and allows to report both the respondent's response and the interviewer's observations. The limitations consists in the difficulty to separate the depressive symptoms from the anxious ones and in the overestimation of the gravity in children suffering from organic pathologies, due to the emphasis given to the somatic symptoms. It is used in studies that evaluate the effectiveness of various treatments. Italian version is not available.
- Mood and Feeling Questionnaire (MFQ; Angold et al, 1995b): it detects depressive

symptoms in the previous two weeks in the 8-18 age group, according to the DSM-III-R criteria (APA, 1987). There is a short and a long version.

In clinical practice, the Child Behaviour Checklist (CBCL) (Achenbach and Rescorla, 2001) is one of the most used questionnaires for assessing the skills, emotional and behavioural problems of children and adolescents. The questionnaire, completed by the parents, is available in two versions depending on the age of the child (preschool 1 ½ -5 years and school 6-18 years). There are also parallel versions of the instrument compiled by the teacher (Teacher Report Form, TRF) for both the age groups and, from the age of 11, by the young people themselves (Youth Self Report, YSR). The questionnaires investigate a broad spectrum of psychopathology in development age through scales that evaluate specific dimensions (withdrawal/ depression, somatic complaints, aggressive behaviour, etc.) and general dimensions (internalizing and externalizing problems). The emotional-behavioural problems are also be investigated through "DSM-oriented" scales, which allow the clinician and the researcher to orientate themselves in the evaluation based on DSM diagnostic classification. DD in developmental age are associated with a significant impairment of family, social and educational functioning. The psychosocial and scholastic problems are useful indicators of the overall functioning of the subject. They can support the diagnosis, but also allow understanding the evolution of the disorder and the possible effectiveness of a treatment, that finally affect the Quality of Life of the child and of the family unit. To explore functioning some of the tools already described may be useful; for example, the CBCL provides for a subscale of social competences.

Other scales used in clinical practice are:

- Clinical Global Impression (CGI; NIMH, 1970): consists of three subscales that evaluate the severity of the disease, the overall improvement and the effectiveness index. It is used to evaluate the trend in the time.
- Children-Global Assessment Scale (C-GAS; Shaffer et al; 1983): measures the severity of symptoms and functional impairment.

1.15 Treatment: the main problem

As for the adult, in the developmental age, the treatment of depression must be chosen on the basis of the characteristics of the pathology (in particular severity and duration) and of the functioning of the subject. Levels of severity are represented in Table 5.

Tab.5 Levels of severity (Brent and Maalouf, 2015)

Level of severity	Number and type symptoms	Impairment
Mild	≤4, no suicidal ideation or psychosis	Able to function in most ways, but takes more effort
Moderate	5-6, suicidal ideation	Impairment in at least one domain
Severe	7, imminent suicidal risk, could have psychosis, mixed features	Unable to function adequately, with impaired self-care

Effective treatments for developmental DD are both psychotherapeutic and pharmacological, although the predictors of response for each of these interventions, and their combination, are not yet fully understood. A combined approach is recommended as the more effective according to the TADS (Vitiello et al., 2006), ADAPT (Wilkinson et al., 2011) and TORDIA trials (Brent et al., 2008). Untreated depression is an important risk factor for SA, chronic and long-term functional impairment (Fombonne et al., 2001). A schematic flow chart for DD treatment is represented in Figure 16 (NICE 2004, AACAP 2007 Ministry Of Health And Social Policy Galicia, 2009).

Non-pharmacological treatment

Considering the current state of art, two types of psychotherapy have proven efficacy on depression in the developmental age even in controlled studies. They are therefore considered as elective treatments for the DD: interpersonal psychotherapy and cognitive behavioral psychotherapy, CBT (Vicari and Vitiello, 2015). Interpersonal psychotherapy (IPT) focuses on the patient's interpersonal functioning and on the role of the patient in the development and maintenance of depressive symptoms. CBT is based on the assumption that emotions and therefore mood are the result of thoughts and behaviors, and that it is possible to modify them by intervening on the way of reasoning and acting when facing stressful events and situations (Vicari and Vitiello, 2015).

Literature show that psychotherapy is effective in the treatment of depression and that this efficacy seems to reduce significatively when the depression is more severe, but if associated with drug therapy it seems to have a possible additional effect, perhaps even protective of the suicidality (Vitiello, 2009).

Although there are currently no evidences of their effectiveness, some psychosocial supports

trategies appear also useful as initial intervention. According to the NICE guidelines for Depression (NICE, 2017), we can identify 3 areas of intervention: self-help capacity implementation (psychoeducation, activation of socialization spaces, support's groups), family support/ education (parent support, family therapy, treatment of affected parents) and socio-environmental interventions (support's groups).

Pharmacological treatment

Pharmacological treatment is indicated: in case of non-response or lack of availability of other approaches, in case of severity of DD (Clinical Global Impressions–Severity score of 4, consistent with accepted guidelines (Birmaher et al. 1998), of high suicidal risk, of recurrent or chronic forms, of comorbidity, of psychotic symptoms, if the psychosocial conditions are disadvantaged, if there is a familiarity with DD. The use of antidepressants in developmental age, in clinical practice generally limited to adolescents, has been increasing during the 1990s, but in more recent years their use has been decreasing, due to the fear that they may favour suicidal thoughts or behaviours during treatment (Olfson et al., 2006).

The use of pharmacotherapy is recommended by some therapeutic guidelines that have found consensus in the scientific community (NICE, 2005; SINPIA, 2007; Ministry Of Health And Social Policy Galicia, 2009).

In this context, Selective Serotonin Reuptake Inhibitors (SSRIs) are generally indicated as first line treatment for depression and anxiety disorders in developmental age. The European Medicines Agency (EMA) and the Italian Agenzia del Farmaco (AIFA) approved fluoxetine for the treatment of moderate or severe depression in people over 8 years of age, who have not responded to 4-6 psychotherapy sessions. Data from randomized, double-blind placebo-controlled trials support the superiority of fluoxetine as a first-choice drug, while weaker confirmations support sertraline, citaloproam, escitalopram, and, limited to adolescence, venlafaxine (Masi et al., 2010). In subjects who do not respond to an SSRI after 4-6 weeks of treatment, a second SSRI (sertraline, citalopram, escitalopram) or, in adolescents, venlafaxine (Masi et al., 2010) may be used. All of these secondary options are used for an unapproved indication or in an unapproved age group, dosage, or way of administration (off-label use).

In subjects who respond positively to the therapy, the pharmacological treatment should be maintained for at least 6-8 months. In the cases of DD with later or less intense response or in relapses, treatment is recommended for 1 year. The risk of onset of symptoms of excitation during antidepressant therapy should be carefully assessed and monitored, especially in the presence of positive family history of BD (Masi et al., 2010).

Generally the most used drugs in the treatment of depression are:

- **Selective Serotonin Reuptake Inhibitors (SSRIs) (fluoxetine, sertraline,**

paroxetine, citalopram, excitalopram, fluvoxamine). They are the first line therapy as for adults, and in particular fluoxetine has been authorized by the EMA (June 2006) and AIFA for moderate and severe depression, in patients over 8 years who have not responded to 4 - 6 sessions of psychotherapy. SSRIs inhibit the reuptake of serotonin with a consequent increase in monoamine levels in the synaptic space. In 3-4 weeks, they desensitize the presynaptic receptors thus determining an increased synthesis of the neurotransmitter and facilitating its release. The serotonin-receptor binding activates a cascade that ultimately regulates gene expression (Garcia et al., 2002; Ivy et al., 2003), modulating the expression of substances (eg substance P or neurokinins), receptors, transporters and neurotrophic factors such as BDNF.

- **Tricyclic Antidepressants, TCA** (amitriptyline, clomipramine, imipramine, nortriptyline). Used before the advent of serotoninergic agents, now progressively abandoned because of their important cardiotoxic effects even at therapeutic doses. They inhibit the re-uptake of 5-HT, noradrenaline and to a lesser extent dopamine, with slightly diversified spectrum of action (eg clomipramine with higher serotoninergic action, desimipramine with greater noradrenergic action). They have also anticholinergic, anti-α1-adrenergic and anti-histaminic action (Vicari and Vitiello, 2015).

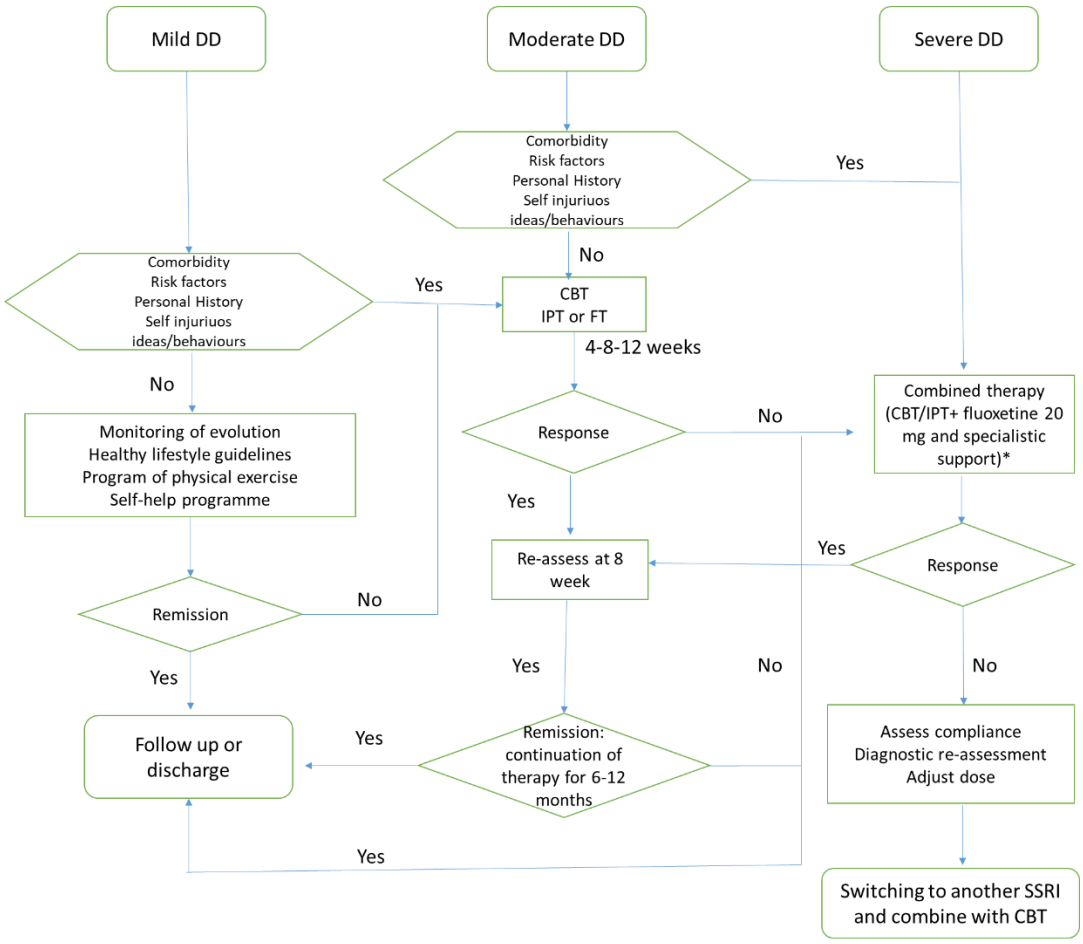
- **Mood stabilizers (lithium, antiepileptics).** There is no indication in the treatment of unipolar depression, while they are the first choice in bipolar depression, in which antidepressants should be used with caution, given the risk of swithing to a maniacal episode (AACAP, 1997-1998; Masi, 2005).

Other new drugs (trials for developmental age not available) are:

- **Nefazodone**. 5-HT and NA re-uptake inhibitor and 5-HT2A receptor antagonist, with lower risk of anxious activation, insomnia and sexual effects, but with less antidepressive potency. Its use is limited by a rare but potentially lethal hepatotoxicity.
- **Serotonin and norepinephrine reuptake inhibitors, SNRI** (venlafaxine, mirtazapine, duloxetine). Venlafaxine, a third-choice drug, inhibits the presynaptic reuptake of serotonin and, at higher doses, of noradrenaline. This double action determines a faster and more powerful antidepressive action. It should be used with caution because it would seem to increase self-injurious events more than any other antidepressant (Mandoki et al., 1997; Emslie et al., 2004a-2004b). Mirtazapine increases the production of monoamines, presenting an additional blocking effect of the 5-HT2A, 5-HT2C and 5-HT3 receptors. This blocking effect reduces some undesired effects (SINPIA, 2007).
- **selective NA and DA reuptake inhibitors, NDRI** (Bupropion). They are used in adults in case of resistance to SSRI.
- **selective NA reuptake inhibitors, NARI** (viloxazine, reboxetine)

- **Serotonin Multimodal Antidepressant, S-MM** (vortioxetine): a study on developmental age is in progress.
- **Others** (agomelatine, trazodone). Interesting studies are published recently on ketamine (NMDA-receptor antagonist).

Despite a large variety of terapeutic options, however, it has been reported that 30–40% of all patients affected by DD who receive a sufficient dose and duration of treatment fail to respond (Maalouf and Brent, 2012). Currently there is no way of knowing in advance which of the patients will respond. This means that many young people are being exposed to an agent that will not be usefuland may have serious adverse events (Amitai et al., 2016). Moreover, the available drugs do not treat the clinical comorbidities associated with the psychiatric pathology, on the contrary, they can even make them worse (e.g. weight gain) (Barbosa et al., 2014)



*Accordinf to the patient's clinical profile, another SSRI could be selected (sertraline, citalopram, escitalopram)

Fig. 16 Flow chart for DD (Adapted from NICE 2004, AACAP 2007 Ministry Of Health And Social Policy Galicia, 2009). CBT Cognitive Behavioural Therapy, IPT Interpersonal Therapy, FT Familiar Therapy

2. Objectives

The primary aim of the present study is to measure plasma levels of BDNF and IL-1β in children and adolescents suffering from depression comparing to a group of healty peers in 3 times (moment of recruitment, 6 months, 12 months).

Secondary aims are: 1) to evaluate the trend of neurotrophin and IL-1β levels after naturalistic psychopharmacological treatment (at 3, 6 and 12 months) in depressed patients, even on the light of clinical psychodiagnostic evalutation; 2) to hypotesize the role of these plasmatic dosages of neurotrophin and proinflammatory cytokine as peripheral "biomarkers" of disease and of early treatment response, finally to improve the diagnostic precision and clinical characterization that permit to target the pharmacological intervention.

Our hypotheses, based mainly on adult literature, is that depressed patients present lower BDNF and higher IL-1β levels than healthy peers with a trend of normalization during psychologic and pharmacological treatment, parallel to the improvement revealed to psychodiagnostic tests. Moreover, we hypothesized that in depressed patients' levels of BDNF and IL-1β, toghether with cinical evaluation, can differentiate some subgroups of patients, for example the responder, from the others, permitting a critical analysis of psychiatric management.

3. Methods

3.1 Sample

From January 2014 until September 2017, every subjects who consecutively had access to the Psychiatric Unit of the Department of Health of Women and Children (University of Padua) for depressive mood disorders was proposed to partecipate to the study.

Inclusion criteria for the depressed group (study group) were: naïve subjects (never treated and never diagnosed), aged between 10 to 17,9 years, with primary clinical diagnosis of mood disorders with prevalent depressive component according to the Diagnostic and Statistical Manual of Mental Disorders, 4th ed., Text Revision (DSM-IV-TR) (APA, 2002). Exclusion criteria for the study group were: a)comorbidity with acute or severe diseases not stabilized, eg oncological diseases, infectious, autoimmune diseases, neurological conditions (in particular epilepsy); b) presence of severe psychiatric comorbidity (active psychosis, post-traumatic stress disorder –PTSD, ASD); c) use of steroid-based therapies, anti-inflammatory drugs, antibiotics or estro-progestins in the 4 weeks prior to the recruitment. Secondary exclusion criteria were: d) use of psychotropic drugs for therapies; e) the inability to read and f) intellectual disability for the impossibility to complete correctly the tests.

A group of heathy subjects matched for age (± 1 year) and sex was also recruited, according

the following exclusion criteria: a) presence of not stabilized acute or severe pathologies; b) presence of psychiatric disorders or suicidality in the subject or in his/her familiar history; c) use of steroid-based therapies, anti-inflammatory drugs, antibiotics, estroprogestants or psychotropic drugs within 4 weeks prior to the recruitment.

After an initial phase of explanation of the project, informed written consent was collected from all parents and all the involved subject.

The study was approved by the Ethical Committee for Scientific Experimentation of the University of Padua (28.01.2014).

3.2 Psychiatric diagnosis

Participants were all assessed for psychiatric disorders in person by a trained, experienced pediatric psychiatrist MD. Psychiatric diagnoses were based on free talks (minimum 2), personal data sheet collection and administration of the following tests:

- the Schedule for Affective Disorder and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): a DSM-IV-TR (APA, 2002) structured diagnostic interview validated in many settings (Kaufman et al., 2004), administered to all parents and subjects who participated to the study. Several psychometric proprieties of K-SADS have already been tested in similar populations and it is suggested that it can be a useful cross-cultural diagnostic measure (Brasil and Bordin, 2010; Polanczyk et al., 2003).
- the Youth Self Report (YSR) and Children Behaviour Checklist 6-18 (CBCL 6-18) (Achenbach and Rescorla, 2001): questionaire completed from all the subjects and their parents respectively. They are reliable tools that comprise more than 100 items grouped into three main scales: internalising, externalising and total problems. Rough score were calculated as T-scores by ASEBA Windows ® software.
- the Children's Depression Inventory (CDI) (Kovacs, 1992), completed by all the involved subjects for the evaluation of the presence and severiy of specific depressive symptoms (cut-off for pathology:18).
- the 21-item Hamilton Psychiatric Rating Scale for Depression (HAM-D; Hamilton, 1980), completed by the psychiatrist for the depressed patients (cut off :8);
- the Multidimensional Anxiety Scale for Children (MASC; March et al., 1997), to evaluate comorbidity with anxiety disorders; it was completed by all the subjects recruited. The questionaire consists of 39 items distributed across four major dimensions (1) physical symptoms (tense/restless and somatic/autonomic), (2) social anxiety (humiliation/rejection and public performance fears), (3) harm avoidance (perfectionism and anxious coping), and (4) separation anxiety.

- the Structured Clinical Interview for DSM-IV axis II disorders (SCID II; First et al., 1997; Ver. 2.0), shortened form, completed by all the depressed patients over 13 years old;
- the Suicide Risk Assessment (SAD PERSON), for the assessment of suicidal risk, completed by the MD for all subjects suffering from depression (Juhnke, 1996 and 1994). This scale was developed in the USA in 1983 as a tool to assess suicide risk following an episode of self-harm (Patterson et al., 1983). It was based on 10 major risk factors, adapted to pediatric population. Even it is not considered a screening instruments, it is useful to obtain central information for evaluation of the risk. It permits to identify 3 class of risk and, associated to clinical evaluation, to guide monitoring choices (admission or not to the hospital).
- projective tests: Machover test (Machover, 1953), Corman test (Corman, 1970), Thematic Apperception Test (Murray, 1943), Rorschach test (according to the Passi-Tognazzo method) (Rorschach, 1921; Passi-Tognazzo, 1994), administered to all children with depression.

Overall clinical severity and improvement were assessed using the CGI-Severity (CGI-S) and CGI-Improvement (CGI-I) subscales of the Clinical Global Impression (CGI, NIMH, 1970), completed by the psychiatrist for every subject involved in the study.

To estimate the general functioning of subjects the Childrens Global Assessment Scale (C-GAS, Shaffer et al.; 1983) was also used. C-GAS is a numeric scale with scores range from 1 (worst functioning) to 100 (better functioning).

3.3 Procedure

Each subject arrived to the services for suspected mood disorder was evaluated with a preliminary meeting in the presence of the parents. After obtained the written informed consent to the clinical evaluation, a careful medical and psychiatric history was collected and a general objective examination was performed, including the measurement of the auxological parameters (body weight- BW- and height -H). Body Mass Index (BMI) was calculated (kg/m^2).

The psychopathological diagnostic evaluation was conducted through at least 2 free interviews and administration of tests at intake. At the end of this phase, if the clinical situation meets the inclusion criteria without exclusion criteria, every subject was proposed the inclusion in the study group, after having read and explained the project and the informed consent form, developed in two versions, one dedicated to the children and one to the parents. To all subjects, routine basal hematochemical tests were performed, to which the plasma dosage of BDNF and of IL-1β were added.

For each depressed subject with a clinical indication to treatment, confirmed by tests scores, a pharmacological therapy with antidepressant drugs (mainly SSRI) was recommended, in association with mood stabilizing drugs (valproic acid, lithium) or atypical neuroleptics (risperidone, quetiapine, olanzapine) if necessary. At the same time, it was recommended a psychotherapy, according to a combined approach.

Subjects were assessed according to a tight schedule of controls at intake, and at 1 (clinical evaluation), 3, 6 (blood and clinical), 9 (clinical evaluation) and 12 (blood and clinical) months. (Fig. 17)

The clinical check consisted of a free talk and the repetition of some tests according to the timing allowed by the test (minimum 6-12 months). Blood sample collection was also used to assess the tolerability of the drug taken.

All assessments were usually carried out on an outpatient modality or, if the subject was in the acute phase of the pathology, during the hospitalization in the Department of Health of Women and Children.

For all the recruited subjects a structured follow-up was maintained and guaranteed with individual interviews to the child and interviews to the parents with variable times and frequencies, depending on the clinical needs.

As for healthy subjects, it was performed a preliminary talk and interview, aimed at the exclusion of neuropsychiatric pathologies. Structured test (K-SADS-PL, CDI, HAM-D, MASC, CBCL and YSR respectively to the parents and the child) were administrated and a blood sample collected at intake. A schedule of checks was then set at 6 months (blood and clinical) and 12 (blood and clinical).

Socio demographic data were obtained using an ad hoc questionaire. Standard techniques and calibrated equipment were used to assess anthropometric measures.

Peripheral blood samples were collected by venipuncture from fasting subjects between 7:30 am and 9 am, upon the arrival at the hospital, after obtaining written informed consent. All individuals had fasted for 10 to 12 h before the procedure.

The sample (5 ml) was collected in potassium EDTA coated tubes (Becton Dickinson, U.S.A.). At the time of intake collection, no patient followed any specific drug therapy. The blood was separated into the plasma components and peripheral blood mononuclear cells (Peripheral Blood Mononuclear Cells, PBMC) with Ficoll Paque Plus (GE Healthcare Life Sciences, Uppsala, Sweden), according to the manufacturer's indications. In particular, samples of each subject were stratified on a volume of Ficoll Paque Plus equal to 1/2 of the volume of blood to be separated and centrifuged at 400g for 40 minutes at room temperature in an ALC PK131 centrifuge (Thermo Fisher Scientific, USA). At the end of centrifugation, the plasma stratified in the upper part, while below it forms a PBMC ring. Below this ring

there is the residual Ficoll and in the lower part stratify the more voluminous erythrocytes and white blood cells. The plasma is then recovered and frozen at -20 ° C until use. PBMCs are collected, washed in 15 ml tubes with a phosphate buffer (phosphate buffer saline, PBS, Life Technologies, U.S.A) at pH 7.4 and then centrifuged at 100g for 10 minutes at room temperature. The PBMC pellet is resuspended, subjected to a new wash again in a 1.5ml tube and pelleted further by centrifugation at 100g for 10 minutes in a Microfuge 22R centrifuge (Beckman Coulter Inc., U.S.A.). The supernatant is removed and the resuspended pellet is stored at -20 ° C for further analysis.In the group of depressed subjects, there is a dropout percentage of more than 50% before completing the 12 months of monitoring.

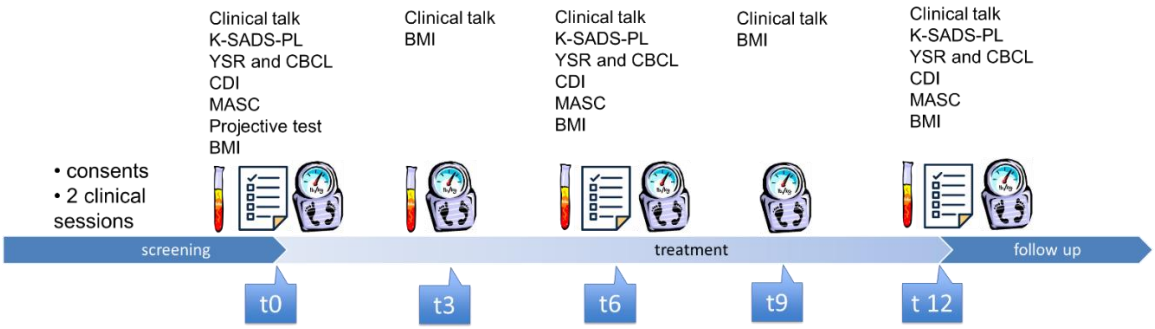


Fig.17 Study time plan.

3.4 BDNFand IL-1β dosage

BDNF and IL-1β levels were quantified in plasma samples at the Molecular Neuropsychopharmacology Laboratory of the Department of Pharmaceutical Sciences of the University of Padua.

BDNF was measured using an ELISA assay (BDNF Emax ImmunoAssay System, Promega, Madison, USA), according to the indications of producer. The assay is used for the specific measurement of the mature BDNF form in a sandwich antibody format. Its minimum sensitivity is 7.8pg / ml and the maximum sensitivity is 500pg / ml.

The assay was performed in 96-well polystyrene plates (Nunc MaxiSorpTM, Thermo Fisher Scientific). To each well were added 100µl of a solution composed by sodium carbonate 250mM and sodium bicarbonate 250mM at pH 9.7. In this solution, the monoclonal anti-BDNF antibody is diluted and the plate was left at + 4 °C for a night, in order to permit the antibody adhere to the plastic. Thereafter the plate was washed three times with a Tris-HCl20mM solution at pH 7.6, NaCl 150mM and Tween 20 at 0.05% (Tris-61 Buffered Saline-Tween 20, TBST) and, in order to blocking all non-specific binding sites for BDNF and anti-BDNF antibody, it was incubated for one hour with 200µl of a blocking solution (unknown proprietary composition).

The plate was then washed three times with TBST and incubated for 2 hours at room temperature with 200µl of the unknown plasma sample and of the standard curve. In particular, the plasma of each patient was tested in serial dilutions in order to obtain consistent results with the linear values of the curve and to avoid enzymatic inhibitions by plasma components. Thereafter the plate was washed 5 times with TBST and each sample was incubated for 2 hours at room temperature with 100 µl of the blocking solution containing the human anti-BDNF antibody diluted 1: 500. At the end, the plate was washed 5 times with TBST and then incubated for one hour at room temperature with the blocking solution containing an anti-IgY antibody conjugated with the peroxidase of Armoracia rusticana (Horseradish Peroxidase, HRP) diluted 1: 200 and subsequently washed for 5 times with TBST. The samples were then incubated for 10 minutes at roomtemperature with 100 µl of a solution containing 3.3',5,5' Tetramethylbenzidine (TMB). Depending on the concentration of BDNF in the well, the samples take different shades of blue. 100µl of 1 N hydrochloric acid were then added to each well to stop the reaction. After acidification, the blue color turns to yellow. Within 30 minutes of acidification, the absorbance at 450nm was read in a plate reader model Victor3 (Perkin Elmer Inc., U.S.A.). The BDNF concentration value of each sample was calculated by extrapolation from the standard curve (Fig.18).

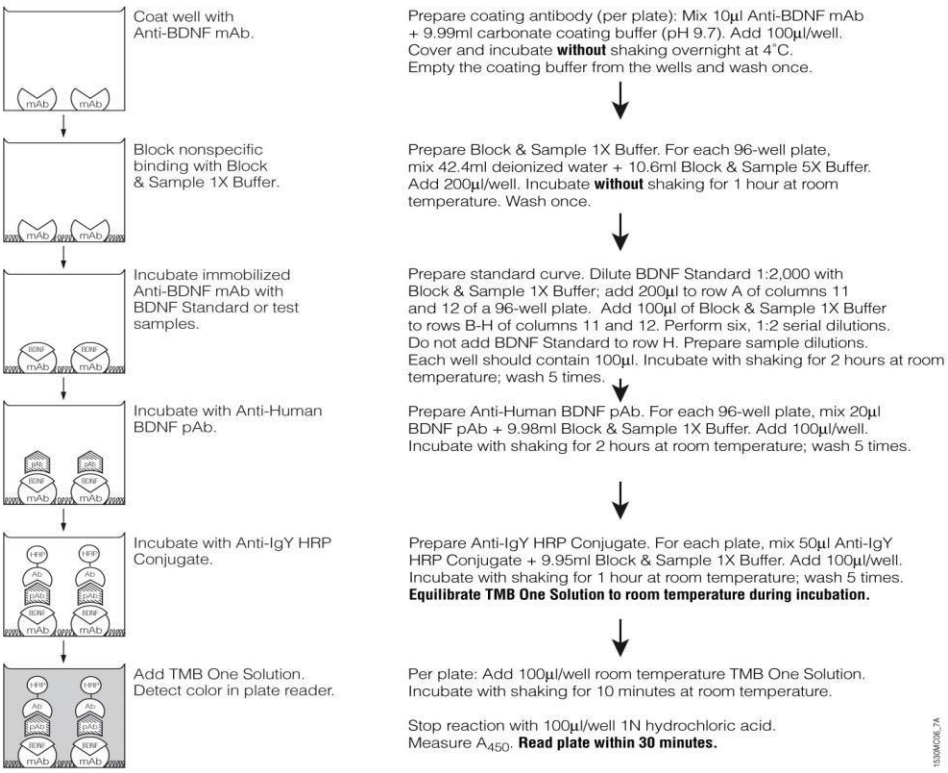


Fig. 18BDNF Emax ImmunoAssay System

Human IL-1β was quantified by commercially available ELISA (enzyme-linked immunosorbent assay) kit according to the manufacturer's instructions (Antigenix America, Huntington Station, NY, USA). Standards with known amounts of IL-1βwere used to convert values into absolute concentrations of the cytokine in pg/mL. The IL-1β ELISA assay kit does not distinguish between the inactive precursor (pro-IL-1β) and the bioactive mature form (as is the case for all commercially available kits), but the specificity for the mature form versus the pro-IL-1β is between 15:1 and 20:1, respectively (personal communication by Rolan Stall, Lundbeak, U.S.A.).

Values not within the linear portion of the standard curves were excluded, generating a truncated distribution.

3.5 Statistical Analysis

In the preliminary phase, subjects with depression were matched for sex and age (±1year) to healty subjects.

Data were analyzed using IBM SPSS statistics vers. 23. Both qualitative and quantitative analyses were run on data obtained. Qualitative analyses were run using descriptive statistics (frequencies, mean scores, standard deviations, and percentages), in order to get an overview of the domains investigated. Subsequently, quantitative analysis were used. Due to the reduced number of subjects, non parametric tests were adopted. Chi-Squared tests were used

to investigate whether the distribution of subjects significantly differed according to a series of variables of interest. The Wilcoxon signed-rank test, an alternative to the paired Student's t-test, and the Friedman test, an alternative to the repeated measures ANOVA, were adopted to investigate whether specific within group variables showed significant changes between the different periods considered. The Mann-Whitney test was run in order to investigate significant differences in between group variables. Finally, Spearman's rank correlation coefficient was used in order to investigate the presence of significant associations between the different variables considered.

A p value< 0.05 was considered to be statistically significant in all analysis.

4. Results

4.1 Subjects and symptoms characteristics: comparison between groups

From January 2014 to December 2017, 18 patientswere selected in the Psychiatry Unit of the Department of Women's and Children's Health of the University of Padua. All patients received an initial diagnosis of mood disorder oriented in a depressive sense according to the DSM-IV-TR (APA, 2002). After a preliminary phase of project explanation, 13 out of 18 depressed patients accepted to participate to the study. Recruited patients were all at the first access to the hospital, without any previous psychiatric diagnosis and untreated. All the patients were caucasian, but in 3 cases (20%) they presented foreign origins (1 China, 2 Colombia). Most of patients were female (12/13, 90%) with an average age of 14 years and 5 months (SD±1,58, range12-17).

In parallel with the group of subjects affected by depression, a homogeneous group of healty subjects (n=12) matched for sex and age was recruited (75% female, average age of 14 years and 5 months [SD±2,23]). All these subjects were Italian.

The group with depressed patient does not differ significantly for sex and age from the group of healthy children, since they were previously matched.

Socio- demographic characteristics of the two groups are shown in Table 6. As shown in theTable, the distributions of the two groups significantly differ for family history of psychiatric diseases, family problems, parents' separation, traumatic events, scholastic problems, social isolation, sleep problems and alchol /substance use.

In both of the groups, the median number of siblings for subject is 1 (range in depressed subjects: 0-4; range in healthy peers: 0-14). As for relevant history information, in the group of depressed patients, the gestational age was 39 weeks on average (SD ±2).

Auxologic charachteristics and BDNF levels in both the groups are detailed in Tables 7 and 8. None of the subjects participating in the study was affected by other chronic diseases.

All depressed patients were treated with psychopharmacological drugs in association to psychotherapy. In detail, 6 patients were treated with SGAs (olanzapine or quetiapine), 4 with mood stabilizers (valproic acid and lithium), 3 with a combination of drugs (2 SGAs + stabilizer, 1 SGAs + SSRI).

Data on the way of admission to pediatric psychiatric services by depressed patients are presented in Table 9.

Tab. 6Socio- demographic characteristics of depressed patients and of healthy peers. ChiSquare Test.

	Depressed group (n=13)	**Healthy group (n=12)**	**Chi Square**	**p**
Family hystory of psychiatric diseases; n	12[a/b]	1	17,62	**.000**
Family problems*; n	12[b]	0	21,30	**.000**
Parents' separation; n	5	0	5,76	**.016**
Mother unemployed; n	2	7	4.99	.025
Father unemployed; n	2	3	.157	.692
Traumatic events; n	9	0	12,98	**.000**
Scolastic problems; n	12	0	21,30	**.000**
Internet use; n	7	9[c]	1,21	.271
Social isolation; n	12	0	21,30	**.000**
Sleep disorders; n	11	0	18,13	**.000**
Alchol /substance use; n	4	0	4,39	**.036**

* including family conflicts; [a] 7/13 (54%) in the maternal family line; [b]in all cases: family history with psychiatric diseases, intrafamiliar problems; [c] on average 2,4hours/day.

Tab. 7Auxologic charachteristics of the groups at intake (T0) and after 12 months. Mann-Whitney Test.

		Depressed patients (n=6)	**Healthy peers (n=12)**	**p**
BMI (Kg/m^2); M±SD	**T0**	19.75 ±3.36	21.73 ±3.08	>.05
	T12	23.33 ±4.01	22.99 ±2.39	>.05

Tab. 8 BMI variation (%) over time in the two groups. Mann-Whitney Test.

	Depressed patients (n=6)	**Healthy peers(n=12)**	**p**
% BMI variation (T0-T12)	14	7	>.05

Tab. 9 Access to services of depressed patients

	N=13
Reason for access; n	
Self-cutting/self harm	2
Attempted suicide	3
Other**	8
Pediatric Emergency Room; n	7
Hospitalization; n	8
Days of hospital stay; median (min-max; SD)	9 (9-60; 19.12)

**Other reasons: scholastic and relational difficulties, abdominal pain, agitation.

Plasma BDNF concentrations are lower in depressed patients compared to heathy subjects at intake (T0), even if they do not reach statistical significance as highlighted by Mann-Whitney test (p>.05) (Tab.10). In the depressed group, ID 112 was not considered because of neurotrophin values outside the standard curve.

BDNF values at T0 in the depressed patients have a median of 15942.99 pg/ml, while in the halthy peers median is 21404.81 pg/ml.

Tab. 10BDNF levels in depressed patients and in healty peers at intake (T0) and after 6 months (T6) and 12 months (12). Mann-Whitney Test.

BDNF (pg/ml); M±SD	Depressed subjects (n=10)	Healthy peers (n=12)	p
T0	16529.60 ± 6064.89	21644,23 ±10974,94	>.05
T6	17215,36±11281,60	14859,23±8430,37	>.05
T12	20634,20±12672,64	17588,85±9355,98	>.05

IL1-β concentrations are higher in depressed patients compared to heathy subjects at intake (T0), even if they do not reach statistical significance (p>.05) (Tab. 11). Il-1-β values at T0 in the depressed patients have a median of 68.64 pg/ml, while in the healthy peers the median is 75.03pg/ml.

Tab. 11IL-1β levels in depressed patients and in healty peers (T0)*

Mann-Whitney Test.

IL-1β (pg/ml); M±SD	Depressed subjects (n=9)	Healthy peers (n=8)	p
T0	133.96± 126.65	88.71±61.96	>.05

T6	56.06 ±5.82	82.85± 45.62	>.05
T12	139.70± 156.36	51.46± 14.75	>.05

* many results missing because out of the measurement curve

Psychopathological scores of diagnostic tests in depressed patients and in healthy peers are presented in Tables from 12 to 21.

Tab. 12 CBCL completed by mothers in the two groups at intake (T0) and after 12 months (T12)

	Time	**Depressed group (n=13) M (SD)**	**Healthy group (n=12) M (SD)**	**Z**	**p**
COMPETENCE AND ADAPTIVE BEHAVIOR					
Activities	T0	6.12 (3.00)	8.46 (2.33)	-2.207	**.027**
	T12	6.52 (2.18)	-	-	-
Social	T0	4.70 (1.79)	7.04 (1.84)	-2.669	**.008**
	T12	5.87 (2.53)	-	-	-
School	T0	3.66 (1.13)	5.42 (0.29)	-3.400	**.001**
	T12	4.66 (0.58)	-	-	-
Total	T0	14.54 (4.07)	20.92 (2.89)	-3.267	**.001**
	T12	16.50 (3.28)	-	-	-
SYNDROME SCALES					
Anxiety/depressive	T0	12.41 (4.87)	2.58 (2.02)	-3.910	.000
	T12	6.50 (4.20)	-	-	-
Withdrawn/depressive	T0	10.66 (2.30)	1.42 (1.62)	-4.137	.000
	T12	6.00 (2.83)	-	-	-
Somatic complaints	T0	5.91 (2.68)	1.75 (2.18)	-3.272	.001
	T12	3.00 (2.16)	-	-	-
Social problems	T0	7.33 (4.03)	1.08 (1.16)	-3.859	.000
	T12	3.25 (4.03)	-	-	-
Thought	T0	9.41 (3.58)	0.92 (1.08)	-4.197	.000
	T12	3.50 (2.52)	-	-	-
Attention	T0	11.42 (4.40)	2.33 (2.10)	-3.978	.000
	T12	5.75 (5.56)	-	-	-
Rulebreak	T0	7.92 (4.83)	2.00 (1.65)	-3.281	.001
	T12	1.50 (2.38)	-	-	-
Aggressive	T0	18.50 (5.87)	3.33 (2.67)	-4.172	.000
	T12	7.25 (5.56)	-	-	-
SUMMARY SCALES					
Internalizing Pr.	T0	29.00 (7.20)	5.75 (5.41)	-4.046	.000
	T12	15.50 (7.14)	-	-	-
Externalizing Pr.	T0	26.42 (9.51)	5.50 (3.87)	-4.161	.000
	T12	8.75 (7.67)	-	-	-
Total problems	T0	89.83 (21.28)	17.42 (12.52)	-4.161	.000
	T12	38.50 (27.23)	-	-	-
DSM-ORIENTED SCALES					
Affective Dis	T0	15.33 (3.31)	1.58 (1.73)	-4.181	.000

	T12	7.50 (3.41)	-	-	-
Anxiety Dis	T0	6.00 (2.33)	1.92 (1.56)	-3.580	.000
	T12	3.75 (2.87)	-	-	-
Somatic problems	T0	3.25 (2.34)	1.25 (1.76)	-1.982	.047
	T12	2.00 (1.41)	-	-	-
ADHD	T0	7.16 (3.13)	2.08 (2.06)	-3.326	.001
	T12	3.75 (3.59)	-	-	-
Oppositional	T0	7.33 (1.87)	1.58 (1.00)	-4.193	.000
	T12	3.75 (3.10)	-	-	-
Conduct	T0	8.75 (4.75)	1.33 (1.37)	-3.927	.000
	T12	1.25 (2.500)	-	-	-
Sluggish_Cogn.	T0	4.17 (1.95)	0.92 (1.31)	-3.533	.000
	T12	1.50 (1.72)	-	-	-
Obs_Compul	T0	6.58 (3.73)	0.92 (0.67)	-4.057	.000
	T12	2.75 (1.26)	-	-	-
Post_Traum	T0	16.83 (3.86)	3.33 (2.23)	-4.182	.000
	T12	8.75 (4.27)	-	-	-

Tab. 13 CBCL completed by fathers in the two groups at intake (T0) and afeter 12 months

	Time	**Depressed group (n=13) M (SD)**	**Healthy group (n=12) M (SD)**	**Z**	**p**
COMPETENCE AND ADAPTIVE BEHAVIOR					
Activities	T0	3.25 (2.17)	7.32 (1.42)	-3.480	.001
	T12	6.20 (2.55)	-	-	-
Social	T0	5.44 (0.92)	6.79 (1.47)	-2.009	.045
	T12	5.67 (3.05)	-	-	-
School	T0	4.27 (0.56)	5.14 (0.59)	-2.667	.007
	T12	4.67 (0.58)	-	-	-
Total	T0	13.06 (2.78)	19.50 (2.01)	-3.558	.000
	T12	16.50 (3.28)	-	-	-
SYNDROME SCALES					
Anxiety/depressive	T0	9.78 (4.12)	2.67 (2.23)	-3.539	.000
	T12	6.67 (5.13)	-	-	-
Withdrawn/depressive	T0	7.44 (3.84)	1.42 (1.56)	-3.539	.000
	T12	6.67 (3.05)	-	-	-
Somatic complaints	T0	5.11 (2.09)	1.08 (1.50)	-3.469	.001
	T12	4.00 (1.00)	-	-	-
Social problems	T0	4.78 (2.95)	1.17 (0.67)	-2.888	.004
	T12	3.33 (4.93)	-	-	-
Thought	T0	7.00 (4.09)	0.50 (0.67)	-3.925	.000
	T12	3.67 (3.05)	-	-	-
Attention	T0	10.11 (3.82)	1.59 (1.68)	-3.752	.000
	T12	7.00 (6.08)	-	-	-
Rulebreak	T0	6.89 (4.20)	1.67 (1.92)	-3.091	.002
	T12	1.67 (2.89)	-	-	-
Aggressive	T0	12.56 (4.50)	2.33 (1.43)	-3.863	.000
	T12	9.00 (5.29)	-	-	-
SUMMARY SCALES					
Internalizing Pr.	T0	22.33 (7.28)	5.17 (4.95)	-3.700	.000

	T12	17.33 (7.50)	-	-	-
Externalizing Pr.	T0	19.44 (5.92)	4.00 (3.13)	-3.812	.000
	T12	10.67 (8.14)	-	-	-
Total problems	T0	68.44 (17.56)	14.83 (11.14)	-3.841	.000
	T12	43.67 (30.86)	-	-	-
DSM-ORIENTED SCALES					
Affective Dis	T0	10.89 (3.72)	1.17 (1.70)	-3.737	.000
	T12	7.33 (4.16)	-	-	-
Anxiety Dis	T0	4.44 (2.74)	1.67 (1.43)	-2.448	.014
	T12	4.00 (3.46)	-	-	-
Somatic problems	T0	3.00 (1.58)	0.75 (1.36)	-2.752	.006
	T12	2.67 (0.58)	-	-	-
ADHD	T0	6.56 (2.65)	1.67 (2.01)	-3.302	.001
	T12	4.67 (3.79)	-	-	-
Oppositional	T0	6.44 (1.67)	1.42 (0.79)	-3.899	.000
	T12	4.67 (3.05)	-	-	-
Conduct	T0	6.00 (2.78)	0.92 (1.08)	-3.683	.000
	T12	1.67 (2.89)	-	-	-
Sluggish_Cogn.	T0	3.00 (1.87)	0.17 (0.39)	-4.008	.000
	T12	2.00 (1.73)	-	-	-
Obs_Compul	T0	4.78 (3.70)	1.25 (1.14)	-2.671	.008
	T12	2.67 (1.53)	-	-	-
Post_Traum	T0	12.56 (4.88)	3.33 (2.23)	-3.688	.000
	T12	10.33 (3.51)	-	-	-

Tab. 14 YSR completed by all the subjects of the two groups at intake (T0) and afeter 12 months

	Time	**Depressed group (n=13) M (SD)**	**Healthy group (n=12) M (SD)**	**Z**	**p**
COMPETENCE AND ADAPTIVE BEHAVIOR					
Activities	T0	6.77 (3.70)	9.33 (2.42)	-1.726	.084
	T12	4.21 (2.27)	-	-	-
Social	T0	5.61 (2.38)	6.92 (1.82)	-1.202	.229
	T12	5.57 (1.94)	-	-	-
School	T0	1.31 (0.63)	2.37 (0.43)	-3.765	.000
	T12	1.75 (0.42)	-	-	-
Total	T0	13.69 (4.90)	18.62 (3.74)	-2.483	.013
	T12	12.00 (2.81)	-	-	-
SYNDROME SCALES					
Anxiety/depressive	T0	15.85 (4.14)	7.17 (4.95)	-3.543	.000
	T12	12.00 (8.14)	-	-	-
Withdrawn/depressive	T0	9.92 (2.40)	2.33 (2.27)	-4.167	.000
	T12	7.29 (4.11)	**-**	**-**	**-**
Somatic complaints	T0	7.00 (3.32)	3.75 (3.07)	-2.307	.021
	T12	5.00 (3.56)	**-**	**-**	**-**
Social problems	T0	9.38 (5.09)	2.83 (2.48)	-3.436	.001
	T12	7.29 (6.37)	-	-	-
Thought	T0	9.61 (4.33)	4.00 (3.98)	-2.927	.003
	T12	5.29 (4.27)	-	-	-
Attention	T0	12.00 (2.48)	6.25 (3.93)	-3.605	.000
	T12	9.57 (3.69)	-	-	-

Rulebreak	T0	7.31 (4.70)	2.67 (2.46)	-3.032	.002
	T12	6.43 (7.32)	-	-	-
Aggressive	T0	13.23 (4.19)	8.17(3.69)	-2.760	.006
	T12	8.43 (6.13)	-	-	-
SUMMARY SCALES					
Internalizing Pr.	T0	32.77 (7.58)	13.25 (9.38)	-3.707	.000
	T12	24.29 (15.11)	-	-	-
Externalizing Pr.	T0	20.54 (7.56)	10.83 (5.54)	-2.996	.003
	T12	14.86 (13.17)	-	-	-
Total problems	T0	89.92 (17.96)	42.08 (23.05)	-3.971	.000
	T12	66.43 (38.30)	-	-	-
DSM-ORIENTED SCALES					
Affective Dis	T0	16.15 (4.32)	4.33 (3.70)	-4.034	.000
	T12	10.71 (7.32)	-	-	-
Anxiety Dis	T0	6.38 (3.15)	3.25 (2.14)	-2.410	.016
	T12	4.715 (3.86)	-	-	-
Somatic problems	T0	4.15 (2.64)	2.33 (2.19)	-1.784	.074
	T12	3.00 (2.31)	-	-	-
ADHD	T0	7.54 (2.07)	5.25 (3.44)	-1.813	.070
	T12	6.71 (2.93)	-	-	-
Oppositional	T0	5.85 (2.07)	3.83 (1.90)	-2.150	.032
	T12	4.86 (2.61)	-	-	-
Conduct	T0	6.92 (4.70)	2.00 (2.00)	-3.229	.001
	T12	4.43 (4.82)	-	-	-
Obs_Compul	T0	8.23 (2.45)	5.63 (5.73)	-2.096	.036
	T12	4.16 (3.43)	-	-	-
Post_Traum	T0	17.54 (3.69)	7.91 (5.85)	-3.345	.001
	T12	11.17 (6.46)	-	-	-
Positive Qual	T0	15.77 (7.63)	18.91 (5.20)	-1.016	.310
	T12	16.83 (4.83)	-	-	-

Tab. 15 C-GAS score in the two groups at intake (T0) and after 12 months

	Depressed group (n=13) M (SD)	Healthy group (n=12) M (SD)	Z	p
T0	5.15 (1.14)	0	-4.525	.000
T6	4.56 (1.13)	-	-	-
T12	4.14 (1.57)	0	-4.111	.000

Tab. 16 CGI- Severity score in the two groups at intake (T0) and after 12 months

	Depressed group (n=13) M (SD)	Healthy group (n=12) M (SD)	Z	p
T0	4.54 (1.20)	1.00 (0.00)	-4.522	.000
T12	3.88 (1.80)	1.00 (0.00)	-3.806	.000

Tab. 17 MASC score in the two groups at intake (T0) and after 12 months

		Depressed group (n=13) M (SD)	Healthy group (n=12) M (SD)	Z	p
Physical Symptoms	T0	16.23 (5.73)	11.50 (5.25)	-1.880	.060
	T12	11.67 (6.98)	-	-	-
Harm Avoidance	T0	12.38 (4.66)	14.83 (2.48)	-1.253	.210

	T12	10.33 (5.71)	-	-	-
Social Anxiety	T0	16.23 (8.48)	11.33 (3.87)	-1.364	.173
	T12	13.33 (9.00)	-	-	-
Separation/ panic	T0	4.38 (4.52)	6.50 (3.58)	-1.940	.052
	T12	4.00 (3.52)	-	-	-
Total Score	T0	47.38 (21.89)	44.17 (8.95)	-0.082	.935
	T12	39.33 (20.61)	-	-	-
Anxiety Disorder Index	T0	14.69 (8.23)	10.58 (4.64)	-0.546	.585
	T12	11.83 (5.78)	-	-	-

Tab.18 CDI scores in the two groups at intake (T0) and after 12 months

		Depressed group (n=13) M (SD)	**Healthy group (n=12) M (SD)**	**Z**	**p**
Emotional tone	T0	9.09 (3.59)	1.58 (1.62)	-3.694	.000
	T12	5.29 (3.15)	-	-	-
Worthlessness	T0	7.45 (4.06)	3.42 (2.23)	-2.576	.010
	T12	5.71 (4.31)	-	-	-
Social relationships	T0	9.18 (4.79)	3.00 (2.04)	-3.156	.002
	T12	6.71 (3.55)	-	-	-
Total Score	T0	25.82 (11.31)	8.00 (4.84)	-3.698	.000
	T12	17.57 (10.16)	-	-	-

Tab. 19 HAM D score in the depressed group (n=13) at intake (T0) and after 12 months

	T0	T12
M (SD)	18.62 (6.87)	14.67 (8.33)

Tab. 20 SCID scores in the depressed group (n=13) at intake (T0) and after 12 months

		M (SD)
Avoidant	T0	3.90 (1.79)
	T12	3.00 (2.83)
Dependent	T0	3.00 (1.49)
	T12	1.50 (1.30)
Obsessive Compulsive	T0	4.90 (1.66)
	T12	3.50 (0.58)
Oppositive	T0	5.70 (1.42)
	T12	3.75 (2.99)
Depressive	T0	5.60 (1.95)
	T12	2.75 (2.36)
Paranoid	T0	4.40 (1.84)
	T12	2.75 (2.75)
Schizotypal	T0	4.30 (2.54)
	T12	2.00 (2.71)
Schizoid	T0	3.90 (1.20)
	T12	3.00 (2.16)
Histrionic	T0	0.90 (1.20)
	T12	0.50 (0.57)
Narcisistic	T0	4.90 (2.56)
	T12	5.50 (4.51)

Borderline	T0	10.10 (3.66)
	T12	5.00 (3.46)
Antisocial	T0	3.80 (3.19)
	T12	2.25 (2.18)

Tab. 21 SadPerson scores in the two groups (n=13) at intake (T0) and after 12 months

	Depressed group (n=13) M (SD)	Healthy group (n=12) M (SD)	Z	p
T0	4.23 (1.48)	0.67 (0.49)	-4.151	.000
T12	4.38 (2.26)	0.25 (0.45)	-3.778	.000

Comparing test scores between depressed and non-depressed subjects at T0, there are significant differences in CBCL, YSR, C-GAS, CGI-S, CDI ($p < 0.05$) and SadPerson ($p < 0.001$) scores. No significant differences were found between the gropus in the MASC scores ($p > .05$).

Scores obtained at psychological tests in the two groups, filled in both by the adolescents and their parents, were subsequently compared on the basis of specifical variablies, as the presence of suicidal ideation, suicide attempt, and traumatic experiences in the patients' clinical history.

Considering the intragroups comparison with respect to the presence of suicidal ideation, in the depressed group, for mother CBCL at T0, there is a statistical significant difference in social competences ($Z=-1.945$, $p=.052$) and thought problems ($Z=-1.955$, $p=.051$). Considering SA, there are no significative differences ($p>.05$); while considering the presence of trauma in personal history, there are statistical significative differences for somatic complaints ($Z=-2.177$, $p=.029$), internalizing problems ($Z=-2.130$, $p=.033$) and post traumatic disorders ($Z= -2.27$, $p=.026$). No differences were found in the healthy group ($p>.05$).

Performing the same analysis for fathers' CBCL at T0, we can find statistical significant differences for DOP score in depressed group, with respect to the history of trauma ($Z=-1.961$, $p=.050$).

The intragroups comparison with respect to the presence of suicidal ideation, for T0 YSR in the depressed group, highlithed statistical significant differences in anxious depressed item ($Z=-2.042$, $p=0.41$), internalizing problems ($Z=-2.054$, $p=.040$) and anxiety problems ($Z=-2.054$, $p=.040$). Considering the presence of SA, there are statistical significative differences for activities ($Z=-2.230$, $p=.026$), total competences ($Z=-2.292$, $p=.022$), anxious-depressed ($Z=-2.158$, $p=.031$) and anxiety problems ($Z=-2.669$, $p=.008$). Considering the history of trauma, there are statistical significative differences for rulebreak ($Z=-2.384$, $p=.017$) and conduct disorders ($Z=-2.037$, $p=.042$).

There are not statistically significant differences with respect the presence of suicidal ideation, SA and history of trauma in CDI and HAM-D scores ($p>.05$).

In the comparison with respect to the presence of suicidal ideation, in the depressed group,

there is a statistical difference in CGI-S at T0 (Z=-2.014, p=.044), while with respect to traumatic events in personal history there is difference for CGI-I (Z=-1.908, p=0.56). No other differences were found.

The intragroups comparison with respect to the presence of suicidal ideation, as regards the depressed group, for MASC at T0, shows statistical significant differences in the harm avoidance (Z = -2.037, p = .042), social anxiety (Z = -2.119 , p = .034) and total score scales (Z = -2.031, p = .042). As for the presence of SA, there are statistical significant differences in physical simptoms (Z=-1.945, p=.052), harm avoidance (Z=-2.080, p=.038) and total score scale (Z=-2.289, p=.022). No differences were found for the presence of traumatic events in personal history.

4.2 Longitudinal Analysis

Observing the trend over time of BDNF plasma levels in subjects suffering from depression we can find after 3 months of therapy a reduction of blood levels of BDNF in 3 subjects (30%) and an increase in 4 subjects (40%). In 1 case there is a stability of the values (Fig. 19). At 6 months,3 subjects present an increase in BDNF levels, while in 3 others situation there is a reduction. At 12 months it is possible to note a rather disomogeneous trend. These changes do not reach statistical significance as highlighted by the application of the Friedman test (p>.05). The analysis performed through Wilcoxon Test considering the two separate intervals (T0-T6 and T6-T12) highlighted the same results (p>.05).

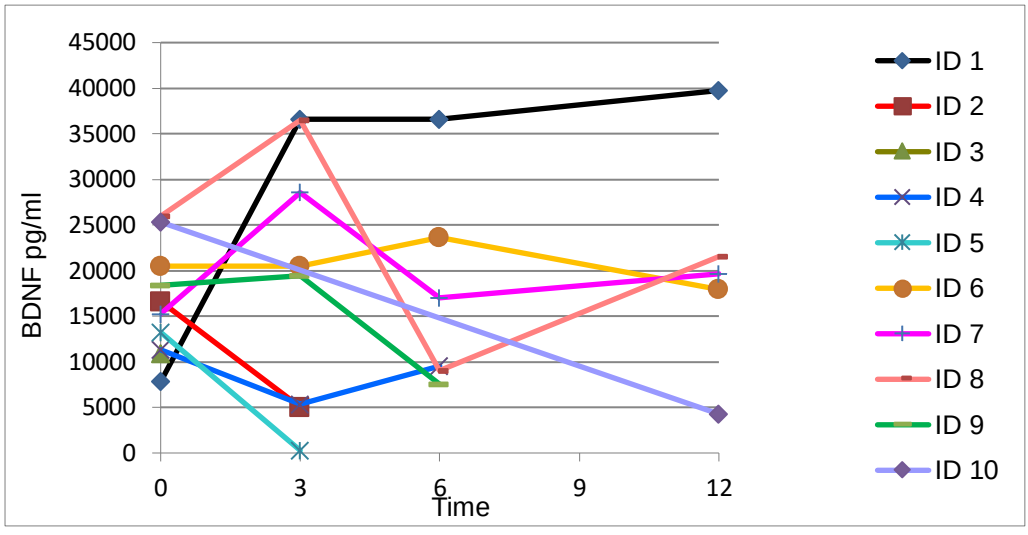


Fig.19: Plasmatic BDNF trend at 3, 6 and 12 months in depressed subjects (5 samples at 12 months, 6 at 6 months, 8 at 3 months, 10 at intakeavailable)

Evaluating the trend over time of plasma BDNF levels in healthy subjects it is notable a homogeneous general trend of reduction at 6 months and a subsequent increase at 12 months.

Only in 3 subjects (ID 101, ID 106, ID 111) there is an increase at 6 months and a subsequent decrease at 12 months (Fig.20).

These changes in trend over 12 months do not reach statistical significance as highlighted by the application of the Friedman test ($p>.05$). The analysis performed through Wilcon test considering the two separate intervals (T0-T6 and T6-T12) produced the same results ($p>.05$). In the statistical analysis, ID12 was eliminated as BDNF values appeared outside the standard curve.

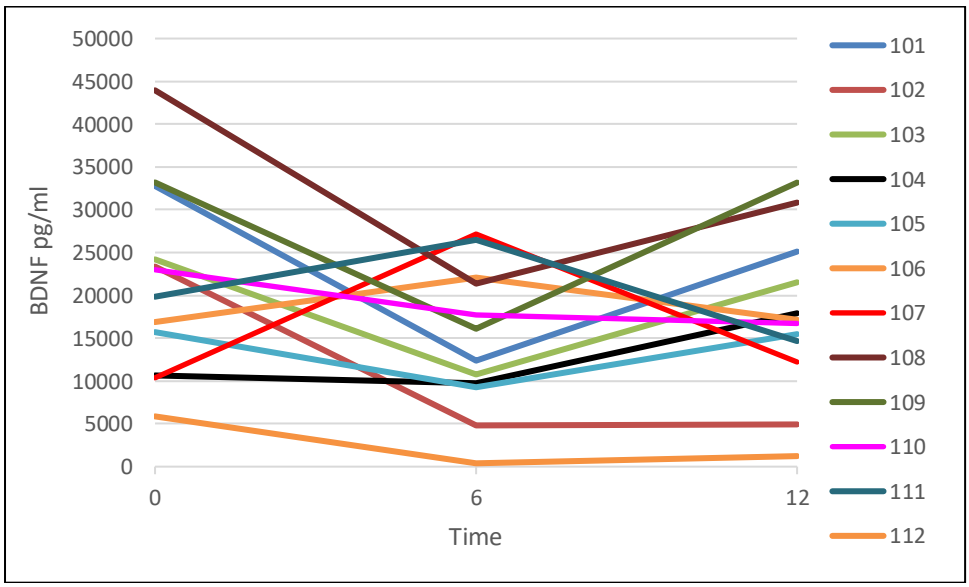


Fig.20: Plasmatic BDNF trend at 6 and 12 months in healthy subjects

IL-1β levels trend over time in subjects suffering from depression is represented in Figure 21. Many data are not represented because under the standard curve of measure.

These changes in the interval T0-T12 can be considered only from a speculative point of view, since the numerosity of the sample do not permit the application of the Friedman test.

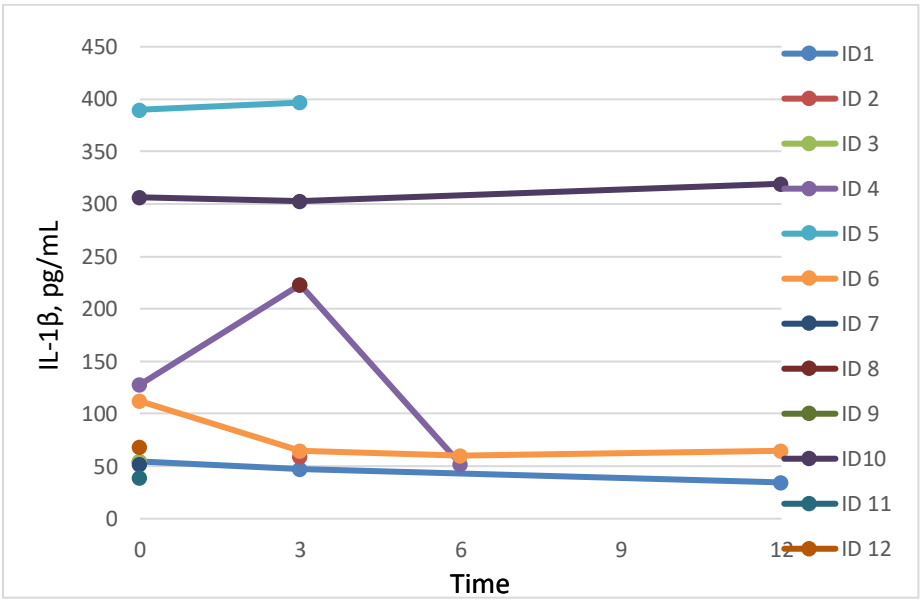


Fig.21: Il-1β trend in depressed subjects at intake and after 3-6-12 months. Many data missing because under standard curve.

The trend of IL-1β over time in healthy subjects is represented in Figure22. Many data are not represented because under the standard curve of measure. Also in this case, changes do not reach statistical significance as highlighted by the application of the Friedman test (p>.05). The analysis performed through Wilcoxon test considering the two separate intervals (T0-T6 and T6-T12) highlighted the same results (p>.05).

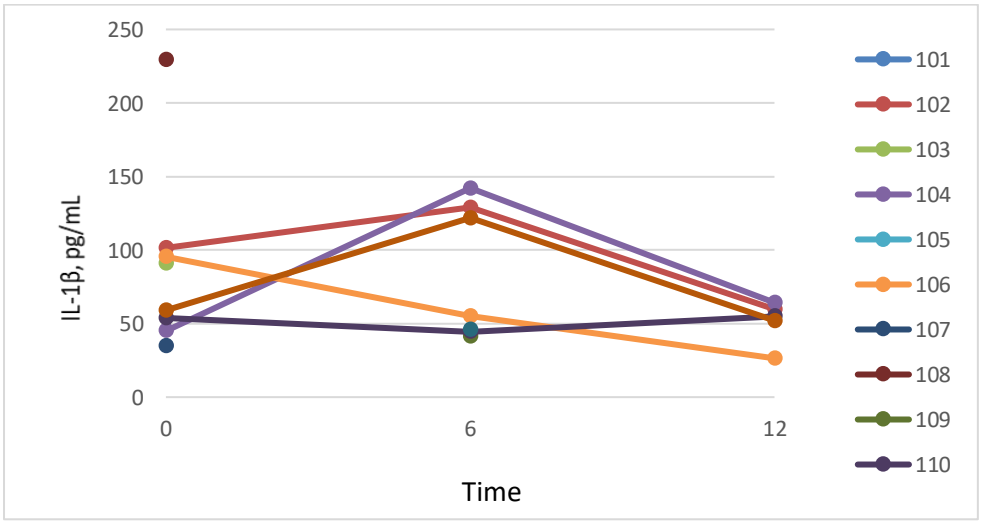


Fig. 22: Il-1β trend in healthy subjects at intake and after 6 and12 months. Many data missing because under standard curve.

As for BMI trend over the 12 months evaluated: the application of Wilcoxon Test to this interval in both the groups do not highlight any statistical significance (p>.05).

As for psychodiagnostic evaluation at T12 in depressed patients, statistical values are reported

in Table 22 and 23.

Tab. 22 Achenbach's tests in depressed group at T12

Test	Subscales	Z	p
CBCL MD			
	Activities	-1.069	.285
	Social	-0.816	.414
	School	-0.447	.655
	Total	-1.604	.109
	Anxiety/depressive	-1.633	.102
	Withdrawn/depressive	-1.604	.109
	Somatic complaints	-1.732	.083
	Social problems	-1.633	.102
	Thought	-1.633	.102
	Attention	-1.604	.109
	Rulebreak	-1.604	.109
	Aggressive	-1.604	.109
	Internalizing Pr.	-1.604	.109
	Externalizing Pr.	-1.604	.109
	Total problems	-1.604	.109
	Affective Dis	-1.604	.109
	Anxiety Dis	-1.604	.109
	Somatic problems	-1.414	.157
	ADHD	-1.342	.180
	Oppositional	-1.633	.102
	Conduct	-1.604	.109
	Sluggish_Cogn.	-1.633	.102
	Obs_Compul	-0.816	.414
	Post_Traum	-1.604	.109
CBCL PD			
	Activities	-1.069	.285
	Social	-0.535	.593
	School	-1.000	.317
	Total	-0.535	.593
	Anxiety/depressive	-1.342	.180
	Withdrawn/depressive	-1.069	.285
	Somatic complaints	-1.633	.102
	Social problems	-0.000	1.000
	Thought	-1.633	.102
	Attention	-1.604	.109
	Rulebreak	-1.069	.285
	Aggressive	-1.604	.109
	Internalizing Pr.	-1.069	.285
	Externalizing Pr.	-1.342	.180
	Total problems	-1.604	.109
	Affective Dis	-1.604	.109
	Anxiety Dis	-1.069	.285
	Somatic problems	-0.000	1.000
	ADHD	-0.447	.655

	Oppositional	-1.604	.109
	Conduct	-1.342	.180
	Sluggish_Cogn.	-1.633	.102
	Obs_Compul	-0.604	.109
	Post_Traum	-1.604	.109
YSR			
	Activities	-1.192	.233
	Social	-0.271	.786
	School	-1.089	.276
	Total	-0.105	.917
	Anxiety/depressive	-0.848	.396
	Withdrawn/depressive	-1.103	.270
	Somatic complaints	-0.597	.551
	Social problems	-1.378	.168
	Thought	-1.951	.051
	Attention	-.940	.347
	Rulebreak	-0.106	.916
	Aggressive	-0.677	.498
	Internalizing Pr.	-1.185	.236
	Externalizing Pr.	-0.406	.684
	Total problems	-0.845	.398
	Affective Dis	-1.016	.310
	Anxiety Dis	-0.595	.552
	Somatic problems	-0.426	.670
	ADHD	-0.947	.344
	Oppositional	-0.180	.914
	Conduct	-0.210	.833
	Obs_Compul	-2.032	**.042**
	Post_Traum	-1.753	.080

Tab. 23 Psychodiagnostic tests in depressed group at T12. Wilcoxon Test.

TEST		Interval	Z	p
C-GAS		**T0-T6**	-2.121	**.034**
		T6-T12	-0.378	.705
CGI-Severity		**T0-T12**	-1.512	.131
CGI -I			-1.732	.083
CGI-E			-1.473	.141
MASC				
	Physical Symptoms		-0.674	.500
	Harm Avoidance		-2.023	**.043**
	Social Anxiety		-1.084	.279
	Separation/panic		-1.753	.080
	Total		-0.944	.345
	Anxyety Dis Index		-1.214	.225
CDI		**T0-T12**		
	Emotional Tone		-1.054	.292
	Worthlessness		-.105	.916
	Social relationships		-.526	.599

	Total		-.762	.446
Hamilton		**T0-T12**	-2.201	**.028**
SCID				
	Avoidant		-0.816	.414
	Dependent		-0.816	.414
	Obsessive-Compulsive		-1.000	.317
	Oppositive		-1.134	.257
	Depressive		-1.069	.285
	Paranoid		-1.069	.285
	Schizotypal		-0.447	.655
	Schizoid		-0.447	.655
	Histrionic		0.000	1.000
	Narcisitic		-1.342	.180
	Borderline		-1.069	.285
	Antisocial		-1.000	.317
SadP			-0.378	.705

The application the Friedman test to longitudinal C-GAS evaluation (T0-T12) do not highlight statistically significant changes of the scores in time (p>.05).

In depressed group there are no statistically significative differences between CBCL and YSR test completed at the intake and after 12 months (p>.05). No differences were also found for CGI, total score of the MASC, SCID, SadPerson (p>.05). C-GAS score from T0 to T6 present a statistically significant change (Z=-2.121, p=.034). From a merely qualitative point of view, we observe that Total scores in the standardized tests (CBCL, YSR, MASC, CDI, CGI, C-GAS) completed by depressed subjects tend to reduce, showing a clinical improvement from T0 (before treatment) to T12 (after treatment), even in absence of a statistical significance.

In healthy group no differences were found with the application of Wilcoxon test to C-GAS score (T0-T12) (p>.05) and to CGI-S, CGI-I, CGI-E (p>.05). SadPerson score, on the contrary, showed a statistically significant improvement during the periods considered (Wilcoxon test; Z=-2.236, p=.025).

4.3 Correlation

BDNF, IL-1β and BMI

In depressed patients, the application of Spearman's Rho Coefficient does not highlight statistically significant correlation between BDNF and IL-1β values (p>.05), however from a merely descriptive point of view values show opposite directions. At T0 BDNF is reduced in depressed subjects, while IL-1β presents higher levels.

In healthy subjects, the levels of IL-1β correlated to BDNF levels (r=.714, p=.047), both measured at T0.

BDNF and IL-1 do not correlate at T3 in depressed group (p>.05), not at T6 and T12 in both

the groups (p>.05). The application of Spearman's Rho Coefficient do not highlight statistically significant correlations between BDNF levels and BMI (p>.05), neither between IL-1β and BMI (p>.05) in both of the groups at T0. In depressed group, BDNF does not correlate to platelets number at T0, too (Spearman's Rho Coefficient, p>.05).

BDNF

To understand whether BDNF levels were related to the severity and to some dimensions of the disease, the relationships between protein levels and psychopathological test scores were assessed in both the groups.

The application of Spearman's Rho Coefficient shows that at T0 higher BDNF levels are associated to higher school skills scores reported by mothers (r= .695, p= .026) in CBCL. A statistically significant inverse correlation between BDNF levels and social problems at T0 (r = -800, p = .005) was also found. Lower levels of social problems are associated with higher levels of BDNF, as reported by the mothers. Others statistically significant inverse correlation were found between: BDNF levels at T0 of depressed subjects and problems of attention (r=-.695, p=.026), rulebreak behaviors (r=-.793, p=.006), aggressive behaviors (r=-732, p=.016), externalizing problems (r=-.754, p=.012), total problems (r= -.794, p=.006), ADHD (r=-.757, p=.001) and conduct disorder score (r=-.760, p=.011) reported by mothers' CBCL.

At T12, higher BDNF levels (T12) are associated to higher scores to anxious/depressed item of CBCL completed by mothers in the same time (r=.900, p=.037). In the same period, higher BMI are associated to higher scores to social competence item of CBCL completed by mothers at T12 (r=.829, p=.042).

In the healthy group, the application of Spearman's Rho Coefficient shows that at T0 higher BDNF levels are associated to lower obsessive-compulsive disorder scores reported by the mothers (r=-.623, p= .031) in CBCL. In this group BMI at T0 is correlated to thoughts problems reported in mothers' CBCL (r= .743, p= .006) at T0.

As for CBCL completed by fathers of depressed subjects: at T0 the application of Spearman's Rho Coefficient shows that at T0 higher BDNF levels are associated to higher school skills scores reported (r= .878, p= .004) in CBCL. A statistically significant inverse correlation between BDNF levels and social problems at T0 (r = -795, p = .018) was also found.

In healthy group, the application of Spearman's Rho Coefficient shows that at T0 higher BDNF levels are associated to lower withdrawal/depressed scores reported by fathers (r= -.606, p= .037) in CBCL T0. Inverse correlation was also found between BDNF levels at T0 and total problems at CBCL completed by fathers of healthy children (r=-.596, p=.041). At T0, BMI correlate to affective disorders (r=.644, p=.024), anxiety disorders (r=.752, p=.005) and slugghish cognitive tempo item (r=.583, p=.047) reported to fathers' CBCL at T0 in

healthy group.

The application of Spearman's Rho Coefficient shows in the depressed group that at T0 BDNF levels inversely correlate to social competences (r=-.839, p=.002), total competences (r=-.802, p=.005), aggressive behaviors (r=-.828, p=.003), externalizing problems (r=-.663, p=.037), and positive qualities (r=-.663, p=.037) reported by depressed adolescents to T0 YSR. In the same analysis BMI inversely correlate to somatic complaints (r=-.714, p=.014), somatic problems (r=-.762, p=.006), obsessive compulsive problems (r=-.729, p=.011). At T6, BDNF inversely correlates to somatic complaints (r=-.821, p=.089), to social problems (r=-.900, p=.037) and affective disorders (r=-.975, p=.005) reported at YSR T12. BDNF levels at T12 do not correlate to any score at T12 YSR.

In YSR completed by healthy at T0 no correlations were found for BDNF. BMI measured at T0 correlate to rulebreak behaviors (r=.865, p=.012), externalizing problems (r=.786, p=.036) and oppositive defiant disorders (r=.811, p=.027) reported by youth at 12 months YSR.

No correlations were found between BDNF levels and C-GAS or CGI at any time (p>.05).

BMI at T0 inversely correlate to ADI score at T0 MASC in depressed group (r=-.641, p=.034). No other correlations were found with subscore of MASC (p>.05).

In depressed patients, BDNF at T6 inversely signiticatively correlate to HAM-D scores at T12 (r=-.900, p=0.37). No other correlations were found (p>.05).

The application of Spearman's Rho Coefficient show in the depressed group that at T6 BDNF levels inversely correlate to the worthlessness subtest at T12 (r=-.900, p=0.37). No correlations were found in healthy group (p>.05).

No correlations were found between BDNF levels and SCID or SadPerson scores in both the groups (p>.05). BDNF levels do not significantly differ with respect to the presence/absence of suicidal ideation, SA, or traumatic events in the subjects' past history (p>.05).

IL-1β

The same analysis were performed for IL-1β and scores in psychodiagnostic tests with the application of Spearman's Rho Coefficient.

In the depressed group, statistically significant inverse correlations were found at T0 between IL-1β levels and attention problems (r = -.766, p = .016), aggressive behaviors (r = -.812, p = .008), externalizing problems (r=-.720, p=.029), total problems (r=-.767, p=.016), ADHD (r=-.874, p=.002), oppositional defiant disorder (r=-.783, p=.013), conduct disorder scores (r=-.717, p=.030) in CBCL completed by mothers (T0). In the same analysis the application of Spearman's Rho Coefficient shows that higher IL1-β levels are associated to higher somatic problems reported by the mothers (r= .819, p= .007) in CBCL at T0.

In the halthy group, IL-1β levels at T0 are inversely correlated with withdrawn/depressive

score at mothers' CBCL (r=-.782, p=.022). In this group IL-1β levels at T6 are inversely correlated with somatic complaints score at mothers' CBCL at T0 (r=-.810, p=.027).

As for CBCL completed by fathers of depressed subjects: at T0 the application of Spearman's Rho Coefficient shows statistically significant inverse correlations between IL-1β levels and social problems at T0 (r = -.800, p = .031), problems of attention (r=-.805, p=.029), rulebreak behaviors (r=-.786, p=.036), affective disorders (r=-.764, p=.046), ADHD (r=-.811, p=.027), conduct disorders (r=-.829, p=.021).

At T0, IL1 levels inversely correlate to social problems reported at CBCL T0 by fathers of healty subjects(r=-.749, p=.033).

The application of Spearman's Rho Coefficient in the YSR completed by depressed group that at T0 does notshow any correlation to IL-1β (p>.05).

In YSR completed by healthy subjects at T0 ,IL-1β correlate to positive qualities (z=.927, p=.003).

No correlations were found at any time for IL-1β and C-GAS, MASC, HAM-D, SCID and SadPerson scores (p>.05).

IL-1β at T0 correlate inversely to CGI severity score (z=-.900, p=.037) and CGI efficacy score (z=-.900, p=.037) reported at T12 in the depressed group.

The application of Spearman's Rho Coefficient show in the depressed group that at T0 IL-1β levels inversely correlate to the subtest emotional tone at T12 (r=-.949, p=0.51). No correlation were found in healthy group (p>.05).

IL-1β levels do not show statistically significant differences considering the presence/absence of suicidal ideation, SA, or traumatic events in the subjects' past history (p>.05).

5. Discussion

The present study aimed to evaluate the usefulness of the dosage of these two molecules in psychiatric diagnosis, monitoring and as early peripheral biomarkers of disease in pediatric patients suffering from depression. BDNF levels, IL-1β and other indexes were measured in the two groups of subjet considered (depresses vs healthy) at intake (T0), after 6 months (T1) and after one year (T2). Moreover, psychological tests were administered both to the adolescents and to their parents. As far as it concerns BDNF levels during T0, we found that young, non-medicated, adolescents affected by mood disorders with depressive features showed lower plasma levels of mature BDNF and higher plasma levels of IL-1β at the first access to psychiatric services, when compared with adolescents without psychiatric disorders. As far as it concerns T6 and T12, depressend patients present BDNF levels higher than healthy

while IL-1β trend show a reduction at T6.

These results are of interest and allow for some speculative observations, despite the lack of statistical significance. It is here assumed that the absence of significant results may be due to the small sample size included in the study.

Considering BDNF, findings are in agreement with previous studies on adults (Brunoni et al., 2008; Sen et al., 2008; Bocchio-Chiavetto et al. 2010; Molendijk et al., 2014) and with three out of only four studies available in pediatric populations (Pandey et al., 2010; Sasaki et al., 2011; Pallavi et al., 2013).

Comparing to the four studies on developmental age subjects, the present work appears relevant for the precise psychiatric characterization of the recruited patients, all preadolescents and adolescents with pathology at onset, with analytical collection of longitudinal prospective data, while literature consist exclusively of transversal studies. The four pediatric studies currently available are not comparable with each other and involve populations that are not characterized by an anamnestic and psychopathological point of view. In Pandey et al. (2010), the BDNF level was performed on platelets, that are one of the main site of protein accumulation, highlighting that in patients with depressive disease, not only there could be a reduced or altered release of the factor from serum platelets (as described in the adult by Karege et al., 2005; Lee et al., 2009), but also reduced storage of neurotrophin. In this study, 14 subjects with mood disorders were studied, without distinguishing partecipants based on the polarity of the episode and with a poor clinical characterization of the sample. The study by Pandey et al. (2010) concluded however for a reduction of neurotrophin in adult and pediatric subjects suffering from depression, without correlation with the severity of the disease. The work was of considerable value because it compared adult and pediatric populations, as well as it associated the dosage of BDNF mRNA of lymphocyte, with an evaluation of any differences related to gender, but it remains an isolated research not comparable with other studies for the method used (dosage in platelets).

In Sasaki et al., 2011, the groups considered are more numerous (30 subjects), but also in this study patients are not characterized, with the recruitment of subjects with different histories, different durations of disease and with a current pharmacological treatment. The authors found a reduction in serum protein levels of BDNF in depressed patients, limited to males, with a correlation with the duration of the disease. In Pallavi et al. (2013), authors explored the dosage of different neurotrophins (BDNF, NGF, NT-3, GDNF) on a large sample of depressed subjects (n = 84) and healthy adolescents. Inclusion criteria for the recruitment of the participants were not clearly defined. Subjects, indeedm may have had different history of psychotropic therapy and duration of the disease. The authors concluded for a

homogeneous reduction of peripheral neurotrophic levels in depressed patients, the most sensitive of which seem to be BDNF, without correlations with any anxiety comorbidities.
In Tsuchimine et al. (2015), the serum dosages of BDNF, polyunsaturated fatty acids and folates were analysed comparing depressed subjects (n = 24) and healthy peers (n = 26). The recruited population was characterized by a diagnosis of naïve MDD. The authors found a reduction in the levels of folate and polyunsaturated fatty acids in depressed patients, while no significant difference in serum BDNF was found; indeed the depressed BDNF levels appear to be even slightly higher.
Therefore, the study presented represent the first study in pediatric age on a sample of subjects at the first diagnosis and without using psychiatric therapies, well psychodiagnostic characterized and compared with a healthy sample, also investigated for any subclinical psychiatric pathology.
To our knowledge, this is the first pediatric study that aims at dosing a neurotrophin in its mature form, established to have a central role in neurogenesis, unlike the proBDNF precursor involved in apoptotic pathways (Hashimoto, 2014).
The preliminary results seem to support "the neurotrophic hypothesis" and "the neuroplastic hypothesis" of depression: the lack of neurotrophic factors regulating brain plasticity could explain the pathophysiology of DD in this delicate age group in which the destruction of obsolete networks in favor of new networks supportive of adolescent changes would be altered. In particular, it is known that the prefrontal cortex reaches its structural maturity only in adolescence; therefore precocious stressors could determine an altered expression of BDNF in an immature substrate, resulting in morpho-functional alterations of the development (Bricolo et al., 2010). According to this, it is interesting to note that in the present study higher BDNF levels at T0 correlate with lower social and behavioural problems (reported by mothers): both social competences and behaviours are functionally regulated by anterior cortical areas (the social brain; Adolphs, 2009) that are precisely the same areas involved in neurogenesis in adolescence. This is also one of the first studies that dosed a proinflammatory cytokine in a pediatric population with DD. In our sample, plasma levels of IL-1β are higher in depressed patients compared with those without psychiatric disorders at the first access to psychiatric services, even though with no statistical significance. This finding is in line with some studies presented in literature (Miklowitz et al, 2016; Henje Blom et al., 2012). Other studies are very heterogeneous as for clinical population, tests used and conclusions. In Spindola et al., 2017, authors studied whole-blood IL-1β mRNA levels, which were found to be down-regulated in MDD patients. The final aim of the paper was to find a direct role of childhood maltreatment in DD. Patients are diagnosed using CBCL only. Blood collection

and psychiatric assessment were performed on different days, introducing a possible bias. In Miklowitz et al, 2016, the groups evaluated (BD patients and MDD patients) were well-characterized using suitable tests. However, authors included patients undergoing active drug treatments. Clinical psychiatric evaluation was well performed in Amitai et al., 2016, but in this study authors compared SSRI responders to non responders, without considering a healthy group. They also excluded subjects with history of trauma. In Scola et al. (2016), the group of patient considered suffer of BD, so one of the main inclusion criteria was the presence of a maniac episode. In our study, all the subjects were affected by depressive disorders, sometimes with mixed symptomatology. None of the patients considered presented maniac episode. In Henje Blom et al., 2012 and in Brambilla et al., 2004, clinical populations are slightly characterized from a psychiatric point of view. Gabbay et al., 2009 focused on suicidal dimension of MDD, founding higher IFN-γ and TNF-α levels in suicidal adolescents with MDD, without differences as for IL-1β.

Our results in a well characterized non-medicated psychiatric clinical population seem to support a role of IL-1β in these patients. Cytokine production may represent an early sign of immune dysregulation during the course of DD in the critical period of life cycle that adolescence is.

It is important to note, that the definition of DD is a crucial aspect. Mood disorders in developmental age are chronic conditions characterized by the recurrence of mood episodes. The first episode often does not permit a clear diagnostic collocation since they can evolve in BD or for mixed characteristics of the episode in these delicate ages of life. As for the literature on developmental age, even in the present study a clear distinction between patients with major depression and patients with mixed symptomatology was not possible, with frequent finding of irritability as symptomatology of onset. However, the administration of a set of standardized tests allowed a quantification of the various depressive components and other dimensions, including confounding effects. BD were firstly excluded, contrary to what was usually found in literature (for the difficulty to differentiate BD patients from depressed ones in pediatric age, they are frequently grouped together).

When referring to MDD, as defined by the DSM-IV or DSM-5 criteria, diagnosis is based on the observation of a cluster of symptoms for adequate length of time. We used well-validated structured interviews and rating scales, as well as experienced clinical raters and rigorous enrollment criteria to assure that MDD was the primary diagnosis of sufficient severity and length to potentially affect BDNF and IL-1β levels.

Nevertheless, it is accepted by researchers that the same diagnosis of MDD comprises a wide range of possible clinical phenotypes, as permutations of the nine diagnostic symptoms (Fried

and Nesse, 2014; Cassano et al., 2017) and that similar clinical presentations could result from different pathophysiological processes (Moylan et al., 2013).

Discussing the results in detail, we obesrved the presence of psychiatric pathology in family history of the majority of depressed patients (90%) and particularly in the maternal line (54%) to confirm the importance of a possible genetic transmission and the centrality of the mother-child bond as a delicate neuroprotective system or vice versa as an element of psychopathological risk (Hayden et al., 2013). Family conflicts are also frequently present. The group of depressed people differs rom the healthy group for the presence of scholastic problems (poor scholastic profit), traumatic events, social withdrawal and the presence of sleep disorders (especially in falling asleep). Eighty five percent of depressed patients complain insomnia with suffering and impairment of Quality of Life. It is well known from literature that sleep disorders represent a strong risk factor for suicide and DD recurrence (Dell'Osso et al., 2010). Furthermore, the hyperactivation of the HPAA frequently observed in depressed patients, appears to be related to sleep dysregulation (Dell'Osso et al., 2010). An interesting study on rats suggested a link between synaptic plasticity and sleep homeostasis, identifying BDNF as the main molecular mediator of this relationship (Huberetal., 2007).
As for traumatic events, it has been reported that the chronic activation of the HPAA induced by stress determine the death of cells in the hippocampal CA3 area and the suppression of neurogenesis in the dentate gyrus, through a dramatic reduction of BDNF expression in these areas. The adrenal steroids would also damage the hippocampal neurons depleting them of glucose, making them be particularly sensitive to increments of excitatory neurotransmitters such as glutamate (Duman et al., 1997).
To obtain more reliable data, patients with clear PTSD symptomatology were excluded in the study, mostly due to the evidences of a reduction of BDNF and increase of IL-1β when these diagnostic symptoms are present (Dell 'Osso et al., 2009; Lu et al., 2013).
The depressed group also differs on a greater use of alcohol and substances, in particular of cannabis.
Considering clinical parameters, the group of affected subjects does not differ significantly from the group of healthy subjects for BMI.
The plasmatic concentration of neurotrophin is not correlated to BMI. There are conflicting data in literature about the possible correlation between BDNF and BMI. Tsuchimine et al. (2015) and Pallavi et al. (2013) showed a relationship between these values, while other authors did not confirm this observation (Iughetti et al., 2011; Lommatzsch et al., 2005; El-Gharbawy et al., 2006). Neurotrophin and its TrkB receptor are in fact also expressed in the

hypothalamic regions considered important for the maintenance of body weight (Mowla et al., 2001; Tapia-Arancibia et al., 2004). Moreover, they can influence the regulation of the leptin signal and hence energy homeostasis (Xu et al.,2016),without any immediate changes after food intake (El-Gharbawy et al., 2006). Haploinsufficiency of BDNF gene is associated with reduction of peripheral blood levels of BDNF, hyperphagia and obesity. Furthermore, the mutation of TrkB receptor was described in children with obesity and developmental delay (Iughetti et al., 2011). As for the proinflammatory cytokine IL-1β, it induces secretion of other proinflammatory cytokines, inhibits pancreatic β-cell function, destroys β-cells, and promotes insulin resistance. Cruz-Mejía et al. (2018) found that IL-1β was positively related with the increase of BMI in a group of adult.

Our results seems to support the opposite way. Children suffering from depression present reduced levels of BDNF, increased levels of IL-1β and reduced BMI at intake (T0), although without statistical significance.

It is important to note that, in this study, after 12 months depressed patients gain body weight (+14%) more than healthy peers (+7%), even if the comparison doesn't reach statistical significance. It is well known that weight gain could be an adverse effect of second-generation antipsychotics (SGAs) treatment (antipsychotic-induced weight gain, AIWG), leading to an increase in frequency of metabolic syndrome and cardiovascular events. Younger patients and patients with a lower baseline BMI are most vulnerable to this effect with a greatest amount of weight gain within the first weeks of treatment (Pillay et al., 2018;Musil et al., 2015).

In our sample, the majority of subjects with depression were treated with atypical antipsychotics (SGAs). This aspect could be influenced on one hand by the risks associated with the administration of antidepressants in young age, as the possibility of hypomanic activation which could potentially increase the risk of suicide (Stone, 2014 ; Gibbons et al., 2014). On the other hand, the choice of using SGAS is supported by the evidence that most of the patients that are diagnosed with mood disorders presented marked symptoms of irritability. Using SGAs permits to target some aspects of the subjects' functioning, as well as to stabilizing mood to particularly target mixed state symptoms (Stahl et al., 2017). However, in this clinical group SGAs showed a reduced efficacy on depressive symptomatology with a potential depressogenic effect, suggesting the need for a different clinical approach, with the use of an antidepressant SSRI as soon as the symptomatology is stabilized (best practice). Unfortunately, actually treatment in depressed and anxious children and adolescents involves trial and error and it can take months to identify an effective therapy for the 30–40% of children and adolescents who do not respond to an initial antidepressant (Amitai et al., 2015).

In light of this, having an ematic biochemical data to support clinical reasoning and individualized therapeutic choices represents an emergent need. Its importance is even more relevant if we consider children with other comorbidity that impede the use of psychodiagnostic tests (i.e. intellectual disability, ASD, severely compromised patients, uncompliant subjects).

All subjects involved in the present study were untreated at the time of recruitment, according to adult literature showing that antidepressive therapy (with SSRI, lithium or SGAs) seems to normalize levels of the BDNF in adult responders (Shen et al., 2014). In our small sample, there are heterogeneous trend in BDNF levels and patients were treated in a"naturalistic" way, so the effect of drugs on plasma neutrophin levels may not be reliable.

BDNF correlates with IL-1β only in healthy subjects at T0, showing contrasting results. From a merely descriptive point of view, in healthy subjects, during the 12 moths of observation, BDNF seems to reduce at T6 and then recuperate at T12 (even under the basal values), while IL-1β gradually reduces.

In depressed patients, the values of BDNF globally increase over time, while IL-1β presents a reduction at T6 and then recuperates until basal levels. Thus, from a speculative point of view, BDNF would seem to be a better pathology's marker, following the trend of clinical observations.

In contrast with literature, no correlation between BDNF or IL-1β and BMI were found in the study, so changes in the values cannot attributable to the variation in BMI.

Contrarily to literature (Begliuomini et al., 2007; Iughetti et al., 2011), the present study showed that plasma concentrations of BDNF are not correlated with platelet levels in the group of affected subjects.

Depressed patients arrived at services referred by the Emergency Room (50%) or through an outpatient visit (50%). In most cases (60%) psychiatric assessment evaluation was requested for other problematic issues (scholastic and relational difficulties, psychomotor agitation, somatic pain) that masked an important impairment of the functioning due to an underlying psychiatric disorder. In 5 cases the contact with the services was caused by self-harm behaviours (self-cutting or suicide attempts). In more than half of the cases (60%) patients were admitted to the Psychiatric Unit of the Department, requiring a period of hospitalization that lasted for an average of 17 days (median 9 days). In the rest of the cases, a strict outpatient monitoring was performed, with weekly or multi-weekly meetings with the patient and his/her parents, since there were no criteria for hospitalization (data compatible with average scores of 4 to SadPerson).

Psychopathological tests are confirmed to be useful diagnostic tools, statistically differing between subjects with depression and healthy peers at the intake (T0). Specifically, there is a significant difference in CBCL (both the one completed by the mother and the one completed by the father), YSR scores (for almost all the items), C-GAS, CGI-S, CDI and SadPerson ($p < 0.0001$) between depressed and non-depressed subjects at T0. Scores on the MASC scale do not statistically differ between the groups (except for separation/panic item). This data could highlight the presence of multiple and heterogeneous components of anxiety (with scores slightly subthreshold) in the healthy group as well.

In the group of depressed subjects, adolescents with higher levels of BDNF seem to have better school performances and less social problems (reported by both mothers and fathers homogeneously). Mothers reported also less problems of attention, rulebreaking and aggressive behaviours. This result is in line with studies that affirm that subjects with reduced peripheral BDNF levels present problems in executive functions with an improvement concomitant with a successful antidepressant therapy (Wagner et al., 2018). After 12 months mothers evidenced that who has higher levels of BDNF presents more anxious problems and that who presents higher BMI has more social competences.

In the group of healthy adolescents, higher levels of BDNF were associated with reduced level of depression, total problems (reported by fathers) and obsessive-compulsive problems (reported by mothers). Parents homogeneously reported that those with higher BMI, would present more psychological problems (thought problems, affective, anxiety).

Globally, having high levels of BDNF seems to have a protective role in some ways in both groups, while a higher BMI could be a risk factor in healthy, according to parents' perception. This last aspect is confirmed also by youth's perception (YSR): they reported more externalizing problems, like oppositive defiant or rulebreak behaviors, in who presented higher BMI at T0.

As for YSR of depressed subjects, they reported reduced social competencies and positive qualities in who has higher BDNF (contrary to what reported by parents) and less aggressive, externalizing, total problems in the same group.

In depressed group, YSR scores on social competences scale show opposite results compared to CBCL: this discordant data is easily explained by the tendency to reduced self-esteem of depressed person.

Depressed adolescents who present higher levels of BDNF at 6 moths of therapy, have less social, somatic and affective problems after 12 months (reported at YSR), less depressive symptoms (reported to HAMD) and worthlessness (at MASC), confirming a sort of "protective" role of BDNF. In this epoch (T6), scores of different tests go in the same

direction, orienting towards a good prognosis at T12 (convergence of indices). Accordingly to what is reported by mother, adolescent with higher BMI at T0 perceive less somatic problems and obsessive-compulsive problems.This data is confirmed also in MASC scores: higher BDNF correlates with reduced anxiety dimensions in depressed group.To summarize: BMI seems to be a risk factor in healthy subjects and a "protective" factor in depressed adolescents.

BDNF levels do not correlate with a peculiar psychopathological functioning (i.e. psychotic, borderline) explored through clinical interviews, SCID and the administration of the Rorschach test, supporting the adequacy of recruitment (we should remember that neurotrophin is altered in personality disorder according to Koenigsberg et al. 2012) (considering however a possible bias linked to the sample size). BDNF levels do not correlate to the severity of the disease estimated with CGAS or CGI, supporting the hypothesis that neurotrophin levels correlate with the presence /absence of disease rather than with its severity, according to what has been observed with respect to the platelet concentrations of BDNF in Pandey et al. (2010).

In literature, some authors found negative correlations between blood concentration of BDNF and psychopathological indexes measured with standardized tests such as MADRS (Karege et al., 2002), HAM-D (Kreinin et al., 2015; Satomura et al. , 2011, Dell'Osso et al., 2010), BDI (Pallavi et al., 2013) and CDRS (Sasaki et al., 2011). Dell'Osso et al., (2010) explained these correlations pointing out that some depressive symptoms, from the neurobiological point of view, are associated with a greater alteration of the HPAA, cortisol exerting a down regulation of BDNF synthesis at the level of the CNS. Interestingly, other studies have not shown any correlation between BDNF levels and severity of depressive symptomatology (Zhang et al., 2008; Pandey et al., 2010; Wang et al., 2011), so in this way, BDNF would represent a state mark of pathology. It is likely that genetic factors, neurotransmitter and hormonal alterations are responsible for such contradictory conclusions.

It is hypothesized that the peripheral dosage of BDNF, associated with clinical and test evaluation, could be useful in the prevention of the consequences of depression, in particular self-harm behaviors and suicide (Grah et al., 2014). The risk of self- harm behaviors is reported and perceived by both the subjects affected and by the clinician in a fluctuating way and therefore less stable than the amount of protein in the peripheral blood can be. The prevention of self-injurious and suicidal behaviours requires both universal and personalized measures for groups at higher risk, therefore the possibility of distinguishing a group of patients at greater risk would allow reasoned and targeted therapeutic choices guided by

different phenomenological characteristics. The identification of these risk groups would allow the early recognition of the DD worsening and an early treatment that finally could avoid the chronicity of the disorder and the structuring of morpho-functional anomalies.
In our study, BDNF levels do not correlate either to suicidal risk (suicidal ideation and SA), nor with personal history of trauma.

At T0 depressed children with suicidal ideation seem to have more social competences but more thought problems (according to mother), less anxious depressed symptoms, internalizing problems and anxiety problems according to YSR (a denial of the problem is possible), more severe scores at CDI-S, reduced harm avoidance, social anxiety and total score of MASC questionnaire. These depressed subjects who presents suicidal ideation have reduced levels of BDNF and the results tend to statistical significativity (p=-06).

Depressed patients who presented a SA are engaged in less activities, perceived less total competences, less anxious-depressed symptoms and anxiety problems (at YSR), reduced physical symptoms, harm avoidance and total score to MASC. These results are in contrast to what is reported in literature in which anxiety is generally considered an independent risk factor for suicide (Nepon et al., 2010; Bilgiç et al., 2017). In our study some dimensions of anxiety, such as social anxiety, appear to be almost protective, according to other recent studies on adults (Abreu et al., 2018).

The evolution from suicidal ideation to SA appear to be somehow prevented by having a richer social support network that finally represents the resilience of the subject (Consoli et al., 2013).

As for the presence of traumatic events in personal history, depressed patients without a history of trauma presented more somatic complaints, internalizing problems and PTSD symptoms according to mother, more DOP problems according to father CBCL. On the YSR, those who presented history of trauma can respect less the rules and have more conduct disorders. At CGI-I subtest, children with a personal history of traumatic events present a reduced improvement during therapy.

The presence of traumatic events in personal history appears to be correlated to behavioral and externalizing problems, therefore with consequences on the irritable side of mood more than on sadness. SAs do not correlate to any psychiatric dimension explored so confirming the difficulties in preventing such dramatic episodes.
Considering psychodiagnostic test, mothers' evaluations seem to be more precise and consistent with clinical judgment, comparing to fathers' and children's ones. This observation confirms the central role of family in detection of DD in developmental age, so highliting the

difficulties of the psychiatrist in case of lack of parents, inadequate parents or in case parents can not recognize depression as a disease, so having a biochemical marker to guide therapeutic approach become again crucial.

During the 12 months' time, psychodiagnostic test did not change significatively, thus highlighting a loss of precision in evaluating some dimensions of DD perceived by the psychiatrist at clinical controls. C-GAS score change significatively from T0 to T6. The item harm avoidance of the MASC and the Hamilton changed significantely, with an improving during time. From a merely qualitative point of view, global scores (externalizing, internalizing and total scores) in the standardized tests (CBCL, YSR, MASC, CDI, CGI-S, C-GAS, HAM-D) completed by depressed subjects tend to reduce, showing a clinical improvement from T0 (before treatment) to T12 (after treatment), even in absence of a statistical significance.

The 3-month trend of the plasma levels of neurotrophin shows an increase in 4 cases, a stability in 1 case and a decrease in 3 cases; from a descriptive point of view the increase, stability or reduction of BDNF levels seem in relation to the clinical course of each subject. The 5 subjects presenting an increase/stability of BDNF (ID1-ID7-ID8-ID9 and ID6 respectively) seem to respond better to a pharmacological therapy (all started) and to a psychotherapy (ID1-ID6-ID7-ID9) from what reported in medical records. In ID6 a reduction of IL-1β could also be seen. Considering the 3 subjects who presented a reduction in BDNF levels (ID2-ID4-ID5): one required a pharmacological shift for a clinical worsening (ID2), while in the other two cases the therapeutic dose of the drug was just reached (ID4-ID5) so suggesting that the reduction of neurotrophin may be due to the latency of the synthesis process (Duman, 2004). In ID 4 an increase in IL-1β could be notable in parallel.

BDNF data are confirmed by the subsequent progress of the patients. At 6 months, only the two depressed subjects who undergo an additional clinical improvement and continue both the pharmacological and the psychological therapies (ID1-ID6) have a further increasing in BDNF levels. Both of these subjects presented a reduction in IL-1β levels even if with a slow trend. The other 3 patients (ID7-ID8-ID9), who had presented an increase in BDNF at 3 months, underwent a reduction of it in association with a clinical worsening that conditioned a modification of the drug therapy (ID8) as well as further potential stressful events such as community admission (ID7) or new hospitalization (ID9). It is known that stress, in particular if prolonged, determines a down-regulation of the expression of BDNF in the brain (Bus et al., 2015). Subsequently two of these subjects (ID7-ID8) presented a slight clinical improvement, which was confirmed by an increase in the BDNF level.

Results are in line with Shen et al. (2014), who asserts that only patients responding to pharmacological treatment show an increase in BDNF levels. In this sense, the level of peripheral BNDF could have a helpful value in reveal early response to treatment.
Since it is a naturalistic study, the non-homogeneous trend in our sample could however be partly explained by the variability of the chosen pharmacological class.
By evaluating the trend over time of plasma BDNF levels in healthy subjects, there is a trend of reduction at 6 months and a subsequent increasing at 12 months. This non-heterogeneous trend could be attributed to multiple variables (climate, physical exercise, learning processes, diet, etc.), but any other speculation is not possible given the small sample size.
Clinical and epidemiological studies have revealed that genetic background, stressful life events (such as childhood maltreatment), or substance abuse are related with the risk of development of mood disorders. It is hypothesized that the first mood episodes are initially triggered mainly by stressful events (Barbosa et al,2014). Mood episodes themselves seem to play a pivotal role acting as a major "toxic player" in the neuroprogression of the illness (Kapczinski et al., 2010).

Although little is known in literature, one of the possible mechanisms underlying the disease progression could be the production of the pro-inflammatory cytokines and other inflammatory markers during mood episodes (Barbosa et al., 2014). Analizing IL-1β dosage in the depressed group, it is possible to observe that it is associated in a non homogeneous way to many dimensions. Parents in depressed group show all a reduction in attention problems and ADHD in subjects with higher levels of IL-1β, with an increasing in somatic complaints. In the healthy group, according to parents' reports, higher levels of the cytokine are linked to lower anxiety/depressive symptoms and lower social problems. According to this, to YSR, adolescents reported higher positive qualities.
In the depressed group IL-1β at T0 inversely correlate to CGI severity score and CGI efficacy score ($z=-.900$, $p=.037$) reported at T12, suggesting that patients with lower levels of the cytokine seem to have more severe forms of depression, in contrast to what is reported in literature on adult groups (Martinez et al., 2012) .
IL-1β does not correlate with suicidal ideation, nor with the SA, nor with a history of traumatic events, contrary to what reported by Lu et al. (2013) who sustained a possible role of childhood trauma on dysregulation of cytokines in adult with MDD.

Similarly to other studies (Cassano et al, 2017), in the present research only peripheral molecules levels were assessed, with the purpose to avoid invasive procedures in pediatric population.

One debating question is related to the extent to which the blood levels of BDNF can reflect BDNF concentrations in the brain. Some authors demonstrated that BDNF can cross the blood-brain barrier, suggesting that the peripheral levels of this neurotrophin may reflect brain levels (Pan et al., 1998). BDNF concentrations in subjects with mood disorders have been studied in serum (Karege et al., 2002; Cunha et al., 2006), plasma (Lommatzsch et al., 2005) and in platelets (Pandey et al. ., 2010), with an increasing debate on what is the best source of neurotrophin dosage. According to Piccinni et al. (2008b) the serum level of BDNF would represent a trait marker, while the plasma level would represent a state marker, the latter being sensitive to antidepressive therapy after only one month of treatment.

In this study we chose to examine plasma BDNF, since the concentration of BDNF in platelet-poor plasma appears to be only minimally influenced by the amount of BDNF stored in the platelets. Plasmatic levels represent therefore a more sensitive and reliable marker of BDNF changes occurring in the brain and at the peripheral level (Lommatzsch et al., 2005). On the other hand, the serum concentration is strongly and variably affected by the amount of BDNF released by the activated platelets during the process to get serum itself. Plasma dosage appears to be feasible in clinical practice because, if the biological material is adequately conserved, it allows delaying the treatment for hours or days. Nonetheless, plasma BDNF also showed high interindividual variability and therefore, the different methodological procedures can help to explain the existence of controversial data in the literature (Karege et al., 2005; Palomino et al., 2006) and the difficult comparison.

Regarding IL-1β, since the debated correlation between plasma and cerebrospinal fluid (CSF) cytokine levels, we cannot exclude the possibility of inflammation of the brain (microglial activation) among the subjects recruited in this study (Bromander et al., 2012; Dellalibera-Joviliano et al., 2003; Hopkins et al., 2012).

For a long time the brain was considered, from an immunological point of view, to be a privileged organ. We now know that proinflammatory cytokines are produced in response to insults not only by immune cells but also by neurons and neural stem cells (Tsakiri et al. 2008; Zunszain et al. 2012). Cytokines can act directly on the brain causing changes known as ‘‘sickness behaviour’’, a coordinated set of subjective, behavioural and physiological changes that develop in sick individuals during the course of an infection (Dantzer, 2004). If the activation of the peripheral immune system is prolonged, the immune signalling to the brain can lead to an exacerbation of sickness behaviour and to the development of depressive symptoms in vulnerable individuals (Dantzer et al. 2008). The therapeutic administration of cytokines and several animal models support this view. Further support for an aetiological role of inflammation in depression comes from longitudinal studies of humans (Gimeno et al.

2008). On the other hand, some observations support an ‘‘after’’ or ‘‘state’’ condition. A recent longitudinal study (Copeland et al., 2012) on young children followed until age 21, suggest that depression precedes inflammation (Zunszain, 2013). This relationship was more obvious after the occurrence of cumulative episodes of depression.

Summarizing there are mixed evidences in the literature on the role of inflammation in major DD. Contradictory findings are attributed to lack of rigorous characterization of study subjects, to the presence of concomitant medical illnesses, to the small sample sizes, and to the limited number of cytokines tested.

In the present study the rigorous methodological criteria aimed at preventing confounding factors (e.g. peripheral inflammation due to medical comorbidity, antidepressant treatments) might have led to the selection bias of a ‘non-inflammatory’ biological phenotype of depression.

The development of new therapeutic targets for depression and more in general for mood disorders in developmental age is of great importance. The immune system (including the relationship between inflammation and function of HPAA) seems to be a promising target for further research direction, in line with recent research on adults with resistant depression (Kiraly et al., 2017). Another interesting therapeutic target is the restoration of ideal BDNF values in the sites considered responsible for depression, that could represent a further therapeutic option in light of the limited pharmacological possibilities available to date for developmental age. Some studies on mice tried to administer directly BDNF intrathecally with some notable adverse effects, mainly related to altered sensory processing and pain (Groth and Aanonsen, 2002) and muscle spasticity (Boyce et al., 2012) that prevent its therapeutic use. More promising seem to be drugs with an indirect effect on BDNF restoration (eg. Cannabidiol, CBD) (Sales et al., 2018).

The blood level of BDNF, reflecting the concentration of the factor in CNS, could be a good peripheral marker of DD, sensitive even if not very specific, allowing a better diagnosis and a more accurate follow up. The dosage would also allow the early detection of responders or resistant patients to pharmacological, psychotherapeutic or combined therapy, according to the study of Tadić et al. (2011) who stated that a failure to normalize BDNF after therapy, associated with unchanged scores at the Hamilton scale are predictors of a therapeutic failure with a specificity of 100%.

In conclusion, after rigorously selecting depressed children and adolescents without significant medical comorbidity, we found no significant associations between plasma BDNF

or cytokines and DD, even if at T0 depressed patients presented lower levels of BDNF and higher levels of IL-1β comparing to healthy peers. At intake BDNF appears to correlate to academic competences, while after 6 months of therapy higher BDNF levels orient to a better prognosis as for depression symptomatology.

The evolution from suicidal ideation to SA appears to be linked to the perception of limited social support (reduced resilience) in adolescents with reduced levels of BDNF.

Depressed children with a history of trauma present more externalizing symptoms, while the dimension of anxiety appears to be somehow protective from suicidal attempts.

The study supports the bio-psycho-social model of DD, highlighting the importance of a balance between neurogenesis, neuroinflammation and cerebral plasticity in the delicate period of developmental age. BDNF seems to be a symptoms or disease biomarker more than a severity index, so the integration of different instruments of evaluations, both clinical and biological (multimodal methods), as well as the integration of different voices (multi-informant approach) seems to represent the"best practice" for pediatric clinical assessment of mental health in line with precision psychiatry. Precision psychiatry represents "an emerging approach for treatment and prevention that takes into account each person's variability in genes, environment, and lifestyle" (National Research Council Committee Washington, 2011): the analysis of data obtained by diverse approaches and techniques produce a set of biomarkers that, when applied to individuals and populations, permit to reach a better diagnosis, endophenotypes, classifications and prognosis (also in terms of response or non response to treatment), as well as tailored interventions for better outcomes (Fernandes et al., 2017).

Further studies are needed to explore the role of BDNF and cytokines in the response of pediatric population to SSRIs, to identify biomarkers for predicting early treatment response, to produce new drug targets, to find preventive strategies and individually target therapy.

6. Study limitations

Results should be interpreted in light of some limitations. First, due to the limited sensitivity of cytokine detection, distribution of IL-1β levels was truncated.

Study limitations are than the characteristics of the sample (reduced numerosity, female prevalence). This is a naturalistic study so the recruitment possibilities are bound to the real access of subjects to clinical services. Females seem to be more prone to access to services and this could be due mainly to two reasons. First, an emergence of a different prevalence of DD in female gender as described in literature for adult age.Secondly, it could be linked to different expression of the discomfort and to a greater request for help from the girls, in who

a certain emotional fragility is more socially accepted.

Another critical point is that BDNF and IL-1β levels could be influenced by many confounding factors (gender, age, sample collection time, etc.) so a possible bias should be hypothesized (Iughetti et al., 2011). In particular, it is important to consider that different climatic conditions linked to seasons can impact on BDNF levels variations (Molendjic et al., 2012), limiting the longitudinal interpretation of the results. To reduce these factors a meticulous and controlled method was used. Levels of BDNF vary with age and specifically they reduce with the increase of age from puberty to late puberty. A possible other bias described in literature (but not considered in the present study) could be due to the high variability of the serum and plasma BDNF in females during menstrual phases (BDNF levels higher in luteal phase than in follicular phase; Iughetti et al., 2011).

Another limiting factor could be the ethnicity of the subjects enrolled, given the observation that the presence of the Val66Met polymorphism, associated with the risk of developing anxiety and DD, has a high genetic variability and is present in 25-32% of Caucasian population and in 40-50% of the Asian one (Hempstead, 2015).

All the previously listed factors can help to explain the variability of data in the literature and the difficult comparability.

A further substantial limitation is high percentage of drop out subjects in depressed group, before completing the 12 months of monitoring. Similar values of "dropout rates" are common in long-term studies on mood disorders (Piccinni et al., 2008), probably for intrinsic difficulties in compliance (families refuse to attend clinical controls when they perceive no clinical improvements or when the depressed subject feels better). To underestand such limited sample numerosity (that is also common in this type of studies), it is necessary to consider the need to follow with scientific rigor tight exclusion criteria and the difficulty in recruiting depressed subjects, who present often resistances in participation in clinical trials due to the still present stigma associated to psychiatric disease and therefore to the difficulties to accept the pathology from patient and families.

Another limitation is the pharmacological treatment since, being a naturalistic study, variability in the use of the pharmacological class and in dosages finally impede comparability with other studies in literature.

Many factors contribute to the variability in cytokine measurement (Cassano et al, 2017), such as stability of each cytokine during the blood/plasma processing time, freeze–thaw cycles and storage. These factors influence both cytokines' detectability and measurement and ultimately produce high variability in cytokines' levels between studies (De Jager et al., 2009). Within our study, variability in cytokine levels was accounted for by testing simultaneously cases

and controls. Furthermore, our rigorous entry criteria developed to limit confounding variables, such as the exclusion of some medical conditions, may have selected an unrepresentative cohort of patients with DD and may have diminished the ecological validity of our comparison.

Patients were required to be free of antidepressants at study entry to examine DD without the influence of antidepressants, which themselves (eg SSRI) may have anti-inflammatory effects (Hannestad et al., 2011). This methodological aspect may have limited levels of severity and chronicity of the sample.

Appendix 1. Major Depressive Disorder (DSM-5; APA, 2013)

A. Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure.
Note: Do not include symptoms that are clearly attributable to another medical condition.
1. Depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad, empty, hopeless) or observation made by others (e.g., appears tearful). (Note :In children and adolescents, can be irritable mood.)
2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation.)
3. Significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day. (Note: In children, consider failure to make expected weight gain.)
4. Insomnia or hypersomnia nearly every day.
5. Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down).
6. Fatigue or loss of energy nearly every day.
7. Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick).
8. Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others).
9. Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide.
B. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.
C. The episode is not attributable to the physiological effects of a substance or to another medical condition.
Note: Criteria A-C represent a major depressive episode.
Note: Responses to a significant loss (e.g., bereavement, financial ruin, losses from a natural disaster, a serious medical illness or disability) may include the feelings of intense sadness, rumination about the loss, insomnia, poor appetite, and weight loss noted in Criterion A, which may resemble a depressive episode. Although such symptoms may be understandable or considered appropriate to the loss, the presence of a major depressive episode in addition to the normal response to a significant loss should also be carefully considered. This decision inevitably requires the exercise of clinical judgment based on the individual's history and the cultural norms for the expression of distress in the contest of loss.
D. The occurrence of the major depressive episode is not better explained by schizoaffective disorder, schizophrenia, schizophreniform disorder, delusional disorder, or other specified and unspecified schizophrenia spectrum and other psychotic disorders.
E. There has never been a manic episode or a hypomanic episode.
Note:This exclusion does not apply if all of the manic-like or hypomanic-like episodes are substanceinduced or are attributable to the physiological effects of another medical condition.

Specify:
With anxious distress
With mixed features
With melancholic features
With atypical features
With mood-congruent psychotic features
With mood-incongruent psychotic features
With catatonia. Coding note: Use additional code 293.89 (F06.1).
With peripartum onset
With seasonal pattern (recurrent episode only)

Appendix 2. Persistent Depressive Disorder (Dysthymia)(DSM-5; APA, 2013)

This disorder represents a consolidation of DSM-IV-defined chronic major depressive disorder and dysthymicdisorder.
A. Depressed mood for most of the day, for more days than not, as indicated by either subjectiveaccount or observation by others, for at least 2 years.
Note: In children and adolescents, mood can be irritable and duration must be at least 1 year.
B. Presence, while depressed, of two (or more) of the following:
1. Poor appetite or overeating.
2. Insomnia or hypersomnia.
3. Low energy or fatigue.
4. Low self-esteem.
5. Poor concentration or difficulty making decisions.
6. Feelings of hopelessness.
C. During the 2-year period (1 year for children or adolescents) of the disturbance, the individual hasnever been without the symptoms in Criteria A and B for more than 2 months at a time.
D. Criteria for a major depressive disorder may be continuously present for 2 years.
E. There has never been a manic episode or a hypomanic episode, and criteria have never been met forcyclothymic disorder.
F. The disturbance is not better explained by a persistent schizoaffective disorder, schizophrenia,delusional disorder, or other specified or unspecified schizophrenia spectrum and other psychoticdisorder.
G. The symptoms are not attributable to the physiological effects of a substance (e.g., a drug of abuse, amedication) or another medical condition (e.g., hypothyroidism).
H. The symptoms caucause clinically significant distress or impairment in social, occupational, or otherimportant areas of functioning.
Note: Because the criteria for a major depressive episode include four symptoms that are absent fromthe symptom list for persistent depressive disorder (dysthymia), a very limited number of individuals willhave depressive symptoms that have persisted longer than 2 years but will not meet criteria for persistentdepressive disorder. If full criteria for a major depressive episode have been met at some point during thecurrent episode of illness, they should be given a diagnosis of major depressive disorder. Otherwise, adiagnosis of other specified depressive disorder or unspecified depressive disorder is warranted.

Specify if:
With anxious distress
With mixed features
With melancholic features
With atypical features
With mood-congruent psychotic features
With mood-incongruent psychotic features
With peripartum onset

Specify if:
In partial remission
In full remission

Specify if:
Early onset: If onset is before age 21 years.
Late onset: If onset is at age 21 years or older.

Specify if (for most recent 2 years of persistent depressive disorder):

With pure dysthymic syndrome: Full criteria for a major depressive episode have not been met in atleast the preceding 2 years.
With persistent major depressive episode: Full criteria for a major depressive episode have been metthroughout the preceding 2-year period.
With intermittent major depressive episodes, with current episode: Full criteria for a major depressive episode are currently met, but there have been periods of at least 8 weeks in at least thepreceding 2 years with symptoms below the threshold for a full major depressive episode.
With intermittent major depressive episodes, without current episode: Full criteria for a major depressive episode are not currently met, but there has been one or more major depressive episodes inat least the preceding 2 years.

Specify current severity:
Mild
Moderate
Severe

Appendix 3. Suicidal Behaviour Disorder (DSM-5; APA, 2013)

A. In the last 24 months, the person has made a suicide attempt.
Note: A suicide attempt is a sequence of behaviors voluntarily undertaken by an individual who, at the time of initiation, expected that his actions would lead him to death. (The "starting moment" is the moment when a behavior took place that involved the method used)
B. The gesture does not meet the criteria for non-suicidal self-injuries ie it does not include acts of self-injuries to the body surface performed to induce relief from a negative emotional or cognitive state or to reach a positive emotional state.
C. The diagnosis is not applied to suicidal ideation or to preparatory actions.
D. The gesture did not begin in a moment of delirium or confusion.
E. The gesture was not implemented exclusively for a political or religious purpose.

Appendix 4 Non-Suicidal Self-Harm Behaviour (DSM-5; APA, 2013)

A. In the last year, in 5 or more days, the individual has intentionally inflicted damage of some kind to her/his body surface capable of inducing bleeding, bruising or pain (eg, cutting, burning, stabbing, hitting, rubbing excessively), with the expectation that the wound will lead to only mild or moderate physical damage (there is no suicidal intentionality).

Note: The absence of suicidal intentionality has been affirmed by the subject or it can be inferred from the fact that the subject has repeatedly performed a behavior being aware, or having learned, that it is not likely to lead to death.

B. The individual is involved in self-injurious activity with one or more of the following expectations:

1. To obtain relief from a feeling or cognitive negative state
2. Resolve an interpersonal difficulty
3. Induce a positive sensation

Note: the desired relief or response is tested during or immediately after the self-injurious gesture, and the subject may exhibit behavioral patterns that suggest a dependence on doing that gesture repeatedly.

4. Intentional self-harm is associated with at least one of the following symptoms:
5. Interpersonal difficulties or feelings or negative thoughts, such as depression, anxiety, tension, anger, generalized discomfort, self-criticism, which occur in the period immediately preceding the self-injurious gesture.
6. Before making the gesture, there is a period of concern difficult to control regarding the gesture that the individual intends to do.
7. Thoughts of self-harm often present, even when the behavior is not put in place.

C. Behavior is not socially accepted (eg, body piercing, tattoos, religious or cultural rituals) and is not limited to inducing scratching injuries or eating nails.

D. The behavior and its consequences cause clinically significant distress or impairment of functioning in the social, educational, employment or other important areas.

E. The behavior does not occur exclusively during psychotic episodes, delirium, intoxication or substance withdrawal. In individuals with a neurodevelopment disorder, behavior is not part of a pattern of repetitive stereotypies. The behavior is not better explained by another mental disorder or medical condition (eg psychotic disorder, autism spectrum disorder, intellectual disability, Lesch-Nyhan syndrome, stereotyped disorder of the self-injurious movement, trichotillomania, excoriation disorder).

Appendix 5 Disruptive Mood Dysregulation Disorder(DSM-5; APA, 2013)

A. Severe recurrent temper outbursts manifested verbally (e.g., verbal rages) and/or behaviorally (e.g.,physical aggression toward people or property) that are grossly out of proportion in intensity orduration to the situation or provocation.
B. The temper outbursts are inconsistent with developmental level.
C. The temper outbursts occur, on average, three or more times per week.
D. The mood between temper outbursts in persistently irritable or angry most of the day, nearly everyday, and is observable by others (e.g., parents, teachers, peers).
E. Criteria A–D have been present for 12 or more months. Throughout that time, the individual has nothad a period lasting 3 or more consecutive months without all of the symptoms in Criteria A–D.
F. Criteria A and D are present in at least two of the three settings (i.e., at home, at school, with peers)and are severe in at least one of these.
G. The diagnosis should not be made for the first time before age 6 years or after age 18 years.
H. By history or observation, the age of onset of Criteria A-E is before 10 years.
I. There has never been a distinct period lasting more than 1 day during which the full symptomcriteria, except duration, for a manic or hypomanic episode have been met.
Note: Developmentally appropriate mood elevation, such as occurs in the context of a highly positive eventor its anticipation, should not be considered as a symptom of mania or hypomania.
J. The behaviors do not occur exclusively during an episode of major depressive disorder and are notbetter explained by another mental disorder (e.g., autism spectrum disorder, posttraumatic stressdisorder, separation anxiety disorder, persistent depressive disorder [dysthymia]).
Note: This diagnosis cannot coexist with oppositional defiant disorder, intermittent explosive disorder, orbipolar disorder, though it can coexist with others, including major depressive disorder, attention-deficit/
hyperactivity disorder, conduct disorder, and substance use disorders. Individuals whose symptoms meetcriteria for both disruptive mood dysregulation disorder and oppositional defiant disorder should only begiven the diagnosis of disruptive mood dysregulation disorder. If an individual has ever experienced a manicor hypomanic episode, the diagnosis of disruptive mood dysregulation disorder should not be assigned.
K. The symptoms are not attributable to the physiological effects of a substance or to another medicalor neurological condition.

Appendix 6

0-3 Classification (2005)

type 1: Major Depression

type 2: Depressive Disorder Not Otherwise Specified.

Diagnostic criteria are the same as for the adult presentation. The proposed modifications for the DSM-IV-TR items, in relation to the early onset, are:

item 2) the loss of interest and pleasure should be observed in playingand in interactions with the adult;

item 7) the presence of feelings ofworthlessness uselessness and guilt must be observed in the game (self punitive actions and games)

item 8) in the younger children the reduced ability to think or to concentrate can manifest itselfas difficulties in problem solving, in answering to the parentand / or in maintaining the attention

item 9) thoughts of death or suicide are considered present if observed in playing, activities or in potentially self harm behaviours.

DC: 0-5^{TM}Classification (2016)

Depressive Disorder od Early Childhood

Disorder of Dysregulated Anger and Aggression of Early Childhood (DDAA)

Other Mood Disorder of Early Childhood

Appendix 7Clinical studies on BDNF and depression in adult population

Author, year, state	Type of study (duration)	N patients (cases vs healty)	Dosage	Testused	Kit ELISA	BDNF	BDNF cases; M±SD	Results
Ga 2016 China	Longitudinal Case control (12 wks)	37 vs 303	serum	EPDS	Promega	ng/ml	n.a	Lower levels of serum BDNF in women with post partum depression
Ihara, 2016 Japan	Longitudinal Case control	1193 vs 106	serum	**H**AM DSBC SCID	Promega	pg/ml	n.a	Reduction of BDNF levels in the follow up of depressed patients compared to controls
Freire, 2016 Brazil	Longitudinal	31	serum	MINI, HDRS	Millipore	ng/ml	n.a	BDNF basal levels higher in ECTresponders compared to ECT non-responders
Martinotti, 2016 Italy	Longitudinal Case control (8 wks)	27 vs 29	serum	SHAPS, HDRS, HARS	M.polis	ng/mL	150.79 ± 42.78	Increased BDNF in patients treated with agomelatine
Nase, 2016 Germany	LongitudinalCase control(8 wks)	76 vs 41	serum	MINI HAMD BID II	Promega	ng/ul	n.a	BNDF reduced in depressed subjects
Salehi, 2016 Iran	Longitudinal (4 wks)	60	plasma	BID HDRS	n.a	pg/ml	n.a	BDNF increases in patients treated with electroconvulsive therapy and physical aerobic therapy
Bus, 2015 Netherlands	Longitudinal	1751	serum	CIDI	Promega	ng/ml	n.a	Low BDNF in subjects with persistent or remitting depression
Buttenschøn, 2015 Denmark	Longitudinal (12 wks)	90	serum	HRSD BDI MADRS	M.polis	pg/ml	n.a	No significative increase in BDNF after treatment
Fornaro, 2015 Italy	Longitudinal case control (8 weeks)	30 vs 32	plasma	HAM	n.a	ng/ml	8.257 ± 1.905	Low BDNF in depressed vs healthy subjects. After duloxetine treatment BDNF increased in early non-responders
Gosh, 2015 India	Longitudinal	60	plasma	HAM	Weldon biotech	pg/ml	850.3 ± 24.92 845.8 ± 32.82	BDNF increased after treatment with fluoxetine and desvenlafaxine
Kreinin, 2015 Israel	Longitudinal case control (8 wks)	51 vs 38	serum	HAM	Promega	ng/ml	n.a.	Low BDNF in untreated subjects. BDNF levels correlate to the severity of depression.
Haile, 2014 USA	Longitudinal (7 days)	22 with resistent depression	plasma	MADRS	DuoSet	ng/ml	n.a.	Ketamine increases BDNF in responsive patients

Ninan, 2014 USA	Longitudinal (12 wks)	427	serum	HDRS	n.a.	n.a.	n.a.	BDNF increased after therapy with Desvenlafaxine
Yoshimura, 2014 Japan	Longitudinal (8 wks)	150	serum	No test	Promega	ng/ml	9.2 ± 6.1	BDNF at t0 and at t4 does not predict response to SSRIs
Munno, 2013 Italy	Longitudinal Case control (2 months)	16 vs 18	plasmaand serum	n.a.	n.a.	n.a.	n.a.	BDNF reduced in plasma of depressed patients, not in serum
Guimarães Barbosa, 2013 Brazil	Cross Sectional Case control	87 vs 58	plasma	MINI, YMRS, HDRS	DuoSet	pg/ml	n.a.	Reduced levels in depressed subjects
Ricken, 2013 Germany	Longitudinal (4 wks)	83	serum	HDRS, MINI	Promega	ng/ml	9.21 ± 4,1	BDNF increased after augmentation with lithium
Karlović, 2013 Croatia	Transversal	122 vs 142	serum	No test	n.a.	ng/ml	37.5 ± 13.3	BDNF allows diagnosis of DDM
Palm, 2013 Germany	Longitudinal (4 wks)	22	serum	HDRS, BDI, CGI	Qua.kine	ng/ml	12,43 ± 4,7	Levels unchanged after transcranial electrical stimulation in resistant depression
Deuschle, 2013 Germany	Longitudinal Case control (28 days)	56	serum	HDRS	Promega	ng/ml	7.29 ± 4.0	No differences between cases and controls; BDNF varies depending on the therapy (venlafaxine vs mirtazapine)
Kurita, 2012 Japan	Cross sectional	38 vs 10	plasma	MADRS	Promega	pg/ml	1827 ± 1340	BDNF levels associated to clinical outcome
Birkenhäger, 2012 The Netherlands	Cross sectional	42	serum	HDRS	Promega	ng/ml	18.0 ± 2.8	BDNF correlate with disease duration
Yoshida, 2012b Japan	Cross sectinal Case control	69 vs 78	BDNF and proBD NF on serum	HDRS SASS	Adipo Biosc	ng/ml	21.09 ± 5.6	Mature BDNF reduced in cases, no differences between groups in the level of proBDNF
Grande, 2012 Spain	Longitudinal (16 wks)	25	serum	YMRS HDRS CGI	n.a.	n.a.	n.a.	BDNF levels increase after therapy with quetiapine
Molendijk, 2011 The Netherlands	Cross sectionalCase control	962 vs 382	serum	IDS CIDI SSI	Promega	ng/ml	n.a.	Reduced levels in untreated patients
Jevtović, 2011 Croatia	Cross sectional	139	serum	HDRS	RayBio	ng/ml	40 ± 13,4	levels do not correlate with severity at HDRS
Tadić, 2011 Germany	Longitudinal (6 wks)	41	serum	HDRS	n.a.	n.a.	n.a.	BDNF and HDRS predict failure of therapy
Wolkowitz, 2011 USA	Longitudinal Case control (8 wks)	30	serum	HDRS CGI	Qua.kine	ng/ml	14.88 ± 5.4	Reduced levels in cases; Increase after therapy

Dell'Osso, 2010 Italy	Cross sectional	30	plasma	HDRS CGI	Promega	pg/ml	2,539 ± 1,616	Levels correlate with disease severity
Pandey, 2010 USA	Cross sectionalc ase control	25 vs 25	Lymphocytes platelets	HDRS CDRS	Promega	ng/mg protein	18,42 ± 6,8	BDNF reduced in the cases
Kang, 2009 Korea	Longitu dinal (8 wks)	243	plasma, polymor phism	HDRS	Qua.kine	pg/ml	n.a.	Levels increase after therapy with mirtazapine
Matrisciano, 2009 Italy	Longitudi nal Case control (6 months)	21 vs 20	serum	HDRS	Promega	ng/ml	42,5 ± 8,3	Reduced levels in cases; increase after therapy
Başterzi, 2009 Turkey	Longitudinal Case control (6 wks)	43	serum	n.a.	n.a.	n.a.	n.a.	No differences between cases and controls
Lee, 2008 Korea	Longitudinal Case control (6 wks)	32 vs 50	plasma	HDRS	DuoSet	pg/ml	698.1 ± 537.7	Reduced levels in cases, increase with therapy
Okamoto, 2008 Japan	Cross-sectional	18	serum	n.a.	n.a.	n.a.	n.a.	Levels increase after response to ECT
Hellweg, 2008 Germany	Longitu dinal (36 days)	40	serum	n.a	n.a.	n.a.	n.a.	Levels change depending on the therapy (paroxetine vs amitriptyline)
Catena, 2007; Piccinni, 2008b Italy	Longitudin al case control (12 months)	15 vs 15	serum plasma	HDRS MADRS	Promega	ug/ml	19,3 ± 8,8	Reduced levels in cases, serum normalized after 1 month of SSRI or TCA
Monteleone, 2008 Italy	Cross-sectional Case control	11 vs 22	serum	n.a	n.a.	ug/ml	29 ± 15,9	Reduced levels in cases
Huang, 2007 Taiwan	Longitudi nal Case control (4wks)	111 vs 107	serum	HDRS	n.a.	ug/ml	10,9 ± 7,1	Reduced levels in cases; increased after antidepressant
Yoshimura, 2007 Japan	Longitu dinalCas e control (8 wks)	42 vs 30	serum	HDRS	n.a.	ug/ml	9,5 ± 7,8	Reduced levels in cases; increased after paroxetine
Aydemir, 2007 Turkey	Cross sectional, Case control	24 vs 26	serum	HDRS	Promega	ug/ml	21,2 ± 11,3	Reduced levels in cases
Aydemir, 2006 Turkey	Longitudi nal case control (6wks)	20 vs 20	serum	n.a.	n.a.	ug/ml	27,7 ± 13,7	Reduced levels in cases; improvement after therapy with citalopram
Gonul, 2005 Turkey	Longitu dinal case control (8 wks)	28 vs 18	serum	HDRS	n.a.	ug/ml	20,8 ± 6,7	Reduced levels in cases, increased after therapy

Gervasoni, 2005 Switzerland	Longitudinal case control	26 vs 26	serum	MADRS	n.a.	ug/ml	22,6 ± 3,6	Reduced levels in cases; increase after therapy
Karege, 2005 Switzerland	Cross-sectional Case control	43 vs 35	serum,plasma	MADRS	Promega	ug/ml	10,1 ± 2,3	Reduced levels in cases
Shimizu, 2003 Japan	Cross-sectionalCase control	16 vs 50	serum	HDRS	n.a.	ug/ml	17,6 ± 9,6	Reduced levels in cases
Karege, 2002 Switzerland	Cross-sectional Case control	30 vs 30	serum	MADRS	n.a.	ug/ml	22,6 ± 3	Reduced levels in cases

EPDS: Edinburgh Postnatal Depression Scale. DSBC: Depression Scale Basic Checklist; SCID: Structured Clinical Interview for DSM-IV; YMRS: Young Mania Rating Scale; SHAPS: Snaith-Hamilton Pleasure Scale; HARS: Hamilton Anxiety Rating Scale; HAMD: Hamilton Depression Rating Scale; HAM: Hamilton Anxiety Scale; BDI II: Beck's Depression Inventory; CIDI: Composite International Diagnostic Interview; CDRS: Children Depression Rating Scale; CGI: Clinical Global Impression; MADRS: Montgomery–Åsberg Depression Rating Scale; HRSD: Hamilton Rating Scale for Depression; BSS: Beck Scale for Suicidal Ideation ; BIS: Barratt Impulsiveness Scale; BHS, Beck HopelessnessScale; MINI: Mini International Neuropsychiatric Interview.

Appendix 8Clinical studies on IL-1 and depression in adult population

First author Year State	Study's type	N patients, average age	Methods	Test used	Kit ELISA	Il-1 levels in depressed M+ SD	Results
Zhang 2018 China Adult	Cross sectional Case-control	50 vs 40	IL-1β, IL-6, LPS	MINI HAMD	Siemens's IMMULITE 1000	n.a.	IL-1b higer in MDD group
Cassano 2017 USA	Cross sectional Case-control	118 vs 118 healthy (M=42 yrs)	Panel of 20 IL and growth factors including IL-1β, IL-6, TNF-α, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-10, IL-13, IL-15, IFN-γ.	SCID, MADRS	Luminex	1.94 ±4.28 pg/ml	No differences
Dahl 2014 Norway	Cross sectional Case-control	50 vs 34 healthy (M=40 yrs)	IL-1β, IL-1Ra, IL-5, IL-6, IL-7, IL-8, IL-10, granulocyte colony-stimulating factor (G-CSF), and IFNγ	MINI, MADRS, IDS	Bio-Rad, Austin, Texas, USA Luminex	n.a. 0.83 pg/ml (median)	Levels of IL-1β higer in MDD group
Hernandez 2013 Mexico	Cross sectional Case-control and Longitudinal	31 vs 30 healthy (M=32 yrs)	L-1β, IL-2, IFN-γ,IL-4, IL-10, and IL-13	MINI, HDRS BDI	n.a.	n.a	Levels increase after SSRI therapy
Lu 2013 China	Cross sectional Case-control	43 vs 22 halthy (M=30 yrs)	Panel of 13 cytokines	SCID, SDS, HAMD	RayBio® Human Cytokine Antibody Array,	n.a. 176.8 pg/ml (median)	Levels of IL-1β higer in MDD patients with child trauma
Fornaro 2013 Italy	Cross sectional Case-control and Longitudinal	30 vs 32 healthy	IL-1β, IL-2, IL-4, IL-10, IL-12, IFN-γ and TNF-α	HAMD	n.a.	15.345 ±3.047 pg/ml	IL-1β level differentiate treatment responders and non responders
Diniz 2010 Brazil	Cross sectional Case-control	23 vs 44	IL-1β	HAMD	n.a.	n.a.	Levels of IL-1β higer in MDD patients
Piletz 2009 USA	Cross sectional Case-control and Longitudinal	n.a.	IL-1β, TNF-α, and other proinflammatory biomarker	n.a.	n.a.	n.a.	Levels of IL-1β higer in MDD patients
Simon 2008 USA	Cross sectional Case-control	49 vs 49	Panel of 29 cytokines	SCID	Beadlyte® Human 22-Plex Multi-Cytokine Detection System, Luminex 100 Total System (Austin, Texas).	42.53 ± 105.19	Levels of IL-1β higer in MDD patients

Hernandez 2008 Mexico	Longitudinal	31 patients	IL-1beta, IL-10, IL-2, IFN-gamma, IL-4, IL-13, and 24-h urine cortisol	HDRS BDI	DuoSet ELISA Development System from R & D systems	12.82±3.84 pg/ml	Levels of IL-1β increase after SSRI treatment
Yang 2007 China	Cross sectional Case-control	23 vs 24	IL-6, IL-1β, TNF-α and leptin	n.a.	n.a.	n.a.	Levels of IL-1β higer in MDD patients
Moorman 2007 USA	Cross sectional	129 patients with heart failure	IL-6, IL-1β, TNF-α	HAMD	n.a.	3.9±8.1 pg/ml	No differenes between depressed and non depressed
Huang 2007 Taiwan	Cross sectional Case-control	42 vs 40	IL-10, IL-1β, TNF-α	SCID HAMD	Amersham Biosciences, London, UK	4.2 ± 7.2	No differences
Leo 2006 Italy	Cross sectional Case-control	46 vs 46	IL-10, IL-1β, TNF-α and a panel of prothrombotic factors	HAMD	Quantikine HS, R&D Systems, Minneapolis, Minn.	1.58±1.23 pg/ml	Levels of IL-1β higer in MDD patients
Pavon 2006 Mexico	Cross sectional Case-control	33 vs 33 (M=33,6ysr)	TNF-α, IL-6, IL-1, IL-2, IFN-gamma, IL-4 and IL-13	HAMD MINI	R&D Systems (Minneapolis, MN	14.15±0.47 pg/mL	Levels of IL-1β higer in controls
Ferketich 2005 USA	Cross sectional	22heart failure patients	IL-6, IL-1β, TNF-α	BDI	ACE Enzyme Immunometric Assay kits (Cayman Chemical Co)	4.4± 2.1 pg/mL	No differences
Thomas 2005 UK	Cross sectional Case-control	19 vs 21	IL-1β	MADRS MMSE	Amersham Biosciences UK Limited, Buckinghamshire, U.K	2.7± 2.5 pg/mL	Levels of IL-1β higer in MDD patients
Miller 2002 USA	Cross sectional Case-control	50 vs 50	IL-6, IL-1β, TNF-α	HAMD	Dade-Behring, Deerfield, Illinois	n.a.	control subjects had signi fi cantly higher levels compared with subjects with depression
Kagaya 2001 Japan	Cross sectional Case-control	12 vs 12 (M=31yrs)	IL-1β, IL-6, sIL-2R and TNF-α	HAM-D POMS	BioSource International ,Camarillo Calif., USA	3.59± 3.89 pg/mL	No differences
Lyness 2001 USA	Cross sectional	37	IL-1β	SCID HAMD CIRS	n.a.	n.a.	IL-1beta level significantly correlated with medical illness burden
Owen 2001 UK	Cross sectional Case-control	20 vs 20	IL-1β	HAMD	n.a.	n.a.	Levels of IL-1β higer in MDD patients

Levine 1999 Israel	Cross sectional Case-control	13 vs 10	IL-6, IL-1β, TNF-α in CSF	n.a.	n.a.	n.a.	Levels of IL-1β higer in MDD patients
Brambilla 1998 Italy	Cross sectional Case-control	10 vs 10 healthy (M=72 yrs)	IL-1β, IL-6	SCID HAMD	immunoradiometric assays (IRMA), using the commercial kits of Medgenix	n.a.	No differences

SCID: Structured Clinical Interview for DSM-IV. MADRS: Montgomery–Åsberg Depression Rating Scale. MINI: Mini International Neuropsychiatric Interview. IDS: Inventory of Depressive Symptomatology.SDS: Zung's Self-rating Depression Scale (SDS). HDRS/HAM-D: Hamilton Depression Rating Scale. BDI: Beck Depression Inventory. POMS: Profile of Mood States. CIRS: Cumulative Illness Rating Scale.

Abbreviations

DD Depressive Disorders
BDNF Brain-Derived Neurotrophic Factor
CNS Central Nervous Syste
OMS Organizzazione Mondiale della Sanità
ICD International Classification of Diseases
DSM Diagnostic and Statistical Manual of Mental Disorders
SINPIA Società Italiana di Neuropsichiatria dell'Infanzia e dell'Adolescenza
TCA, ATC Tricyclic Antidepressants
SSRI Selective Serotonin Reuptake Inhibitors
CRH Corticotrophin Releasing Hormone
BD Bipolar Disorders
SA Suicide Attempt
CBT Cognitive Behavioral Theraphy
MDDMajor Depressive Disorder
NE Norepinephrine
ECT Electroconvulsive therapy
NT Neurotrophine family
5HT 5-idrossitriptamin
5-HIAA 5-Hydroxyindoleacetic acid
PKC Protein Kinase C
EMA European Agency for the Evaluation of Medicinal Products
SNRI Serotonin and Norepinephrine Reuptake Inhibitor
PTSD Post Traumatic Stress Disorder
SGAsSecond Generation Antipsychotics
ELISA Enzyme-Linked ImmunoSorbent Assay
SD Standard Deviation
HPAA Hypothalamic–Pituitary–Adrenal Axis

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