International PhD Program in Neuropharmacology XXVI Cycle

Role of psychometric tools in the diagnosis and pharmacological treatment of Major Depression and Alzheimer's disease

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PhD thesis
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Acknowledgements

I am grateful to my tutor, Prof. Filippo Caraci, for the guidance he showed me throughout my dissertation writing.

I express my sincere gratitude to Professor Filippo Drago, for introducing me to the field of neuropsychopharmacology and for his precious suggestions and support.

I also would like to thank the Coordinator of the International PhD Program in Neuropharmacology, Professor Salvatore Salomone, for its organizative support.

I owe sincere and earnest thankfulness to Professor Santo Di Nuovo, for introducing and guiding me in to the field of research, for his essential contribution to the work presented in this thesis, for his precious and continuous support and for believing in me.

I am also grateful to:
my dear colleague and friend Silvia Platania, for her precious human and scientidic support;
Nina Popovic and the other collaborators of Prof. Vieta's of the Hospital Clinic, for their kind wellcome in Barcelona and for sharing their knowledge.

It has been a pleasure to share the latter three years with my colleagues of the International PhD Program in Neuropharmacology.

Finally, a special tiribute goes to my family, example of infinite love, that allowed me to reach this goal, and to Fabio, who has continuously supported me with love and patience.

List of abbreviations

AD	Alzheimer's disease
ADAS	Alzheimer's Disease Assessment Scale
ADL	Activities of Daily Living
APOE	Apolipoprotein E
APP	β-Amyloid Precursor Protein
Aβ	β-amyloid peptide
BD	Bipolar Disorder
BDI-II	Beck depression inventory- II
BDNF	brain-derived neurotrophic factor
BES	Binge Eating Scale
CBA	Cognitive Behavioural Assessment
CBI	Caregiver Burden Inventory
CBS	Cornell-Brown Scale
COPE	Coping Orientation to Problems Experienced
CSF	cerebrospinal fluid
EOAD	early-onset familial AD
FAB	Frontal Assessment Battery
GWAS	genome-wide association studies
HC	healthy controls

HDRS	Hamilton Depression Rating Scale
IADL	Instrumental Activities of Daily Living-
ICD-10	International Classification of Diseases
IDS	Inventory of Depressive Symptomatology
IL-1A	interleukin 1A
IL-1B	interleukin 1B
IL-1RN	interleukin 1 receptor antagonist
LOAD	late-onset AD
MADRS	Montgomery-Åsberg Depression Rating Scale
MADRS	Montgomery–Åsberg Depression Rating Scale
MAOIs	monoamine oxidase inhibitors
MCI	Mild Cognitive Impairment
MDD	Major Depressive Disorder
MMPI-2	Minnesota Multiphasic Personality Inventory-2
MMSE	Mini-Mental State Examination
MoCA	Montreal Cognitive Assessment
MODA	Milan Overall Dementia Assessment
MRI	magnetic resonance imaging
MTHFR	methylenetetrahydrofolate reductase
NGF	Nerve Growth Factor
PAI	Personality Assessment Inventory

PET	positron-emission tomography
PSEN1	presenilin 1
PSEN2	presenilin 2
PUFA	polyunsaturated fatty acids
QOL-AD	Quality of life in Alzheimer's disease
SNP	single nucleotide polymorphisms
SNRIs	serotonine-noradrenaline reuptake inhibitors
SSREs	serotonin reuptake enhancer
SSRIs	selective serotonin reuptake inhibitors
TCAs	tricyclic antidepressants
TGF-β1	transforming growth factor-beta 1

Introduction

Psychometrics is the science of psychological measurement. One part of the psychometrics is concerned with the objective measurement of skills and knowledge, abilities, attitudes, personality traits, and educational achievement. Another part of the psychometrics is concerned with statistical research bearing on measurement theory. Psychometrics, then, involves two major research tasks: i) the construction of instruments and procedures for measurement; and, ii) the development of theoretical approaches to measurement.

While the classical definition of measurement adopted in the physical sciences, is that the measurement is "the estimation or discovery of the ratio of some magnitude of a quantitative attribute to a unit of the same attribute", the measurement in the social sciences is "the assignment of numerals to objects or events according to some rule" (Michell, 1997).

Theory and measurement are reciprocally related. Theory defines the content of a construct and describes the relation among constructs. Measurement of constructs can then help to revise and refine theory development.

Psychometric validation of instruments is arguably among one of the most important aspects of developing a strong empirical foundation for any field (Cook and Beckman, 2006; Stichter and Conroy, 2004). Despite this, psychometrics is frequently absent from implementation science articles (Cook and Beckman, 2006; Weiner, 2009).

Reliability and validity are the most basic and necessary psychometric properties that allow for accurate interpretation of data (Cook and Beckman, 2006; Downing and Haladyna, 1997; Osipow, 1991).

Different fields of application have been described for psychometrics. Several studies have used psychometric instruments for evaluating the clinical efficacy of a psychotropic drug suggesting the relevance of psychometrics in clinical psychopharmacology.

One of the major field of interest has been the evaluation of antidepressants drug efficacy by using old and new psychometric tools.

Efficacy trials with drugs often fail to show significant treatment effect even though efficacious treatments are investigated. This failure can be attributed, among other factors, to the lack of sensitivity of psychometric tools (Santen et al., 2008). In the studies on the effectiveness of antidepressants the Hamilton Depression Rating Scale (HDRS) is regarded as the 'gold standard'; nevertheless, some studies suggest that this tool is not sensitive enough in assessing changes in depression. Therefore, studies that use different psychometric instruments are needed. According to this scenario, Helmreich et al. (2011) conducted a study to investigate whether the Inventory of Depressive Symptomatology (IDS) is more sensitive than the HDR in detecting changes in depression symptoms in depressed patients. The authors found that both scales are well able to assess depressive symptomatology. However, the IDS surpasses the HAMD in detecting small changes especially in the core symptoms of depression. In another study Murrough et al. (2013), evaluated the rapid antidepressant efficacy of ketamine in a large group of patients with treatment-resistant major depression by the Montgomery-Åsberg Depression Rating Scale (MADRS). Herrera-Guzmán et al. (2010), studied the effects of selective serotonin reuptake inhibitors (SSRIs) and serotonine-noradrenaline reuptake inhibitors (SNRIs) treatments on affective symptoms as well as on the performance of working memory, attention and executive functions in patients with Major Depressive Disorder (MDD) by combining Hamilton Depression Rating Scale with an extended neuropsychological battery. Leombruni et al. (2009), found that duloxetine may be a successful option to reduce binge eating and depressive symptoms in binge eating disorder patients using the Binge Eating Scale (BES) and the Beck depression inventory (BDI).

The Montgomery–Åsberg Depression Rating Scale (MADRS) is a ten-item diagnostic questionnaire which psychiatrists use to measure the severity of depressive episodes in patients with mood disorders. It was designed in 1979 by British and Swedish researchers as an adjunct to the HDRS which would be more sensitive to the changes brought on by antidepressants and other forms of treatment than the Hamilton Scale was. There is, however, a high degree of statistical correlation between scores on the two measures (Heo et al., 2007).

Kongsakon and Bunchapattanasakda (2008) found that MADRS was highly sensitive in detecting superior clinical efficacy of escitalopram compared to fluoxetine and venlafaxine in MDD patients.

The Beck Depression Inventory is a self-assessment scale designed by Aaron Beck 1961 for the assessment of the severity of depressive symptoms. This scale can be used in combination with HDRS or MADRS. Bould et al. (2012), by using the Beck Depression Inventory as the outcome measure at 12 weeks, did not detect any difference in terms of clinical efficacy between reboxetine and citalopram in treating fatigue as a core symptom of depression, whereas other tools such as HDRS seemd to suggest that reboxetinewas more effective in treating depression with high levels of fatigue.

Talarowska et al. (2011), found that the higher the degree of hypochondria and hysteria symptoms, measured by the MMPI-2 test at the onset of therapy in patients with depressive disorders, the higher severity of depression was found after 8 weeks of therapy with SSRI agents, measured by the HDRS scale. Uher et al. (2011), to investigate whether subtypes of depression predict differential outcomes of treatment to SSRIs and nortryptiline (a tricyclic antidepressant) in MDD, combined MADRS, 17-item of Hamilton Rating Scale for Depression (HDRS-17) and the Beck Depression Inventory (BDI). The authors found that Melancholic, atypical or anxious depression, are not sufficiently robust differential predictors of outcome to help clinician choose between SSRI and tricyclic antidepressants. There is therefore the need to investigate other predictors of outcome with the use of many psychometric instruments.

Recent studies suggest that psychometric tools are relevant not only in the diagnosis of and treatment of MDD, but also in evaluating the effectiveness of pharmacological treatment in other neuropsychiatric diseases, such as bipolar disorder and dementia, where is essential to use multiple psychometric tools to obtain an early diagnosis as well as to monitor clinical changes observed during treatment.

Many studies have shown that affective disorders such as MDD and bipolar disorders diseases are characterized by neurocognitive deficit. Cognitive dysfunction is increasingly recognized as a symptom in different psychiatric disorders including schizophrenia, major depressive

disorder, and bipolar disorder (Etkin et al., 2013). Despite the many available cognitive assessment instruments, consensus is lacking on their appropriate use in clinical trials.

Pharmacological treatments for bipolar disorders have been associated with neurocognitive side-effects (Geddes and Miklowitz, 2013). More long-term studies are needed to better understand the impact of atypical antipsychotics on neurocognitive functioning in patients with bipolar disorder (BD), both in monotherapy and in association with other drugs. On the other hand it should be underlined that cognitive dysfunction in bipolar disorder has been demonstrated in cross-sectional studies independently from the effects of pharmacological treatments; however, there are few data regarding the longitudinal course of cognitive performance in bipolar disorder.

In this regard Sole et al. (2011) suggest that neurocognitive deficits are present across the bipolar spectrum (bipolar disorder -I and bipolar disorder -II), but they seem slightly more severe in bipolar disorder -I. A careful analysis of the cognitive function, in fact, is very important to evaluate the efficacy of long-term therapy with antidepressants or mood stabilizers and also useful to predict which drug treatment is more suitable.

Affective disorders and in particular major depression have been recently identified as new risk factors for the development of mild cognitive impairment and Alzheimer's disease (AD) (Modrego et al., 2004; Caraci et al. 2010). Common genetic factors have been found between AD and depression such as the choline acetyltransferase 4G to A polymorphism (Grünblatt et al. 2009), the brain-derived neurotrophic factor (BDNF) Val66Met functional polymorphism (Borroni et al. 2009), and Transforming-Growth Factor-ß1(TGF-ß1) (Bosco et al., 2013; Caraci et al. 2012). Recent studies also suggest common pathophysiological mechanisms between depression and AD, including chronic inflammation, hyperactivity of the hypothalamic-pituitary-adrenal axis, and an impairment of TGF-ß1 and BDNF signaling (Leonard, 2007; Borroni et al., 2010; Caraci et al., 2010).

The precise relationship between depressive symptoms and the risk to develop AD is still uncertain. Some studies suggest that depression is a prodromal feature of dementia (Wetherell et al., 1999), whereas other

studies have shown a monotonic increase in the risk to develop dementia as a function of the number of depressive episodes (Kessing et al., 2004; Dotson et al., 2010). A neurobiological and clinical continuum has been suggested between late-life depression, mild cognitive impairment (MCI), and dementia (Wuwongse et al., 2010).

According to this clinical continuum between depression, MCI and AD new strategies of neuropsychological assessments should be explored by using multiple psychometric tools able to detect both affective and cognitive symptoms from depression and MCI to early AD.

Aim of the present Doctorate Thesis is to discuss recent evidence on the role of psychometric tools in the diagnosis of MDD and AD and in the evaluation of pharmacological treatment, focusing on a new combination of psychometric tools useful to detect early cognitive deficits both in MDD patients and MCI patients with an high risk to convert into AD.

Chapter I

Psychometrics and pharmacological treatment in Major Depressive Disorder

Diagnosis of depression

Major Depressive disorder is one of the most prevalent and life-threatening form of mental illnesses and a major cause of morbidity worldwide. It is estimated that 5 to 20% of the general population suffers from it, including people with mild depressive episodes (Kessler et al., 2005).

Epidemiological studies have shown that depression is estimated to affect 350 million people. The World Mental Health Survey conducted in 17 countries found that on average about 1 out of 20 people reported having an episode of depression in the previous year (World Health Organization, 2008).

There are multiple variations of depression that a person can suffer from: (1) depressive episode, involving symptoms such as depressed mood, loss of interest and enjoyment, and increased fatigability, categorized as mild, moderate, or severe; (2) bipolar affective disorders, typically consisting of both manic and depressive episodes separated by periods of normal mood (Grosso et all. 2014).

The previous version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV - TR) described the Major Depression as a chronic disorder characterized by symptoms of the duration of at least two weeks and causing a significant impairment in social and occupational functioning.

Unlike in DSM-IV, in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). DSM-5 the chapter "Depressive Disorders" has been separated from the previous chapter "Bipolar and Related Disorders." The new version of Manual of Mental Disorder

describe Major Depression as condition characterized by the following criteria:

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 understandableor considered appropriate to the loss, the presence of a major depressive episode inaddition 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 context 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 hypomanie episode.
Note: This exclusion does not apply if all of the manic-like or hypomanic-like episodes
are substance-induced or are attributable to the physiological effects of another medical
condition.

(DSM-V, APA 2014)

Depression is associated with significant disability (Murray and Lopez, 1997) and with excess mortality particularly increasing the risk of

cardiovascular diseases (Rivelli and Jiang, 2007). In addition, depression is associated with dysregulation of circadian rhythms, high incidence of sleep disorders, and anxiety (Grosso et al., 2014).

The main symptom manifestations of Major Depression are anxiety and somatization, increase or decrease in body weight, cognitive disorders, psychomotor slowing and difficulty sleeping. In addition to mood disorders, depression is often accompanied by cognitive impairment that are identified in the domains of attention, learning, memory, and execute functioning (Lee et al., 2012).

The treatment of major depression has changed in recent years. Although additional factors, such as demographic, psychological, and biological factors, have been suggested to influence the profile and severity of neuropsychological deficits, the etiological mechanisms are not fully understood yet (Beblo et al., 2011). Even though current antidepressant drugs, such as SSRIs and SNRIs, provide a useful therapeutic tool, particularly when long lasting and with moderate or severe intensity, depression may become a serious health condition. In about 20% of cases depression follows a chronic course with low rates of remission, especially when adequate treatment is not available. The recurrence rate for those who recover from the first episode is around 35% within 2 years and about 60% at 12 years. The recurrence rate is higher in those who are more than 45 years of age (Grosso et al., 2014).

Neuropsychological assessment in MDD

Different psychometric tools are available in literature to assess symptoms of MDD and for monitoring drug treatment in this patients (Gelenberg, 2010).

However, an open question in psychopharmacology is the need to identify valid psychometric tools that can: i) measure the severity of depressive symptoms before and at the end of drug treatment; ii) detect the different patterns of response of antidepressants with different pharmacodynamic profile in order to improve the evaluation of the depressed patient before and after treatment.

In fact, has been observed by some authors that in efficacy trials the lack

of response to treatment could be attributed to a reduced sensitivity of psychometric tools adopted (Santen et al. 2008).
One of the most commonly used scales in clinical trials is the Hamilton scale for depression, validated in 1960 (Hamilton, 1960).
The Hamilton Depression Rating Scale - HDRS provides a simple way to quantitatively assess the severity of the patient's condition and to document changes in those conditions over time and after therapy. The assessment can be done in several ways, in relation to the purpose which it is proposed, but one must not forget that "the scores are only a particular way of recording the judgment of the evaluator" (Hamilton, 1967). For the evaluation the last few days and up to one week before the interview has to be taken into account.
The original 1960 version contains 17 items to be rated (HDRS-17), but four other questions are not added to the total score and are used to provide additional clinical information. Each item on the questionnaire is scored on a 3 or 5 point scale, depending on the item, and the total score is compared to the corresponding descriptor. Assessment time is estimated at 20 minutes. A score of 0-7 is considered to be normal. Scores of 20 or higher indicate moderate, severe, or very severe depression, and are usually required for entry into a clinical trial. Questions 18-20 may be recorded to give further information about the depression (such as whether diurnal variation or paranoid symptoms are present), but are not part of the scale.
Given the heterogeneity of the items, scores in the factors are generally used.
One of the most used has been proposed by Cleary and Guy, (1977) who have isolated 6 factors:

- Fatt. I: Anxiety / Somatization, composed of 6 items (10, 11, 12, 13, 15, and 17);
- Fatt. II: Weight, composed of 2 item exploring weight loss (16A and 16B);
- Fatt. III: Cognitive disorders, consisting of 6 items (2, 3, 9, 19, 20 and 21);
- Fatt. IV: Diurnal variations, composed of 2 item that explore these symptoms (18A and 18B);
- Fatt. V: Psychomotor retardation, composed of 4 items (1, 7, 8 and

14);
- Fatt. VI: Sleep disorders, consisting of 3 item (4, 5 and 6).

However, this instrument it is not sufficient to assess both cognitive and affective symptoms of depression and is not a sensitive parameter that can describe the complexity of the response to treatment with antidepressant drugs (Santen et al., 2008).
Others methods of psychometric analysis are available to detect specific clinical effects exerted by antidepressants, with different pharmacodynamic profile, on the different psychopathological dimensions of major depression in order to assess both affective and cognitive symtoms. Some of these are tools described below:

Beck's Depression Inventory (BDI-II)
The Beck Depression Inventory is a self-assessment scale designed by Aaron Beck in 1961 for the assessment of the severity of depressive symptoms and feelings associated with it.
The BDI-II is the third and most updated version of this tool, consisting of 21 multiple-choice questions aimed at detecting the severity of depressive disorders in the last two weeks.
The score shows the absence of depressive content if it is between 0-13, a mild depression if it is between 14-19, moderate depression if it is between 20-28 and severe depression if it is between 29 to 63.

Montgomery - Asperg Depression Rating Scale (MADRS)
The scale was created by Montgomery et al. in 1979 with the aim to obtain a tool more sensitive to changes in symptoms during treatment.
The MADRS consists of 10 articles that explore, through what the patient reports, humor, depression, tension, sleep disturbance and appetite, difficulty concentrating, fatigue, loss of sensitivity, pessimistic thoughts and suicidal ones. The overall rating, namely the sum of the scores of the ten elements, expresses the overall severity of symptoms.

Other tools
Many other tools are useful for assessing the severity of depression and for the monitoring of therapy, such as the Cognitive Behavioural

Assessment (CBA: Sanavio et al., 2002) which explore all the symptoms of MDD (including cognitive and somatic symptoms), whereas other instruments are essential to assess dysfunctional attitudes (Coping Orientation to Problems Experienced- COPE: Sica et al., 2008), quality of life (Activities of Daily Living – ADL: Katz et al., 1963; Instrumental Activities of Daily Living- IADL: Lawton & Brody, 1969), and personality traits (Big Five Questionnaire: Caprara et al., 1993; Rorschach test: Exner, 1993).

These tools are useful to investigate aspects related to the person in order to improve the diagnosis and treatment of depression.

Other tools are useful for the evaluation of the comorbidity of depression, such as Minnesota Multiphasic Inventory-II (MMPI-II: Butcher et al., 1989) or Personality Assessment Inventory (PAI: Morey, 2007), both useful to assess mental health problems in psychiatric and medical settings.

Pharmacological treatment of MDD

A wide variety of drugs with different mechanisms of action and pharmacological properties are currently available for the treatment of depressive disorders (Spina et al. 2012). Older or first-generation antidepressants include monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants (TCAs), which became available for therapy in the 1960s. Newer antidepressants (also referred to as second- and third-generation antidepressants) include several different classes of drugs that were developed mainly in the 1980s and 1990s, starting with selective serotonin reuptake inhibitors (SSRIs). All these antidepressant therapies are effective for treating depression, but each compound has important safety and tolerability concerns. The SSRIs are at present the most widely used agents throughout the industrialized world.

The treatment of major depression in the last fifty years was substantially based on the monoamine hypothesis. The diverse types of antidepressant treatment have different effects on the serotonergic and noradrenergic system and it is also possible that antidepressant drugs exert their therapeutic effect through more than one mechanism.

The ‘modern’ antidepressant era began in 1987 with the introduction of fluoxetine, which, like other successive SSRIs, became widely popular due to its relatively lower burden of adverse effects, safety in overdose and ease of use when compared with previously available medications, such as tricyclic antidepressants and monoamine oxidase inhibitors (MAOIs). After fluoxetine, five other SSRIs have emerged and are used in the treatment of depression; no additional SSRIs are currently in development (Connolly and Thase, 2012).

In addition to the SSRIs, several serotonin and norepinephrine reuptake inhibitors (SNRIs) have also been introduced, with a goal of producing an activity profile similar to that of the TCAs, while also providing the wide therapeutic index that made the SSRIs so popular. A number of medications with more novel approaches have also been introduced, but analogously with the SSRIs and SNRIs (as well as the prior generation of medications) these agents act primarily through the modulation of serotonin and norepinephrine transport (Connolly and Thase, 2012).

Currently available antidepressants generally demonstrate comparable efficacy in placebo controlled trials, with rates of complete remission of depressive symptoms ranging from about 30 to 45% in controlled trials (Fava, 2003). Literature meta-analyses highlighted that sertraline, mirtazapine, venlafaxine and escitalopram offer small benefits in efficacy over other antidepressants, though these advantages have been on the order of about a 5% benefit in response or remission rates (Bauer et al., 2009; Montgomery et al., 2007). Although this level of advantage may be important from a population standpoint (millions of people are treated for depression worldwide each year), the resultant number needed to treat (NNT) for benefit (~ 20 patients) is higher than generally deemed to have clinical significance.

Relatively few trials have directly compared current firstline antidepressants with the TCAs, that is, the previous standard of therapy. However, meta-analyses of the head-to-head studies directly comparing these medications, have found overall similar efficacy among outpatients, with some evidence of greater efficacy for TCAs versus SSRIs in the small number of inpatient studies (Anderson, 1998; Anderson, 2000)

As the efficacy of newer treatments is similar to older antidepressants, the success of the currently first-line agents is primarily due to their enhanced tolerability and safety profiles.

Among the current generation of first-line drugs, tolerability is overall similar, though there is some evidence of marginal advantages with individual agents.

For overall tolerability, a 'multiple treatments meta-analysis' in 2009 found that among the 12 first-line agents, escitalopram, citalopram, sertraline and bupropion had a significant advantage in tolerability over other agents, though the absolute difference was relatively small with none showing an odds ratio of more than 1.2 in its favor (Cipriani et al., , 2009).

For the SSRIs overall, dropout rates due to adverse effects have been shown to be very similar to (and often statistically indistinguishable from) placebo in clinical trials (Deshauer et. al., 2008). Nonetheless, tolerability remains an important concern in clinical practice: as many as 50% of subjects may discontinue antidepressants within a 6-month period and patients' concern over adverse effects is a main reason for discontinuation (Hunot et al., , 2007). Between agents, the side effect profiles differ somewhat. Nausea is twice as common with SSRIs as with placebo, and also more common than with bupropion or mirtazapine (Thase et al., 2005; Papakostas et al., 2008). Insomnia is also more than twice as likely with SSRIs than placebo; bupropion, venlafaxine and duloxetine show similar rates, while insomnia is less common with mirtazapine (Thase et al., 2005; Papakostas, 2010). On the other hand, somnolence may occur, again more common with SSRIs and SNRIs than placebo; bupropion shows less somnolence than mirtazapine (Thase et al., 2005; Papakostas, 2010). Sexual side effects remain a significant concern with modern antidepressants as well, and are likely underreported. SSRIs and SNRIs reliably show significantly higher rates of sexual adverse effects than placebo, and also higher rates than mirtazapine; bupropion has a very low rate of sexual side effects (Papakostas, 2010).

So, while current antidepressants enjoy a greater tolerability than the drugs they replaced, tolerability remains a potentially valuable area for pharmacological advances and, as much as with the drugs introduced in

the past 30 years, an effective antidepressant that improves on current drugs' tolerability would likely be very successful.

The potential for drug interactions represents another important issue in the evaluation of antidepressants. Multiple drug therapy is common in clinical psychiatry practice and antidepressants are often combined with medications used to treat concomitant psychiatric, neurological or somatic disorders. Polypharmacy carries the risk of drug-drug interaction. While certain drug combinations may be used advantageously, in many cases they may be harmful resulting in either decreased efficacy or increased toxicity. There are two basic types of drug interactions, pharmacokinetic (when absorption, distribution, metabolism or excretion are affected) or pharmacodynamic (when target organ or receptor sites are involved). The available antidepressant medications differ considerably in their potential for pharmacological interactions. First-generation antidepressants have been associated with a significant risk of potentially harmful pharmacodynamic drug interactions which has contributed to a gradual decline in their utilization in clinical practice, as is the case for MAOIs. In addition, TCAs have a relatively high potential for pharmacodynamic interactions as they bind to multiple receptors types (muscarinic cholinergic, α_1-adrenergic, H_1-histaminergic receptors). Although SSRIs have been considered for many years an homogeneous class of antidepressants, they are not equivalent in their potential to inhibit Cytochrome P450 isoenzymes, thus causing clinically relevant pharmacokinetic interactions with other medications (Spina et al., 2012). Although adverse drug interactions are often predictable, the use of antidepressants with a low potential for drug interactions is desirable, especially in elderly patients, who may take many medications simultaneously.

Chapter II

Psychometric tools and pharmacological treatment in Mild Cognitive Impairment and Alzheimer's Disease

Mild cognitive impairment and Alzheimer's disease: from neurobiology to diagnosis and treatment

Neurobiology and genetics of Alzheimer's disease

Alzheimer's disease is a neurodegenerative disorder characterized by the presence of β-amyloid in the senile plaques, intracellular aggregates of tau protein in the neurofibrillary tangles, and progressive neuronal loss (Hardy, 2009). AD is mainly characterized by memory loss, with disoriented behaviour and impairments in language, comprehension, and spatial skills able to interfere with the quality of life and normal daily activities. Neuropsychiatric symptoms, such as depression, psychosis and agitation are also frequent in people with AD, and are a common precipitant of institutional care (Ballard et al., 2008).

AD is the most frequent form of dementia in the population under 65 years of age. In AD, genetic variability has an incidence of about 70% (Fang et al., 2012). Three decades of genetic research in AD have led to the identification of rare, disease-causing mutations in genes encoding for the β-Amyloid Precursor Protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2) (Combarros et al., 1999; Bertram et al., 2010). These mutations cause the majority of early-onset familial AD (EOAD) through an increased production of β-amyloid (Aβ), the accumulation of which is widely thought to trigger both synaptic dysfunction and neurodegenerative phenomena in the AD brain (Hardy, 2009; Lacor, 2007). However, more than 90% of AD cases are of the late-onset form

(LOAD), which typically manifests in people older than 65 years and seem to have a separate and largely undescribed genetic etiology.

Genetics of LOAD is known to involve several genetic risk factors among which the Apolipoprotein E (APOE) gene seems to be the major recognized genetic determinant. Different studies have demonstrated the essential role of the APOE gene (with the APOE4 allele increasing risk and the APOE2 allele decreasing risk) both in familial late-onset and sporadic AD patients (Corder et al., 1993; Genin et al., 2011). Different efforts have been made to identify others genetic factors involved in the pathophysiology of LOAD (Wu and Hu, 2006). Association studies for specific candidate genes, selected because of their known biological function relevant to AD, have been performed for over 350 single nucleotide polymorphisms (SNP) of different genes including interleukin 1A (IL-1A), interleukin 1B (IL-1B), interleukin 1 receptor antagonist (IL-1RN) (Griffin and Mrak, 2002; Grimaldi et al., 2000), and methylentetrahydrofolate reductase (MTHFR) (Nishiyama et al., 2000; Coppedè, 2010). Unfortunately, data obtained in association studies have not been always replicated in different samples for different reasons such as the reduced sample size and the single-stage study design which have generally been too small for the moderate effect sizes and the substantial locus heterogeneity that we now know underlie LOAD. Replication studies that utilize a large design population are needed to confirm the association between the identified SNP and the risk to develop LOAD.

Genome-wide association studies (GWAS) represent a new and successful approach to find new candidate genes in AD (Lambert and Amouyel, 2011). We have recently participated to systematic, high-throughput genomic approaches which identified new genetic determinants involved in the pathophysiology of LOAD such clusterin (*CLU)* and complement component receptor 1 (*CR1)* (Lambert et al., 2009; Hollingworth et al., 2011). These recent studies confirm the central role of genes associated with a defect in peripheral β-amyloid (Aβ) peptide clearance, suggesting that the amyloid cascade hypothesis could be relevant not only in the AD monogenic forms (EOAD), but also in the common and late-onset forms of the disease.

According to the “amyloid cascade hypothesis”, oligomeric species composed of aggregated ß-amyloid (Aβ) are believed to cause synaptic

dysfunction and, finally, neurodegeneration in the AD brain (Hardy, 2009). Recent studies suggest that genetic deficits of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), and transforming-growth-factor-β1 (TGF-β1) might also contribute to increase the vulnerability of AD brain to the neurotoxic activity of Aβ (Cotman, 2005; Caraci et al., 2011).

Recently, a specific dysfunction of the TGF-β1 signaling pathway has been demonstrated in AD patients (Caraci et al., 2012; Tesseur et al., 2006) with a reduced expression of TGF-β type II receptor in neurons in an early phase of the disease. AD patients also showed a reduction in the plasma levels of the active (25 kDa) and inactive (50 kDa) forms of TGF-β1 (Mocali et al., 2004; Juraskova et al., 2010) as well as a reduced secretion of TGF-β1 from circulating peripheral blood mononuclear cells (Luppi et al., 2009).

The central role of TGF-β1 dysfunction in AD pathophysiology has been validated also with *in vitro* and *in vivo* models of AD, where the deficiency of TGF-β1 signaling is associated to the Aβ pathology and neurofibrillary tangle formation (Caraci et al., 2012; Wyss-Coray, 2006).

A recent study investigated the role of TGF-β1 codon 10 polymorphism as a genetic risk factor both for MCI and AD in 198 healthy controls (HC), 193 patients with LOAD and 48 patients with MCI (Arosio et al., 2007). Interestingly the authors found that the CC genotype of TGF-β1 gene significantly increased the risk to develop LOAD, independently of the APOE4 status. On the other hand the genotype and allele frequencies of +25 C → G SNP were similarly distributed in AD subjects, MCI subjects and controls. Interestingly in MCI converted to AD, after a 4-year follow-up, the percentages of both +10 C allele and CC genotype were higher than in stable MCI. The authors also showed that the CC genotype of TGF- β1 gene was associated both with reduced serum level of TGF-β1 and an increased conversion of MCI patients into AD. This was the first study which examines the role of TGF-β1 gene variants in the preclinical phase of AD, demonstrating that an impairment of TGF-β1 signaling can contribute to promote the transition from MCI into AD. Because of the small size of the sample, additional studies are needed to confirm these results in a larger sample, focusing in particular on

multiple-domain amnestic MCI patients who are at high risk to develop AD.

We have recently examined TGF-β1 +10 (T/C) and +25 (G/C) SNPs and allele frequencies in a case-control study with 131 sporadic AD patients and in 135 healthy age- and sex-matched controls. We found that allele frequencies of codon +10 polymorphism showed a significant difference between AD patients and controls. Interestingly we observed a different distribution of the +10 (C/C) genotype between LOAD patients and controls, but not between EOAD patients and controls (Caraci et al., 2012). The homozygous state for the C allele was associated with an increased risk of LOAD (more than twofold) regardless of the APOE4 genotype.

We also examined in this study the influence of the +10 (T/C) and +25 (G/C) polymorphism on the onset of AD-related depression in LOAD patients. TGF-β1 is known to be involved in the pathogenesis of depression (Adler et al., 2006; Caraci et al., 2010) and depressive disorders occur in about 30-40% of AD patients influencing the clinical evolution of the disease (Starkstein et al., 2005; Shim and Yang, 2006). We found that LOAD patients with the +10 C/C genotype showed >5-fold risk to develop depression, independently of a history of depression. A significant correlation was also found in LOAD patients between the number of TGF-ß1 +10 C alleles (0, 1, 2) and the severity of depressive symptoms as assessed by the Hamilton Rating Scale for Depression (HAM-D) (Caraci et al., 2012).

Depression is known to be a risk factor for the development of AD (Ownby et al., 2006), and the presence of depressive symptoms significantly increases the conversion of MCI into AD (Modrego and Ferrández, 2004). As observed with the +10 (T/C) and +25 (G/C) polymorphism of the TGF-ß1 gene, other genetic variations of neurotrophic factors, such as the brain-derived neurotrophic factor (BDNF) Val66Met functional polymorphism, increase the risk to develop depression in AD patients (Borroni et al., 2009) and also determine a higher risk of disease-progression in patients with MCI (Forlenza et al., 2010). It might be interesting to examine whether the +10 (T/C) functional polymorphism of TGF-β1 acts synergistically with the BDNF Val66Met functional polymorphism in increasing the risk to develop

depressive disorders in MCI and/or the following risk of conversion into AD.

According to DSM-IV TR, the dementia is a disorder of intellectual functions characterized by impairment of memory in the short and long term, and at least one of the following cognitive deficit: aphasia, apraxia, agnosia, or alteration of executive functioning (DSM-IV-TR, 2002).

It's very important to specify that cognitive deficits must be sufficiently severe to cause impairment in social or occupational functioning and must represent a deterioration from a previous level of functioning.

Spinnler (2005) defines dementia as a complex behavioral modification, resulting in defects in cognitive and psychiatric, whose evolution is systemically worse.

According to the author the key feature of the mental deterioration is based on three aspects, the trend progressive, the cognitive impairment and the inability in dealing with situations of everyday life.

In DSM 5 dementia and memory disorders are included within the category of neurocognitive disorders. Within this category, the DSM 5 recognizes a less severe cognitive impairment, a mild neuro cognitive disorder that refers to less disabling diagnosis of full-blown dementia.

Although the boundaries between the two conditions are not fully defined, there are important reasons to consider these two groups separately.

Mild Cognitive Impairment

The term "Mild Cognitive Impairment" (MCI) was introduced in the International Classification of Diseases (ICD-10) of the OMS to define the decline of mental function that does not meet the criteria for dementia (Petersen, 1995). It is an intermediate state between normal ageing and dementia.

The diagnosis of MCI is done through genetic testing, neuroimaging and neuropsychological assessment. Through the neuropsychological assessment, in fact, it is possible to detect early signs of the syndrome, and specify the subtypes based on the prevalence or less of memory disorders and to the concomitance of other deficits: amnesic or not amnesic, in both cases with the possibility of "single domain" or "multiple domain" depending on whether they are involved - either individually or

in combination - other language deficits, executive function, visual-spatial perception (Petersen, 2004). According to Petersen (2003) amnestic type is that predisposes more to the development of AD, although recent studies suggest that executive dysfunction significantly contributes to increase the risk to develop dementia (Caraci et al. 2014).

The DSM 5 recognizes a less severe cognitive impairment that refers to less disabling diagnosis of full-blown dementia and puts this disorder among neurocognitive disorders.

About half of the subjects with MCI develops within three years of diagnosis, a full-blown dementia (Petersen, 2003; Amieva et al., 2004).

The prevalence of MCI in the elderly population ranges from 3% to 6% depending on the criteria and methods used for the diagnosis (Kivipelto et al., 2001; Petersen, 2003).

MCI has become a new focus for trials to prevent or delay progression to Alzheimer's disease (Christa Maree Stephan, 2013).

Much effort is now directed to identify the most sensitive biological and neuropsychological markers which can predict the progression from MCI to AD. These biomarkers will be essential in the future to develop clinically efficacious disease-modifying drugs. Secondary-prevention trials with disease-modifying drugs might have a great socio-economic impact in the next decades because a delay in the onset of AD symptoms by 3.5 years would reduce AD prevalence by 1/3 (European Brain Council).

Neuropsychological evaluation in MCI and AD

Neuropsychological tools in combination with biological markers play a central role in the early diagnosis of Alzheimer's disease, especially in patients with amnestic MCI which show the highest risk to develop AD.

Because of the variety of symptoms and behavioral aspects associated with Dementia, the diagnosis of this disease requires different methods of evaluation, involving imaging techniques and neuropsychological assessment.

The battery of tests commonly used in the neuropsychological assessment of patients for the diagnosis of dementia is the Mental Deterioration Battery (MDB). It consists of seven tests to provide information on the

efficiency of different functional areas of cognition: verbal and visual-spatial, episodic memory, logic skills, visual-constructional ability (Spoletini et al., 2006).

The MDB, however, does not evaluate the executive functions, and is therefore not suitable for the differential diagnosis between the various forms of dementia.

Other commonly used tools for the diagnosis of dementia are the Milan Overall Dementia Assessment (MODA) and the Alzheimer's Disease Assessment Scale (ADAS: Rosen et al., 1984 Ed. En., 1996), consisting of a scale that assesses cognitive function (ADAS-cog) and a scale that describes the clinical aspects (ADAS-noncog) (Castellano and Di Nuovo, 2011).

The test most commonly test used for screening in Dementia is the Mini-Mental State Examination (MMSE: Folstein et al., 1975). The MMSE consists of 12 items exploring the following cognitive functions: temporal and spatial orientation, immediate memory, attention and calculation, memory, recall, language and visual-constructional praxis. This test is also used for the evaluation of MCI and to differentiate clinical subtypes of MCI (Diniz et al., 2007).

Another tool to assess global cognitive functions is the Montreal Cognitive Assessment (MoCA: Nasreddine et al., 2004). This tool assesses different cognitive domains: attention and concentration, executive functions, memory, language, visual-constructional skills, abstraction, calculation and orientation. It is a cognitive screening with high sensitivity and specificity for the detection of MCI.

According to a study by Larner in 2012 MoCA is more sensitive than the MMSE in detecting cognitive impairment. However, both the scores of the MMSE and the MoCA, while allowing for rapid identification of risk, are not sufficient for a detailed analysis of specific cognitive functions and do not allow for an accurate differential diagnosis.

Over the tools for the evaluation of global cognitive functioning there are many neuropsychological tests to assess specific cognitive functions. Some of these are described below:

Rey's 15 Words Test

It is an explicit verbal memory tests devised by Rey (1958) with the aim

to quantify the ability of immediate and delayed recall. The evaluation of the test of immediate and deferred are considered separately.

Verbal Memory Span

This measurement tests of memory span and verbal has been used in several batteries of Wechsler (Wechsler, 1981). The Digit Span consists of two different tests:

1. Digits Forward, which consists in the repetition of digits forward;
2 Digits Backward, which consists in the repetition of digits in reverse.

The test provides information about the performance of the auditory short-term memory and attention.

Corsi block-tapping test

The Corsi block-tapping test (Roy et al. 2000) measure the span of visual spatial memory, the amount of visual-spatial information that you can hold in short term memory.

Frontal Assessment Battery

Frontal Assessment Battery (FAB) is a short battery for screening of cognitive functions designed by Dubois et al. (2000). It is made up of 6 subtests that explore: conceptualization, mental flexibility, motor programming, sensitivity to interference, control of inhibition, environmental autonomy.

Modified Wisconsin Card Sorting Test (WCST)

Modified Wisconsin Card Sorting Test (Nelson, 1976) assess problem solving skills, flexibility in the choice of strategies and persevering. Is mainly used to explore the executive functions.

Coloured Progressive Matrices (PM)

The Coloured Progressive Matrices (Raven, 1947) highlights analytical ability independent of previously learned concepts. Spatial skills and reasoning are the main cognitive domains examined with this test.

Besides the tools that assess cognitive function and clinical aspects of dementia, there are tests that assess psychiatric symptoms associated with

AD such as HDRS, quality of life and caregiver burden such as Cornell-Brown Scale for Quality of Life in Dementia (CBS: Ready et al., 2002), Quality of life in Alzheimer's disease (QOL-AD: Logsdon et al., 2002) and *Caregiver Burden Inventory* (CBI**:** Novak and Guest, 1989).
In addition, the test Activities of Daily Living / Instrumental activities of daily living (ADL: Katz et al., 1963; IADL: Lawton et al., 1969) assess the loss of ability to perform both basic and instrumental activities of daily living.

Current pharmacological strategies in MCI and AD

Current treatment of AD includes cholinesterase inhibitors (donepezil, rivastigmine, galantamine), used for mild to moderate AD, and the NMDA receptor antagonist, memantine, approved for the treatment of moderate-severe AD, but no for MCI (Klafki et al., 2006; Mangialasche et al., 2010). These drugs mainly provide symptomatic short-term benefits without affecting the underlying pathogenic mechanisms of the disease [4], though a neuroprotective potential has also been proposed (Nordberg, 2006; Wu et al., 2009). Developing disease-modifying drugs, able to counteract the progression of AD, is one of the biggest challenges of modern pharmacology. The pathophysiological process of AD begins many years before the clinical diagnosis is set; the optimal time for disease-modifying drug treatment may therefore be in the presymptomatic stage of AD, where the disease is still hidden. Recently, the criteria for the clinical diagnosis of AD have been revised by the National Institute on Aging and the Alzheimer's Association workgroup (McKhann et al., 2011) and new criteria incorporate biomarkers to identify early stages of AD, susceptible of being treated with disease-modifying drugs (Albert et al., 2011; Sperling et al., 2011).

Disease-modifying drugs in MCI and AD: definition and implications for drug development

A disease modifying drug is an agent that slows the progression of structural damage, such that its effect is persistent and can be detected even after stopping the treatment, because the cumulative pathological changes would be less severe in the treated group compared to the control

(placebo) group. In contrast, the definition "symptomatic drug" refers to an agent that does not alter the progression of the disease, but only decrease (palliate) the severity of symptoms. The symptomatic effect is usually reversible, indeed, if the treatment is interrupted, the treated group might be indistinguishable from the control (placebo) group. Definition and validation of appropriate biomarkers and clinical outcome scales are of paramount importance for assessing efficacy of new supposedly disease modifying drug treatments for AD. Agents that target the underlying pathophysiology of AD are expected to have greater effect on biomarker levels and disease progression before any substantial irreversible functional loss occurs (Siemers, 2009). Biological markers of AD may be divided into different classes according to the "amyloid" hypothesis. Biomarkers of brain Aβ amyloidosis include both reduction in $A\beta_{42}$ in cerebrospinal fluid (CSF) (Cummings, 2011) and positron-emission tomography (PET) evidence of Aβ deposition, using a variety of specific ligands (Fagan et al., 2006). Elevated tau in CSF seems related to neuronal injury, but is not specific for AD. However, the association of elevated tau with low levels of $A\beta_{42}$ in CSF is considered the most informative biomarker of AD. Furthermore low $A\beta_{42}$ in CSF together with elevated tau might help in predicting the progression of patients with Mild Cognitive Impairment (MCI) to AD (Albert et al., 2011).

Other biomarkers are less specific, tracking indices of synaptic dysfunction and neuronal injury, such that PET measurement of fluorodeoxyglucose 18F (FDG) uptake and magnetic resonance imaging (MRI) of brain atrophy (Cummings, 2011) All these biomarkers may be very helpful in early detection of AD-related brain dysfunction. In fact, studies conducted in carriers of AD genetic risk factors, have demonstrated the presence of Aβ accumulation in CSF, positive PET amyloid imaging, FDG-PET hypometabolism and functional MRI abnormalities up to a decade before the clinical onset of AD (Sperling et al., 2011; Cummings, 2011). These biomarkers need to be prospectively tested for predictive accuracy; moreover, specific cutoff values need further validation in clinical practice. Neuropsychological and neurobehavioural tools to detect the earliest clinical manifestations of AD might be particularly useful in monitoring the response to disease-modifying therapies in amnestic MCI patients presenting a prominent

impairment in episodic memory and positive biomarkers (Albert et al., 2011). Because AD is slowly progressing, demonstrating the effectiveness of a disease-modifying treatment might require years. Most clinical studies examine 18-24 months of active treatment compared to placebo, but should provide data informative for a much longer period of time, given that patients are likely to take these medications for many years in clinical practice.

Up to now no disease modifying drugs are available for AD; several have been tested, down to phase 3, but none has yet reached approval. The failure of clinical trials with disease modifying drugs rises a number of questions, spanning from methodological flaws to fundamental understanding of AD patho-physiology and biology. Some problems may arise from: publication bias that favors positive results (van der Worp and Macleod, 2011; Dirnagl, 2006); biomarkers and clinical outcomes utilized in animal models that substantially differ from human studies; time course of treatment in relation to development of disease, i.e. clinical studies enroll symptomatic patients, where some degree of neurodegeneration is already in place.

Since the original Alzheimer's description (Alzheimer et al., 1995), Aβ production and deposition has been considered as responsible for most of the pathological mechanism of AD, because it was documented in amyloid plaques of AD subjects by *post-mortem* analysis. This view is referred to as "Aβ hypothesis". The Aβ hypothesis has recently been challenged by the observation that Aβ clearing is not necessarily accompanied by cognitive improvement (Hardy, 2009; Extance, 2010; Green et al., 2009); the physiological role of Aβ peptides, encoded also in genome of normal (healthy) population, just begins to be unraveled and might be involved in basic mechanisms of cognition and memory, such as LTP (Puzzo et al., 2011). Proper folding and aggregation state of Aβ, rather than its absolute concentration, seems to be the determinant of neuronal toxicity (Malchiodi-Albedi et al., 2011) in AD; while assessing Aβ folding and aggregation state *in vitro* or, *post mortem*, in brain, is achievable (Galvin, 2011; Grasso, 2011), this is not feasible, at present, in living human brain, which makes very difficult the use of parenchymal Aβ as AD biomarker.

Recently published new diagnostic criteria are focused on the presymptomatic stage of the disease. These new criteria will have strong implications for drug development in AD and there is an emerging consensus that future trials on disease-modifying drugs will be therefore focused on prevention rather treatment of AD.

Chapter III

Role of omega-3 fatty acids in the treatment of depressive disorders: a comprehensive meta-analysis of randomized clinical trials

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Published on Plos One 2014 May 7;9(5):e96905. doi: 10.1371/journal.pone.0096905

Abstract

Background. Despite omega-3 polyunsaturated fatty acids (PUFA) supplementation in depressed patients have been suggested to improve depressive symptomatology, previous findings are not univocal.

Objectives. To conduct an updated meta-analysis of randomized controlled trials (RCTs) of omega-3 PUFA treatment of depressive disorders, taking into account the clinical differences among patients included in the studies.

Methods. A search on MEDLINE, EMBASE, PsycInfo, and the Cochrane Database of RCTs using omega-3 PUFA on patients with depressive symptoms published up to August 2013 was performed. Standardized mean difference in clinical measure of depression severity was primary outcome. Type of omega-3 used (particularly eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) and omega-3 as mono- or adjuvant therapy was also examined. Meta-regression analyses assessed the effects of study size, baseline depression severity, trial duration, dose of omega-3, and age of patients.

Results. Meta-analysis of 11 and 8 trials conducted respectively on patients with a DSM-defined diagnosis of major depressive disorder (MDD) and patients with depressive symptomatology but no diagnosis of MDD demonstrated significant clinical benefit of omega-3 PUFA treatment compared to placebo (standardized difference in random-effects model 0.56 SD [95% CI: 0.20, 0.92] and 0.22 SD [95% CI: 0.01, 0.43], respectively; pooled analysis was 0.38 SD [95% CI: 0.18, 0.59]). Use of mainly EPA within the preparation, rather than DHA, influenced final clinical efficacy. Significant clinical efficacy had the use of omega-3 PUFA as adjuvant rather than mono-therapy. No relation between efficacy and study size, baseline depression severity, trial duration, age of patients, and study quality was found. Omega-3 PUFA resulted effective in RCTs on patients with bipolar disorder, whereas no evidence was found for those exploring their efficacy on depressive symptoms in young populations, perinatal depression, primary disease other than depression and healthy subjects.

Conclusions. The use of omega-3 PUFA is effective in patients with diagnosis of MDD and on depressive patients without diagnosis of MDD.

Omega-3 polyunsaturated fatty acids (PUFA) eicosapentaeoic acid (EPA) and docosahexaenoic acid (DHA) have been demonstrated to be effective in cardiovascular disease (CVD) prevention due to their anti-inflammatory and cardio-protective effects [1]. Recently, new therapeutic indications for omega-3 PUFA have been proposed, such as treatment for certain forms of mental illness, including depressive disorders [2]. Indeed, some psychiatric diseases as depression may share certain pathophysiological mechanisms with CVD, namely increased production of pro-inflammatory cytokines, endothelial dysfunction, and elevations in plasma homocysteine levels [3-5]. The positive effects of omega-3 PUFA on depression may depend on their physiological abundant content in the human nervous system and their involvement in neurogenesis and neuroplasticity [6]. Moreover, their anti-inflammatory capacity may counteract inflammatory processes occurring in depression [7,8]. Several ecological, cross-sectional, and prospective studies supported such hypotheses by reporting an inverse association between omega-3 intake and prevalence of depression [2]. Further clinical studies demonstrated lower concentration of omega-3 PUFA in plasma or red blood cell membranes of depressed subjects [9-13]. All together, these observations suggest a correlation between omega-3 PUFA and depressive disorders, justifing the rationale of a number of randomized controlled trials (RCTs) of omega-3 PUFA supplementation for the treatment of depressive disorders. The overall analysis of these studies from previous meta-analyses suggested a general benefit of omega-3 PUFA on depressive symptoms, despite certain variability in results weakened the possible validity of the findings. Indeed, results of such studies are not univocal, jeopardizing the evidence of therapeutic implications of omega-3 PUFA in depressed patients. It has been suggested that the heterogeneity between studies may depend on clinical and methodological issues, such as severity of baseline depression and methods of assessment and diagnosis of depression. Some important issues regarding therapeutic regimen have been explored in more recent meta-analysis, reporting that the positive effects of omega-3 PUFA on depressive symptoms appeared to depend more on EPA administration rather than DHA, severity of depression, and study quality [14]. However, some concerns regarding these findings still persist [15,16]. The analyses previously conducted

focused on the effects of omega-3 PUFA supplementation on depressive symptoms, but features associated with the pathophysiological nature of the depression occurring in the patients and their comorbidity status were often lacking. It is reasonable to believe that the biological effects of omega-3 PUFA may result effective in certain subtypes of depressive disorders rather than in others due to the different type of depression or clinical phenotype of the patient. Despite a full understanding of the processes leading to the depressive status is lacking, primary psychiatric disorders, such as major depression disorder (MDD) and bipolar disorders, are specific psychiatric conditions as recognized in the American Psychiatric Association's revised fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) [17], marking out specific depressive symptoms that should be present as inclusion criteria to determine MDD diagnosis. The mental health examination may include the use of rating scales, such as the Hamilton Rating Scale for Depression [18], the Beck Depression Inventory [19], or the MARDS [20] for MDD, and the bipolar spectrum diagnostic scale [21] for bipolar disorders. These psychiatric diseases have indeed specific biological causes and are often known to be treated with and respond to different pharmacological interventions [22]. Another specific pathological condition is perinatal depression, which indicates the occurrence of depressive and other mood-associated symptoms during pregnancy and lactation, with a range of 5-25% of women developing post-partum depression [23]. Pregnancy and lactation are challenging periods due to a higher demand of omega-3 PUFA from the fetus and the newborn, respectively, and a low DHA status may induce depressive symptoms [24]. Despite the fact that it is not clear if the depressive status is caused by or simply precipitated by pregnancy and lactation conditions, it is however likely associated with these conditions rather than with the aforementioned causes of MDD. Similarly, psychiatric disorders occurring in young populations need special attentions because major differences between adult and juvenile depression have been well-documented, despite the reasons for such dissimilarities are not clear [25]. Actually, there is very limited evidence upon which to base conclusions about the relative effectiveness of psychological interventions or antidepressant medication, but effectiveness of these interventions cannot

be fully established [26]. Finally, the occurrence of depression secondary to a different primary disease, for instance schizophrenia, Alzheimer's disease (AD), Parkinson's disease, and CVD, may raise doubts on the pathophysiological mechanisms that cause the depressive symptomatology. In addition, despite it is of interested to examine the role of omega-3 PUFA on potential mood depression in healthy subjects, it is important to underline that preventive and therapeutic pathways may differ each other. Thus, altogether, the choice in previous meta-analyses to pool together studies with such different baseline conditions, in which depression occurred, may have affected the quality of the studies as well as utilizability of the results [27,28]. Moreover, the last meta-analysis included studies up to 2010 [29]. Thus, the aim of this study was to update the current knowledge about the overall clinical efficacy of omega-3 fatty acids (particularly EPA and DHA) in previous and more recent RCTs published in the last years, minimizing, from a clinical point of view, the differences among the populations of patients included in the studies, finally focusing on patients with a DSM-defined diagnosis of MDD.

Methods

A comprehensive search on MEDLINE, EMBASE, PsycInfo, and the Cochrane Database systematic Reviews of all RCTs using omega-3 PUFA on patients with depressive symptoms published up to August 2013 was performed. Articles of potential interest were identified by using the following search terms: "omega-3", "polyunsaturated fatty acids", "PUFA", "trial", "EPA", "DHA", combined with the following terms: "depression", "depressive disorder", "depressed mood", "bipolar", combined with "perinatal", "post-partum", "CVD", "schizophrenia", "Parkinson", "Alzheimer", "diabetes", "angina". Among the 192 articles retrieved, RCTs were identified and screened by reading the abstract and, when necessary, the full text, in order to select those articles relevant for the analysis. The reference list of the relevant reports was also inspected to identify any additional trials not previously identified. The process of identification and inclusion of trials is summarized in Figure 1. Inclusion criteria were the following: (i) studies conducted on humans; (ii) randomized design; (iii) placebo controlled; (iv) use of omega-3 PUFA

supplement which relative amount could be quantified; (v) exploring changes in depressive symptoms as primary or secondary outcome. Exclusion criteria were the following: (i) studies reporting insufficient statistics or results; (ii) adopted a dietary intervention design. Study quality was measured in a 13-point scale including the Jadad criteria [30] and specific information regarding (i) registration of RCT before conducting the study, (ii) adequate blinding of the researchers, (iii) the use of an intention-to-treat analysis, (iv) control for patients' diet (i.e., number of servings of fish), (v) assessment of compliance through measurement of plasma fatty acids, (vi) significant differences at baseline, (vii) adequate sample calculation, whether (viii) depression was the primary outcome, and (ix) number and reasons of withdrawal were mentioned. Data were abstracted independently from each identified trial by GG and SM using a standard data abstraction form. This process was independently performed by two researchers and discordances were discussed and risolved.

Out of 59 originally selected studies, one [31] was excluded because of having a non-randomize non-placebo controlled design; two [32,33], because there was used a dietary intervention design; five [34-38], because the depressive status was reported as a categorical variable rather than a rating scale; two [39,40], because an inadequate or poorly comparable rating score of depression was used; two [41,42], because poorly comparable omega-3 PUFA or placebo preparations. This selection strategy resulted in a final selection of 47 studies eligible to be included in the present systematic review.

The clinical outcome of interest was the standardized mean difference in the change from baseline to endpoint scores on a depression rating scale, in patients taking omega-3 PUFA supplements *vs.* patients taking placebo. Preferred rating scales for measuring depression severity were the Hamilton Depression Rating Scale (HDRS), either the 9-item short form, 17-item, 21-item or 25-items scales, and the Montgomery Asberg Depression Rating Scale (MADRS) [20,43,44]. When available, HDRS scores from each study were used. If the HDRS was not available we used the MADRS. If neither HDRS nor MADRS data were available, we used the clinician rated measure of depression that the investigators identified as their primary outcome.

Among selected RCTs lacking in data, such as means and/or standard deviations (SDs), the data of one study [45] were provided by authors; SDs and 95% confidence intervals (CIs) of five studies [46-50] were retrieved from graphs; data of one study [51] were medians; and data of three studies [52-54] were imputed from data from all other trials using the same measure for depression as described elsewhere [55]. Eight studies [56-63] were finally excluded from the meta-analysis due to lacking data, resulting in a total number of 39 studies to be included in the analysis.

Effects due to participant diagnosis were investigated by grouping studies according to the most relevant clinical characteristics of the population on which they were conducted, as follows: (i) Depressed patients (including DSM-defined diagnosis of MDD and general assessment of depression without clinical visit); (ii) Bipolar disorder patients (including bipolar disorder during pregnancy); (iii) Children or adolescents with depression or bipolar disorder; (iv) Women with perinatal depression (including DSM-defined diagnosis of MDD and prevention of post-partum depression); (v) Mild-cognitive impairment or AD patients; (vi) Schizophrenic patients; (vii) Parkinson's disease patients; (viii) Patients with concomitant CVDs; and (ix) Healthy subjects.

Data regarding type of diagnosis, number of subjects enrolled in the trial, on-going therapy, (TRATT.) type of supplement used in the intervention, type of placebo, daily dose, duration of the intervention, outcome measures, and information to retrieve the study quality were collected. Those RCTs reporting more than one dose of omega-3 PUFA [54,64-68] or more than one formulation (i.e., EPA or DHA separately) [48,51,69], were considered as separate studies in the pooled analyses. One study [70] enrolled different populations (MDD and non-MDD patients), thus each population was also included in the meta-analysis as a separate study.

Statistical analysis

Continuous data were reported as mean and SDs and listed in descriptive tables. All depression scales' means and SDs at baseline and end of follow-up period of both intervention and control groups were combined [71] and the standardized mean effect for all trials was calculated by using

Hedges adjusted *g* in order to correct for small sample bias [72]. Both random- and fixed-effects models were used to estimate the overall effect size. Heterogeneity was investigated by using Higgins' I^2 statistic [73,74]. When heterogeneity between results of the studies exists, the random-effect models were preferred.

Possible publication bias for the analysis regarding RCTs conducted on MDD patients (MDD group, n = 11) and those not diagnosed with DSM-IV criteria (non-MDD group, n = 9) was investigated by drawing a funnel plot to look for funnel plot asymmetry [71] and meta-regression based on study size. Meta-regression was performed using linear regression, with the effect size (SMD) of trials as the dependent variable and the variables of interest as the independent variable. The generic inverse variance method was used to weight trials. Effects due to severity of depressive symptoms, age of patients, and study quality were also investigated by using meta-regression based on standardized baseline depression scores, mean age of the study participants, and our modified Jadad scores of the studies, respectively. The effects of trial duration, EPA and DHA dose in omega-3 preparations, and the use as mono or adjuvant therapy were also examined. Particularly, the qualitative analysis of the type of supplementation used was investigated grouping the studies in those using mainly EPA (EPA >50% of the dose) and mainly DHA (DHA >50% of the dose). A further analysis was computed by splitting the grouping in mainly EPA, pure EPA, mainly DHA, and pure DHA supplementation. As well, the therapeutic approach was investigated by grouping studies using omega-3 in monotherapy or as adjuvant therapy together with antidepressant drugs. The quantitative analysis of the dose was computed by a meta-regression analysis of the EPA and DHA doses used.

Random- and fixed-effects models, forest and funnel plots, and Higgins' I^2 statistics were performed in Review Manager (RevMan) version 5.2 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration), meta-regression analyses were performed in SPSS version 17 (SPSS Inc., Chicago, IL, USA).

Results

Overall studies

The most relevant features of the 47 studies included in this systematic review and meta-analysis are displayed in Table 1. Considerable differences among studies were found for all characteristics examined. The average quality of the studies was about 9 over a maximum score of 13 (range 5-13). The mean length of the trials was about 16 weeks (range 4-160), 36 studies used a mixed intervention with EPA+DHA, 14 pure EPA and 4 pure DHA. The average dose of EPA+DHA was 1.39 g (range 0.63-6.2 of EPA and 0.27-3.4 of DHA), whereas 1.93 g (range 1-6) and 0.86 g (range 0.22-2) were the average doses of pure EPA and DHA, respectively (Table 1). The most of RCTs used the Hamilton Depression Rating Scale [46,48,49,54,57,58,63,65,69,75-84], 10 studies [46,49,56,68,79,85-89] used the Beck Depression Inventory, and 13 studies [47,50,54,62,64,67,77,84,90-94] the Montgomery-Asberg Depression Scale as the main outcome measure. Among the studies not included in the quantitative analysis ,due to lack of data, one was conducted on patients with obsessive-compulsive disorder [57] and one on patients with chronic fatigue syndrome [56], both reporting no relevant effects of omega-3 fatty acids compared with placebo; four studies conducted in bipolar depressed patients [58,59,61,63] reporting that there were no significant differences on any outcome measure between the EPA and placebo groups; one study on diabetes mellitus patients with MDD [62] reporting no effect of omega-3 fatty acids on depression severity; and one on older adults with mild cognitive impairment suggesting that increased intakes of DHA and EPA can reduce depressive symptoms and the risk of progressing to dementia [60].

Depression (MDD and non-MDD groups)

A total of 19 studies were included in the first pooled analysis conducted in patients with depressive symptoms (Figure 2). Among them, 11 trials were conducted in patients with a DSM-defined diagnosis of MDD, including 8 studies conducted in adults [46,48,70,76-78,83,84] and 3 studies in elderly patients [53,95,96]. The pooled standardized difference in means using a fixed-effects model for the MDD group was 0.47 SD (95% CI: 0.29, 0.66), which suggests a beneficial effect of omega-3 fatty acids on depressed mood compared with placebo in patients with diagnosis of MDD. The pooled standardized difference in means in a

random-effects model was 0.56 SD (95% CI: 0.20, 0.92). The remaining 8 were those conducted on patients with an assessment of depression but not rigorously diagnosed according to the DSM criteria, and included patients with depressive symptoms despite on-going treatment [54,69,79,97], women with borderline personality disorder [91], patients with recurrent self-harm [49], people with mild to severe depressed mood not taking medications [86], post-menopausal women with psychological distress and depressive symptoms [70], and subjects with a history of at least one major depressive episode [89], whereas two studies were excluded due to lack of data [56,57]. Despite patients pooled in this analysis were not homogeneous in terms of health status, all studies clearly reported to have included subjects with no other psychiatric or neurological illnesses such as AD, Parkinson's disease, as well as no history of any end-stage diseases, CVDs, or any unstable medical conditions, thus to make them comparable each other for our purposes. Similar results were found for this group of patients (standardized mean difference – fixed effects-model: 0.15 SD, 95% CI: 0.01, 0.30; random-effects model: 0.22 SD, 95% CI: 0.01, 0.43). For both MDD and non-MDD groups, there was evidence of heterogeneity (MDD group, I^2 = 71%, P <0.001; non-MDD group, I^2 = 46%, P = 0.04).

The overall analysis including both groups was conducted to assess whether results were different considering a mood-improving effect on depressive symptoms in patients with non-organic, metabolic, nor genetic-related neurodegenerative disease. The pooled standardized difference in means using a fixed-effects model was 0.27 SD (95% CI: 0.16, 0.39), and the pooled standardized difference in means using a random-effects model was 0.38 SD (95% CI: 0.18, 0.59). However, there was evidence of heterogeneity (I^2 = 65%, P <0.001). To test this heterogeneity, a funnel plot was drawn and is shown in Figure 3.The funnel plot did not show considerable evidence of asymmetry. Meta-regression of study effect size, based on study size, did not present significant association (regression coefficient = -0.108, 95% CI: -0.224, 0.012; P = 0.066) indicating no role of the sample size in determining the results of the analysis.

A meta-regression analysis was performed of standardized mean depression scores on baseline depression scores to test whether the gravity of depression at baseline may play a role in the efficacy of omega-3 fatty supplementation. The analysis showed no relation between baseline depression scores and efficacy for all studies (regression coefficient = 0.019, 95% CI: -0.009, 0.047; *P* = 0.167) as well as for MDD patients (regression coefficient = 0.008, 95% CI: -0.053, 0.068; *P* = 0.787) and non-MDD (regression coefficient = 0.019, 95% CI: -0.017, 0.054; *P* = 0.270) separately. Even taking into account the comparison of studies using the same depression scale (HDRS), no significant relation between baseline depression scores and efficacy was found (data not shown).

Analysis conducted to explore the role of type (namely, the administration of mainly EPA or DHA supplementation) and dose (separately for EPA and DHA) of omega-3 supplement used showed that the use of mainly EPA within the preparation, rather than DHA, appeared to influence final clinical efficacy (standardized mean difference – fixed effects-model: 0.46 SD, 95% CI: 0.31, 0.61; random-effects model: 0.50 SD, 95% CI: 0.27, 0.72) (Figure 4). Despite heterogeneity fallen by 55%, it remained significantly high (*P* = 0.002). When the analysis was split in mainly EPA, pure EPA, mainly DHA, and pure DHA supplementation, both the EPA preparations were significant (for pure EPA, standardized mean difference – fixed effects-model: 0.40 SD, 95% CI: 0.19, 0.61; random-effects model: 0.43 SD, 95% CI: 0.18, 0.68) and the heterogeneity fallen to 28% (*P* = 0.19). This result indicates that despite the overall heterogeneity represented an underlying true difference in effect sizes across studies, it may be strongly affected by type of formulation of omega-3 fatty acids used.

The meta-regression analyses exploring the role of the dose of omega-3 fatty acids revealed that the total dose of DHA were unrelated to efficacy (regression coefficient = -0.066, 95% CI: -0.471, 0.603; *P* = 0.801), whereas the dose of EPA formulation resulted related to efficacy both for all MDD plus non-MDD patients (regression coefficient = 0.477, 95% CI: 0.084, 0.869; *P* = 0.02). However, when the analyses was repeated separately for each group, the association remained significant only for

MDD patients (regression coefficient = 0.746, 95% CI: 0.100, 1.392; *P* = 0.028) whereas lost significance for non-MDD patients (regression coefficient = 0.215, 95% CI: -0.288, 0.718; *P* = 0.359).

No relation between study size (regression coefficient = -0.109, 95% CI: -0.231, 0.012; *P* = 0.075, baseline depression severity (regression coefficient = 0.026, 95% CI: -0.007, 0.060; *P* = 0.116), trial duration (regression coefficient = -0.058, 95% CI: -0.153, 0.038; *P* = 0.223), age of patients (regression coefficient = 0.013, 95% CI: -0.10, 0.036; *P* = 0.879), and study quality (regression coefficient = -0.142, 95% CI: -0.357, 0.072; *P* = 0.183) and omega-3 PUFA efficacy was found, despite study quality almost reached significance when considered only for RCTs conducted on patients with MDD (regression coefficient = -0.403, 95% CI: -0.857, 0.052; *P* = 0.077). On the contrary, fixed- and random-effect models of RCTs grouped by use of omega-3 PUFA as mono- or adjuvant therapy revealed a significant effect when they were used in combination with standard antidepressant therapy (standardized mean difference – fixed effects-model: 0.26 SD, 95% CI: 0.09, 0.44; random-effects model: 0.39 SD, 95% CI: 0.06, 0.71).

Bipolar disorder

In our systematic review we collected 7 trials conducted on patients with bipolar disorder (both type I and II) [58,59,61,63,65,75,80] (Table 1). The only three studies pooled for the analysis included one study [65] that accounted for more than 70% of the weight of the analysis, that together with others [75,80] resulted in a significant effect of omega-3 fatty acids in ameliorating depressive symptoms in adults with bipolar disorder (standardized mean difference – fixed effects-model: 0.73 SD, 95% CI: 0.39, 1.07; random-effects model: 0.74 SD, 95% CI: 0.38, 1.10; I^2 = 9%, *P* = 0.35) (Figure 5).

Depression or bipolar disorder in children and adolescents

Among the studies conducted on depression occurring in youth, one study [98] documented a positive effect of omega-3 fatty acids in improving the mood of children diagnosed of MDD and one study conducted on adolescents at high risk of psychosis [92] reported that omega-3 fatty

acids significantly reduced positive symptoms, negative symptoms, and improved functioning compared with placebo, but no significant effect was observed on depressive symptoms.

Perinatal depression

There were six trials aiming to explore the effects of omega-3 PUFA on perinatal depression. We distinguished between those studies conducted on pregnant women with MDD [82,93,99] (Figure 6) and those on apparently healthy women (primary prevention) [51,68,85] (Figure 7). However, both analyses led to inconclusive results (MDD in pregnancy, standardized mean difference – fixed effects-model: 0.08 SD, 95% CI: -0.29, 0.45; random-effects model: 0.24 SD, 95% CI: -0.73, 1.21; prevention of post-partum depression, standardized mean difference – fixed effects-model: 0.05 SD, 95% CI: -0.24, 0.15; random-effects model: -0.05 SD, 95% CI: -0.24, 0.15). Only one study [82] concluded that omega-3 fatty acids might have therapeutic benefits in depression during pregnancy. Besides the clinical efficacy of omega-3, in regard to the safety issue, it is important to underline that omega-3 fatty acids supplementation was well tolerated and no adverse effects were reported on the subjects treated and newborns in all studies.

Depression as secondary outcome

Among the trials conducted in patients with primary disease other than depression, those conducted on AD or mild cognitive impairment [50,81] (Figure 8), schizophrenia [54,90] (Figure 9), and CVDs [87,94,100] (Figure 10) reported inconclusive results, whereas the only study conducted on Parkinson's disease patients in comorbidity with MDD [47], including those treated with antidepressants and those without, reported improvement in depressive symptoms and indicate that the intake of omega-3 PUFA can be used as adjuvant therapy in Parkinson's disease patients. However, in one study conducted on schizophrenic patients with persistent ongoing symptoms [54], the authors reported a large placebo effect in patients on typical and new atypical antipsychotics and no difference was observed between active treatment and placebo, but in patients on clozapine, there was a clinically important and statistically

significant effect of 2 g/day omega-3 PUFA treatment on the PANSS and its sub-scales.

Depressive symptoms in healthy subjects

The trials conducted on healthy subjects aimed to explore potential beneficial effects of omega-3 fatty acids as mood improving medicaments in the general population (Figure 11). Among the tot studies included [52,66,67,88,101,102], the overall analysis showed a nearly null effect of this supplement on depressive symptoms in healthy subjects (standardized mean difference – fixed and random effects-model: 0.00 SD, 95% CI: -0.13, 0.13).

Discussion

We demonstrated that the use of omega-3 PUFA as therapeutic agents was effective in patients with diagnosis of MDD and on depressive patients without a diagnosis of MDD, whereas inconclusive results were found for patients with other pathological conditions (namely schizophrenia and AD) as well as in healthy subjects and perinatal depression. The analysis of the studies on bipolar disorder showed a positive effect of the omega-3 PUFA, but the evidence is weakened due to the exclusion from the quantitative analysis of three studies that may affect the overall effect of the supplement. When the studies conducted on patients with MDD or those on patients with depressive symptoms but not rigorous evaluation by health professionals were pooled together, a general positive effect of omega-3 PUFA was found.

As previously reported [15], the studies that mostly negatively influenced the pooled results of the non-MDD patients included non-homogenous individuals, since their enrolment was in settings such as general practice surgeries, shopping malls, and university freshman fairs [86], newspaper, radio and television advertising, and flyers posted [70], and through a Community Mental Health Service, general practices, and advertisements in community newspapers [79]. Despite the idea of a widely available low cost supplement that could assist those being treated for a current depressive episode in a community setting is highly desirable, a lack of rigor in patients' selection may lead to the inclusion of subjects with

normal emotional states, eventually affecting the results and, thus, challenging the model's credibility. It is noteworthy that negative results came out mostly from studies sharing this methodology [70,79,86]. Moreover, as reported by the authors [79,86], both experimental and control groups improved significantly, usually indicative of a major placebo response which is expected to exert a meaningful clinical effect in the treatment of such "subthreshold" depressed subjects [103]. A recent meta-analysis demonstrated that the relative efficacy of the active drug compared to placebo in clinical trials for MDD is highly heterogeneous across studies, with a worse performance in showing a superiority of the drug *versus* placebo for studies with placebo response rates ≥30% [104]. Thus, the studies quality decreased when placebo response rates were not mantained below this critical threshold that may depend on the non-homogenous depressive "phenotypes" of the subjects enrolled. The non-MDD group also included four studies conducted on patients with depressive disorders despite ongoing antidepressant therapy [54,69,79,97]. These results should be considered with caution, because these studies may include those "non-responder" subjects that generally fail to reach remission with the first anti-depressant therapy and have higher relapse rates and poorer outcomes than those who remit [105]. Studies conducted in this subgroup of patients can explain not clearly favorable effects of omega-3 PUFA on depressive symptoms in these studies and puzzling results.

Previous meta-analyses included all RCTs with little distinction among population groups, leading to controversial results, such as overall benefit [106,107] and negligible effects [27,28] of omega-3 PUFA against depressive symptoms, especially due to the high heterogeneity of studies. The following studies improved some methodological issues (i.e., better definition of inclusion criteria, especially in the distinction between the definition of MDD and other depressive disorders) and focused attention on specific aspects of omega-3 administration (i.e., dosage, EPA:DHA ratio) leading to the conclusion that administration of EPA, rather than DHA, is responsible for the beneficial effects of omega-3 PUFA intake as therapeutic agents in patients with depressive disorders [108,109] and supplements containing EPA ≥ 60%, in dose range from 200 to 2200 mg

EPA in excess of DHA, were effective against primary depression . On the contrary, the last meta-analytic study [14] reported small, non-significant benefit of omega-3 PUFA for the treatment of MDD, generally in contrast with the aforementioned previous meta-analyses, but some methodological issues in study selection have arisen [15,16]. Taking into account that pathophysiological processes of depressive symptoms involved in MDD patients are likely to be very different from those in patients with depression occurring in other clinical conditions (i.e., bipolar disorder, pregnancy, primary diseases others than depression) and in non-homogenous patients (i.e., community sample of individuals), we used a different approach to analyze the RCTs using omega-3 PUFA supplementation against depressive symptoms, grouping the studies by type of diagnosis of depression and taking into account any possible health condition that may influence the onset of the depression as well as the response to therapy. Other meta-analyses reported that the more severe was the depression, the more likely omega-3 PUFA supplementation would reduce depressive symptoms. We failed to demonstrate such a result, and we consider this finding as a surrogate of our observation that, overall, the efficacy of omega-3 PUFA was mostly related to a specific DSM-based diagnosis of MDD. Hence, this latter has been translated in a correlation of efficacy to more severe symptoms whereas, according to our results, we hypothesized that this efficacy may be more related to the specific pathophysiological processes of the MDD rather than to its severity. Compared with previous meta-analyses, the differences of findings may depend on the additional number of RCTs published since the publication of the last study [37,40,53,60,61,66,68,69,84,89,92,94-97,100-102], the increasing number of participants which vary the overall weight of previous studies, the requirement for public registration of trials resulting in an increase of general studies' quality and may be responsible for the decreased evidence of publication bias.

Since the pathophysiological mechanisms and the therapeutic approach for bipolar disorder differ from those of MDD [22,23], when previous analyses included and pooled findings of studies conducted on these groups of different patients, they led to inconclusive results. It has been

hypothesized that the efficacy of omega-3 PUFA may be different in the depressive phase rather than the maniacal episode [110], and recent systematic analysis of trials focused on this topic showed positive effects of omega-3 PUFA as an adjunctive treatment for depressive but not mania in bipolar disorder patients [111,112]. Thus, we separately grouped the studies conducted on patients with bipolar disorder and explored efficacy of omega-3 PUFA in ameliorating the depressive symptoms, finding a significant efficacy of the supplement in two [65,75] out of the three trials. Despite the positive results, it is noteworthy to underline that we had to exclude, due to missing of data, four studies [58,59,61,63] conducted on bipolar patients reporting poor effect of the omega-3 PUFA intervention, thus weakening our findings. There is a need of well-designed, high quality studies, which may clarify the potential effects of omega-3 PUFA supplement in patients with rigorously diagnosed bipolar disorder.

Regarding the substantial inefficacy of the omega-3 PUFA in patients with primary diseases other than depression, it may be possible that these studies are more likely to suffer from publication bias, since depression was often a secondary outcome. Despite this methodological issue, the effects of the omega-3 PUFA may have been also affected by factors particularly related to the primary disease. Regarding the studies conducted on patients with CVDs, the analysis included very heterogeneous populations, namely patients with coronary heart disease [87], with diabetes mellitus [94], and post myocardial infarction [100], that may have been responsible for the inconclusive results. Moreover, it has been recently reported that supplementation of EPA in diabetes mellitus patients with comorbid MDD poorly affect biological risk factors for adverse outcome observed in this category of patients [113]. The RCTs conducted on patients with mild cognitive impairment or AD revealed poor efficacy of omega-3 PUFA in ameliorating the depressive symptoms. It has been reported that molecular mechanisms and pathways that underlie the pathogenesis of depression (i.e., impairment in the signaling of some neurotrophins such as Transforming-Growth-Factor-β1 and Brain-derived-neurotrophic-factor) are also involved in the pathogenesis of AD [114,115], thus the omega-3 PUFA supplementation

may not be the optimal pharmacological approach for this specific group of patients [116-118]. The two trials (including different dosages) conducted on schizophrenic patients with persistent ongoing symptoms resulted in limited effects of the omega-3 PUFA on patients' affective states. These results may be attributable to some psychotic symptoms (i.e., negative symptoms) that may directly influence (i.e., improve) depression-rating scores. Moreover, these patients were receiving different types of antipsychotics such as first- and second-generation antipsychotics that may differently affect (positively or negatively) the final effects of omega-3 PUFA on depressive symptoms. Finally, depression examined as secondary outcome could suffer by changing of the measurement depending on the improvement (or worsening) of the underlying primary disease.

Regarding the different efficacy of EPA compared with DHA and EPA-DHA combinations, the analysis of RCTs grouped according to type of omega-3 PUFA administered confirmed the findings of previous meta-analysis and substantial stronger pooled results of studies using EPA rather than DHA. However, as previously reported [109], the aforementioned methodological issues may have biased the results in favor of efficacy for EPA-containing preparations suggesting that the reported benefits on depressive symptoms in this group of studies may not therefore be definitively attributed only to the EPA content of the supplementation regimen and also that further studies are needed in this field. Whether EPA, rather than DHA, is effective in ameliorating depression in specific groups of patients, the different effects of these classes of omega-3 PUFA is a challenge to be explained convincingly, since DHA is a major structural component of neuronal membranes, and we can hypothesize that increasing its nutritional availability would have beneficial effects on brain function, rather than EPA, which is present at levels several hundred-fold lower [120]. Possible explanations of the beneficial role of EPA are the following: (i) the anti-inflammatory effects of EPA-derived eicosanoids [121] and its oxidized derivatives [122] (ii) its efficacy at reducing the inflammatory cytokines tumor necrosis factor-alpha (TNF-α), IL-6, and IL-1b [123] through inhibition of the activity of nuclear factor kappa-B (NF-kB) [124]; (iii) *in vivo* evidence of a more

effective anti-inflammatory action of dietary EPA compared with DHA [125]. Moreover, DHA has been reported to be poorly incorporated in the human brain [126], and EPA may facilitate an increase in brain DHA levels after its conversion [127]. Finally, EPA supplementation has been associated with N-acetyl-aspartate increase in brain, a marker for neuronal homeostasis, suggesting its role as a neuroprotective agent [80]. Together with the inflammation theory of depression [8], chronic intake of omega-3 fatty acids has been reported to play an important role in neuronal structure and function [128]. However, such hypotheses are not completely exhaustive and further research is needed to better identify the specific molecular mechanisms underlying clinical efficacy of omega-3 PUFA (both EPA and DHA) in preventing or ameliorating depression.

The studies excluded from this systematic review were not comparable in terms of methodology used, and their exclusion was needed in order to reduce differences among RCTs and improve data quality (i.e., reduce selection bias). On the other hand, these trials may still be directly relevant to the topic of the present study, and a specific discussion (e-discussion) may strengthen conclusion retrieved from this meta-analysis. Moreover, we discussed in a specific section of the e-discussion about the studies quality and potential sources of heterogeneity.

The main limitation of this study was the inability to control all the many potential sources of heterogeneity. Despite the fact that a logical grouping of trials was performed, a non-modifiable degree of heterogeneity, due to specific characteristics of all trials included, still weakened the pooled analysis of these studies. However, compared with older studies, the inclusion of the updated RCTs strengthened the conclusions of the effects of omega-3 PUFA intake on depressive disorders.

To sum up, trials conducted in individuals with a diagnosis of MDD provided evidence that omega-3 PUFA supplementation has beneficial clinical effects on depressive status. Evidence of their efficacy was provided also for patients with bipolar disorder, whereas no evidence was found for individuals included in the other diagnostic groups. According to our findings, in RCTs with omega-3 PUFA supplementation in healthy

subjects and patients with schizophrenia, AD and CVD seems to result ineffective.

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Chapter IV

Searching for disease modifying drugs in AD: can we combine neuropsychological tools with biological markers ?

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# International PhD Program in Neuropharmacology

Published on CNS Neurol Disord Drug Targets. 2014 Feb;13(1):173-86

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Keywords: Alzheimer's disease, MCI, biological markers, neuropsychological tests, disease-modifying drugs, risk factors.

Abstract

Drug discovery processes in Alzheimer's disease (AD) have been directed in the last ten years to develop "disease-modifying drugs" able to exert neuroprotective effects in an early phase of AD pathogenesis. Unfortunately several candidate disease-modifying drugs have failed in Phase III clinical trials conducted in mild to moderate AD for different methodological difficulties such as the time course of treatment in relation to development of disease as well as the appropriate use of validated biological and neuropsychological markers.

Mild cognitive impairment (MCI) has been considered a precursor of AD. Much efforts are now directed to identify the most appropriate and sensitive markers which can predict the progression from MCI to AD, such as neuroimaging markers (e.g. hippocampal atrophy and amyloid PET imaging), CSF markers (i.e. association of elevated tau with low levels of $A\beta_{42}$) and neuropsychological markers (i.e episodic memory deficits and executive dysfunction).

Recent studies demonstrate that the combination of these different biomarkers significantly increases the chance to predict the conversion into AD within 24 months. All these biomarkers will be essential in the next future to analyze the clinical efficacy of disease-modifying drugs in MCI patients at high risk to develop AD.

In the present review we analyze recent evidence on the combination of neuropsychological and biological markers in AD as a new tool to track disease progression in early AD as well as the response to disease-modifying drugs.

Alzheimer's disease: from the pathogenesis to disease-modifying drugs

Neurodegeneration is the main histopathological feature of Alzheimer's disease (AD), a devastating disorder affecting more than 24 million people worldwide, with 3 million new cases of this condition arising every year [1]. The incidence rate for dementia increased exponentially with age, with the most notable rise occurring through the seventh and eighth decades of life [1]. Because of the increasing life expectancy, the

number of affected individuals is predicted to grow to 42 million in the year 2020 [2].

AD is mainly characterized by memory loss, with disoriented behaviour and impairments in language, comprehension, and spatial skills. Neuropsychiatric symptoms, such as depression, psychosis and agitation are also frequent in people with Alzheimer's disease, and are a common precipitant of institutional care [3]. AD is characterized by the presence of ß-amyloid (Aβ) in the senile plaques, intracellular aggregates of tau protein in the neurofibrillary tangles (NFT), and progressive neuronal loss [4]. According to age at onset, two major types of AD are generally differentiated: early-onset forms beginning before the age of 65 (EOAD) and late-onset forms beginning after 65 (LOAD). Three decades of genetic research in AD have led to the identification of rare, disease-causing mutations in genes encoding for the β-Amyloid Precursor Protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2) [5]. These mutations cause the majority of early-onset familial AD through an increased production of Aβ, the accumulation of which is widely thought to trigger neurodegenerative phenomena in the AD brain. Moreover, genetics of LOAD includes other risk factors, such as Apolipoprotein E (APOE) variants, with the APOE4 allele increasing risk and the APOE2 allele decreasing risk, both in familial late-onset and sporadic AD patients [6, 7].

Genetic studies in patients with familial AD, as well as evidence from transgenic animal models, suggest a primary role for Aß in the pathogenesis of AD [8, 9]. According to the "amyloid hypothesis", accumulation of the 42 a.a. long Aß protein, resulting from an excessive production (derived from missense mutations in specific genes linked to familial AD) or from a reduced cerebral clearance (as observed in sporadic AD especially in ApoE4 carriers), might lead to the aggregation of monomeric Aβ(1-42) species into higher molecular weight oligomers [10]. Aß oligomers are believed to exert toxic effects on synaptic and cellular functions, finally leading to neurodegeneration and cognitive as well as neuropsychiatric symptoms in AD patients [9, 10]. Although both amyloid‐ β and tau have been separately studied for different years with regard to their role in AD pathogenesis, recent evidence suggests that the

two major histopathological hallmarks of AD, i.e. Aβ deposits and NFT, containing hyperphosphorylated tau, lie along the same pathological cascade and also that tau-related events are essential for AD pathogenesis [11]. Aβ accumulation precedes and drives tau hyperphosphorylation, which in turn increases the vulnerability of neurons to the toxic effects of Aβ [11]. Furthermore tau aggregates are associated with both cognitive neuronal losses and are better indicators of disease progression [12].

The mainstay of current management of patients with AD involves cholinesterase inhibitors (i.e., donepezil, rivastigmine, galantamine), which are used for mild to moderate AD, as well as the N-Methyl-D-aspartate (NMDA) receptor antagonist, memantine, which is approved for the treatment of moderate-severe AD [13]. Unfortunately, these drugs provide mainly symptomatic short-term benefits, without affecting the underlying pathogenic mechanisms of the disease, such as Aß oligomers toxicity or tau hyperphosphorylation [14]; much effort is therefore now directed to develop disease-modifying drugs, able to counteract the progression of AD. A disease-modifying drug is an agent that slows the progression of neurodegenerative phenomena in AD, such that its effect is persistent and can be detected even after stopping the treatment, because the cumulative pathological changes would be less severe in the treated group compared to the control (placebo) group [15].

In the last ten years several "potential disease-modifying drugs" able to exert neuroprotective effects in preclinical models of AD have been developed. Unfortunately these drugs have failed in Phase III clinical trials, especially in mild to moderate AD. Different factors might explain the failure of these trials. So far, clinical trials on disease-modifying drugs have been limited by the difficulty of making an early diagnosis of AD. In AD, neurodegeneration precedes overt symptoms by several years; therefore, neuroprotective drug treatments initiated after the onset of symptoms, have been tested when their time window of maximal efficacy had been already expired. However, the scene seems now less pessimistic. The recent National Institute of Aging (NIA) and Alzheimer's Association (AA) work group (NIA-AA) revision of the diagnostic criteria enlarges the window for the detection of the disease to the early stages of the disease and there is an emerging consensus that future trials

on disease-modifying drugs will be therefore focused on prevention rather than on treatment of AD [16-18].

Mild cognitive impairment (MCI) has been considered as a precursor of AD, although only a proportion of individuals with MCI patients (50-70%) develop dementia within the next 5 to 7 years [19], suggesting that clinical MCI symptoms can also stem from non-AD related etiologies. MCI is characterized by a progressive cognitive decline in memory (amnestic MCI) or other cognitive domains (non-amnestic MCI). The criteria for MCI diagnosis require the report of subjective cognitive impairment by patients themselves or their relatives in addition to cognitive impairment as assessed by neuropsychological tests, without affecting social functioning and instrumental activities of daily living [20]. Conversion rates from MCI to AD range from 4% to 23% in community-based and 10% to 31% in clinic-based samples [21]. This variability may be related to differences in diagnostic criteria and sample selection. Recently revised criteria for the diagnosis of AD from NIA-AA work group do not consider mandatory anymore the presence of a deficit in the episodic memory domain, but require the presence of an impairment in amnestic or non amnestic cognitive abilities [18]. The main difference for the clinical diagnosis of AD, as opposed to MCI, is the presence of impaired social functioning and instrumental activities of daily living [18]. According to the recent published criteria (2011), the NIA-AA work group has recommended purely clinical and neuropsychological criteria for the diagnosis of AD dementia and MCI, but a dual clinico-pathological definition has also been proposed, as research diagnostic criteria only, by combining clinical testing with biomarker measurements. Along this line "MCI due to AD" include subjects with the core clinical criteria of MCI, which also show pathophysiology of AD as confirmed by biomarkers based on imaging and cerebrospinal fluid (CSF) measures [16, 17].

MCI due to AD is a research construct which should be validated in future studies conducted in epidemiological community-based populations. This construct might represent the future target of disease modifying therapies.

Successful prediction of conversion from MCI to AD is a fundamental step for the enrichment of clinical trials of disease modifying therapies

which aim to slow or prevent AD. Much efforts are now directed to identify the most appropriate biochemical, neuroimaging and neuropsychological markers which can predict the conversion from MCI to AD [22].

In the present review we will discuss recent evidence on biological and neuropsychological markers in AD and the possible combination of these biomarkers as a new strategy to improve both the diagnosis of AD in an early phase of the disease (i.e. MCI due to AD) as well as to monitor the response to disease-modifying drugs in future clinical trials.

Biological markers in AD

Identification of sensitive and specific biological markers for AD is one of the major challenges in the field of neuroscience. Definition and validation of appropriate biological markers are of paramount importance for assessing efficacy of new supposedly disease modifying drug treatments for AD. Potential disease-modifying drugs for AD are likely to be most effective when given in non-demented subjects and should be therefore tested in the pre-symptomatic stage of AD with the aid of biological markers related to the underlying cerebral AD pathology.

Drug discovery processes in the last ten years have been directed to identify disease-modifying drugs endowed with a neuroprotective activity. These drugs have been studied in transgenic models of AD, where they have demonstrated a neuroprotective effect preventing Aβ-induced neurodegeneration and tau hyperphosphorylation only when administered in an early phase of the disease [15]. On the other hand most of these drugs have been tested in clinical trials in AD patients with a too advanced stage of the disease when substantial neuronal and synaptic loss has occurred and therefore their time window of maximal neuroprotective efficacy had been already expired. This translational disconnect between preclinical animal models and clinical outcome might be resolved in future clinical trials only selecting “MCI due to AD” patients which show pathophysiology of AD as confirmed by biological markers, where it may be possible to initiate effective disease-modifying treatments before the onset of clinical dementia. This would be consistent with the medical approach adopted in other chronic diseases such as diabetes and

congestive heart failure, i.e. secondary prevention , not waiting until there is significant organ failure before initiating therapy.

According to this scenario biological markers might become essential in the near future to predict disease progression prior to the development of overt dementia [23]. Furthermore, disease-modifying drugs for AD that target the underlying pathophysiology of AD are expected to have greater effect on biomarker levels and disease progression if used before the occurrence of substantial neurodegeneration [22].

Different biological markers are actually available to detect the development of AD dementia such as neuroimaging biomarkers, genetic biomarkers (i.e Apo E4 allele) and CSF-derived biomarkers [22]. The Alzheimer's Disease Neuroimaging Initiative (ADNI) is a five year research project that began in 2004 and was designed to study the rate of change of cognition, function, brain structure and biomarkers in cognitively normal elderly, persons with MCI and AD. This project, together with other important longitudinal studies, has provided the means to begin mapping the interrelationships among biomarkers and cognitive and clinical disease features, improving early diagnosis of AD as

well as other aspects of trial design [22-25]. Neuroimaging biomarkers are used to detect *in vivo* brain changes associated with neurodegeneration and cognitive decline in AD patients as well in MCI patients with an high risk of conversion in AD [26]. According to the "amyloid hypothesis" Aβ accumulation precedes and drives tau hyperphosphorylation as well as synaptic dysfunction and neuronal injury [4]. Some neuroimaging techniques can detect Aβ accumulation and deposition (PET amyloid imaging), whereas others techniques, such as fluorodeoxyglucose positron-emission tomography [(FDG)-PET], structural and functional magnetic resonance imaging (MRI), can detect synaptic dysfunction and neuronal injury in an early phase of AD pathogenesis [22, 25]. PET is a valuable tool to visualize and quantify Aβ deposition in AD patients in an early phase of the disease, using a variety of specific ligands such as [^{11}C]-labeled Pittsburgh Compound-B (PiB) or [^{18}F]-labeled tracers such as 2-(1-(6-[(2-[^{18}F]fluoroethyl)(methyl)amino]-2-naphthyl)ethylidene)malononitrile ([^{18}F]FDDNP) [27-29] which is the only currently available radiotracer to image both amyloid aggregates and

neurofibrillary tangles, or [^{18}F]florbetapir, recently approved by Food & Drug Administration (FDA) [30]. [^{18}F]florbetapir may help to identify individuals at increased risk for progressive cognitive decline [31]. Recent longitudinal studies conducted for 2-3 years have demonstrated that patients with MCI converted to AD during follow-up had greater [^{11}C]PiB retention in different brain regions (i.e. anterior and posterior cingulate, lateral frontal cortex and temporal cortex) compared to non-converters [32, 33], and the presence of Apo E4 allele is significantly associated with faster conversion rates in PIB-positive subjects with MCI [32].

FDG-PET uses [^{18}F]FDG to measure brain glucose metabolism and cerebral blood flow in AD brain. A specific pattern of hypometabolism, a marker of synaptic dysfunction, has been demonstrated in the temporo-parietal region of the cortex of AD patients, a region which has a critical role in episodic memory function (Table 1). Consistent with the "amyloid hypothesis" an inverse correlation exists between amyloid deposition and hypometabolism in the temporal cortex of PiB-PET (+) subjects [34]. Interestingly a similar pattern of hypometabolism has also been found in MCI patients which progress to AD within 1-15 years with a rate of 80-90 % [35]. When combined with the presence of the APOE4 allele, the risk of progression as well as the accuracy of prediction significantly increase, underscoring the importance of the joint evaluation of different biomarkers to identify MCI patients with an high risk of progression to AD [25, 36, 37].

Several studies have demonstrated that Aβ accumulation and deposition in AD brain is associated with grey matter atrophy already in the presymptomatic of stage of AD [25]. Structural Magnetic Resonance Imaging (MRI) is the imaging technique which allows the detection of selective atrophy in the hippocampus of mild AD patients and, most importantly, already in subjects with MCI [38]. In particular, a specific pattern of neurodegeneration, including medial temporal lobe, posterior cingulate and orbitofrontal cortex, has been found to predict the progression to AD in MCI patients recruited in the ADNI project [39]. Functional MRI (fMRI) has been used in the last years to evaluate abnormal cognitive task-related changes in brain activity and basal brain activity during resting state in AD patients as well as MCI patients [25].

An hyperactivation within medial temporal lobe is predictive of cognitive decline in MCI patients [40]. fMRI might represent an important imaging biomarker for identifying the subgroup of MCI individuals at highest risk of cognitive decline for potential inclusion in disease modifying clinical trials, but further studies are needed to validate this imaging technique in large community-based populations.

Other biological markers, such as cerebrospinal fluid (CSF)-derived biomarkers, have been discovered in recent longitudinal studies and are becoming essential to improve the diagnosis of AD and to identify the subgroup of MCI patients with an underlying pathophysiology specific for AD [22] (Table 1). CSF-derived biomarkers include CSF $A\beta_{1-42}$ concentrations as well as CSF total tau (t-tau), phospho-tau [(p-tau(181)]. Different studies have been conducted in the last years to analyze the correlation between CSF markers measured during life in AD patients and neuropathological hallmarks of AD (plaque deposition, NFT and neurodegeneration) observed at autopsy [22, 23]. A challenge for the widespread use of these CSF biomarkers is the high variability in the assays used to measure these analytes which has been ascribed to multiple pre-analytical and analytical test performance factors, including patient preparation, CSF sample acquisition, processing and storage [41]. Much efforts are now directed to estimate and monitor variability of measurements, quantify batch-to-batch assay variations, and identify sources of variability [42].

Biomarkers of brain Aβ amyloidosis include a reduction in $A\beta_{1-42}$ in CSF [23, 43]. An inverse correlation has been found between low CSF $A\beta_{1-42}$ concentrations and brain Aβ deposition in AD patients as assessed by PiB-PET-Amyloid imaging [44]. Aggregation of monomeric Aβ(1-42) species into higher molecular weight aggregates might explain both the reduction of $A\beta_{1-42}$ in CSF as well as the increased deposition. Direct detection of $A\beta_{1-42}$ oligomers in AD brain would require development, in the next future, of specific ligands.

In CSF, elevated tau seems to reflect the degree of neuronal and axonal degeneration and damage, but is not specific for AD and positively correlates with the amount of tissue damage and poor clinical outcome in acute brain disorders [45]; on the other hand, elevated p-tau (181) correlates with the development of NFTs in the brain [43, 46, 47]. The association

of high levels of total tau and p-tau (181) with low levels of $A\beta_{42}$ in CSF is considered the most informative biomarker of AD, able to discriminate AD patients from elderly healthy control subjects with 80% to 90% sensitivity and specificity [22, 48] (Table 1). Furthermore, low $A\beta_{1-42}$ in CSF together with elevated tau and p-tau181 have a high predictive value to identify MCI patients with higher risk of progression to AD, as confirmed by studies conducted in the ADNI project [22, 49].

Longitudinal studies are also essential to assess whether or not these biomarkers change with disease progression prior to the development of overt dementia. A recent prospective longitudinal study conducted in 128 subjects with dominantly inherited AD has demonstrated that CSF $A\beta_{1-42}$ levels declined 25 years before expected symptom onset, whereas increased CSF concentrations of tau protein as well as an increase in brain atrophy were detected 15 years before expected clinical onset [50].

Much efforts are now directed to characterize the precise timing of AD-related biomarker trajectories over the extended course of the disease with the aim to validate CSF-derived biomarkers as surrogate markers for treatment efficacy in clinical trials for disease-modifying therapies [51]. A recent meta-analysis suggests that combination of $A\beta_{1-42}$ and CSF tau for selecting MCI patients due to AD pathology in a fictitious clinical trial would reduce sample size by 67% and trial costs by 60%, compared to a trial with unselected MCI subjects [52].

According to amyloid hypothesis, Aβ accumulation is an early event in AD pathogenesis which precedes and promotes both tau hyperphosphorylation and neuronal degeneration [4]. Along this line CSF levels of $A\beta_{1-42}$ have been found to be already fully decreased at least 5 to 10 years before conversion to AD dementia, whereas total tau and p-tau181 seem to be later markers [51, 53]. Recent studies also suggest that amyloid markers can be used to identify MCI-AD, whereas injury markers, such as increased levels of total tau and p-tau181, may predict rapid progression to dementia [54]. Further studies are needed both in EOAD and LOAD patients to confirm that CSF biomarkers are sensitive indicators of presymptomatic disease and, most importantly, to understand whether CSF-derived biomarkers could replace autopsy confirmation of AD plaque and tangle pathology as the "gold standard"

for diagnosis as well as for assessing the efficacy of AD treatment in the near future.

Novel candidates biological markers have been proposed, such as putative markers of synapse loss and/or neurodegeneration, neuroinflammatory molecules (such as Tumor Necrosis Factor-α) and hyperhomocysteinemia, which significantly increased the risk of progression from MCI to AD [55]; future long-term studies are needed to validate these biomarkers in epidemiological community-based populations [22].

Neuropsychological and functional measures in AD

Neuropsychological tests are essential for the diagnosis of mild AD, but are also essential to assess cognitive dysfunction in MCI and in particular to identify the subgroup of MCI patients with a high likelihood to develop AD, according to the recently revised clinical criteria [16].

Neuropsychological tools can be used to detect the earliest clinical manifestations of AD, buy they might be also particularly useful in monitoring the response to disease-modifying therapies in MCI due to AD [56] (Table 1). This section will not examine all the instruments available in clinics for the diagnosis of AD, but will mainly focus on the most sensitive and specific neuropsychological tests, which might be used in future clinical trials both in early phase of AD and/or in MCI patients with an high risk of conversion in AD. Neuropsychological tools in AD include instruments for the evaluation of cognitive deficits such as screening tests [i.e. Mini-Mental State Examination (MMSE) and Alzheimer's Disease Assessment Scale-Cognitive Behavior section (ADAS-Cog)], as well as specific tools for assessing neuropsychiatric symptoms, such as Neuropsychiatric Inventory Questionnaire (NPIQ) combined with instruments evaluating normal activities of daily living (ADL)/instrumental activities of daily living (IADL). ADAS was developed to evaluate the intensity of cognitive and non-cognitive symptoms in AD patients [57]. The scale is composed of two parts with a maximum score of 120 points. One is cognitive (ADAS-Cog) and includes items 1-11, with a maximum score of 70, while the other assesses behavior disturbances, including items 12-21 with a maximum

score of 50 [58]. The main areas of the cognitive domains evaluated in the ADAS-Cog are memory, language, praxia and command understanding. A high score indicates a poor performance. When compared to MMSE, 2.5 points of cognitive worsening at ADAS-Cog correspond to 1 point of worsening observed in the MMSE, in patients with a score between 23 and 11 (mild to moderate AD) [58]. An increase of 6-8 points is expected in AD patients after 18 months, which is the time required in new clinical trials designed to study disease-modifying drugs [15]. ADAS-Cog is the most widely used test in clinical trials dealing with AD and recent simulations utilizing ADNI data indicate that ADAS-Cog is able to detect slowing of progression with manageable group sizes [59]. Nevertheless, several concerns have been found about its use in early-stage disease, because the scale has well-known ceiling effects that limit its use in MCI and early AD [60].

The typical design of clinical trials for symptomatic treatments in AD is a randomized, double-blind, placebo-controlled, parallel group study comparing changes in two primary endpoints: one reflecting the cognitive domain (for example changes in ADAS-Cog subscale) and the other preferably reflecting the functional domain of impairment (ADL, IADL) [61]. Overall global change of severity scale ratings, or alternatively, of ADL scales, are required co-primary outcomes in regulatory or registration trials for drugs for dementia.

Impact of AD on everyday activities can be evaluated using the Clinical Dementia Rating Scale (CDR). This scale can be used by the physician to assess performance in five different cognitive domains based on a semi-structured interview of both the patient and the informant [62]. The global CDR score ranges from 0 to 5, where a score of 0 means no cognitive impairment and a score of 5 corresponds to severe dementia. In MCI patients a score of 0.5 has been used in clinical research studies. An alternative instrument, frequently used in 6-months clinical trials for AD to assess global change, is the Alzheimer's Disease Cooperative Study-Clinician's Global Impression of Change rating (ADCS-CGIC), which is an example of a Clinician's Interview-Based Impression of Change with caregiver's input (i.e., a “CIBIC+”) [63].

Diagnostic tools discussed in this section are essential for the diagnosis of mild AD, but they might be also useful in large prospective community-

based cohort studies designed to evaluate the potential benefits of dietary factors and or natural products in preventing age-related cognitive decline and/or AD [64, 65]. As discussed in the previous section, the pathophysiological process of AD begins many years prior to clinically obvious symptoms, and the concept of a presymptomatic or preclinical stage of AD is becoming more widely accepted.

Due to the particularly long pre-dementia phase of AD a long-term follow-up of 20 years might be essential to detect significant differences in cognitive decline assessed by MMSE or more specific neuropsychological tools in healthy subjects [65]. Different long-term prospective studies have recently demonstrated differences on cognitive tests in individuals who ultimately developed dementia ten years or more prior to confirmed diagnosis [66, 67]. Ideally, primary prevention studies should be conducted in individuals at risk for AD prior to the presence of any biomarkers suggestive of pathology. According to this methodology it is now possible to assess the potential neuroprotective efficacy of drugs or natural product such as Gingko Biloba which have failed in the treatment of AD, because they have been tested in a too advanced stage of the disease (mild to moderate AD) over a relatively short follow-up (18 months). A recent large population-based study conducted in France with 20 years of completed follow-up has demonstrated that cognitive decline on MMSE, verbal fluency and visual memory in a non-demented elderly population is lower in subjects treated with Gingko Biloba extract EGb761 compared to the 'neither treatment' group [65]. The authors demonstrate that this effect is specific for EGb761 which has also been studied in preclinical models of AD and is known to prevent both Aβ aggregation and Aβ oligomers formation [68].

The same methodological approach used in this large population-based study should be adopted in the next future in other primary prevention trials designed to examine the neuroprotective efficacy of other “potential neuroprotective” natural products such as omega-3 [69] or the benefits of folic acid and B12 which are known to reduce hyperomocysteinemia, a known risk factor both for MCI and AD [70].

Neuropsychological tests in MCI

Cognitive deficits can be assessed in MCI patients through the use of different psychometric tools [71]. There is fair evidence to support the use of new screening tests that can detect MCI and mild dementia with higher sensitivity (>or=80%) than MMSE [72]. MMSE is the most widely used screening tool for dementia [73]; however, it is more useful for moderate and severe dementia and probably not sensitive or specific enough for diagnosis of MCI, with different studies showing 70% sensitivity and specificity using a cutoff of 26 or less for cognitive impairment [74]. The Montreal Cognitive Assessment (MoCA) was recently proposed as a cognitive screening test for milder forms of cognitive impairment, having surpassed the well-known limitations of the MMSE [75, 76]. The MoCA is a brief cognitive screening tool with high sensitivity and specificity for detecting MCI as currently conceptualized in patients performing in the normal range on the MMSE. The main areas of the cognitive domains evaluated in MoCA are executive function, attention, orientation, memory, language and visuoconstructional function [76]. Recent studies demonstrate that MoCA is superior to MMSE as a global assessment tool, particularly in discerning earlier stages of cognitive decline [77, 78] and also achieves significantly superior values in comparison with MMSE for sensitivity, specificity, positive predictive value, negative predictive value, and classification accuracy, with an optimal cut-off of below 22 for MCI and below 17 for AD [79]. Further studies are needed to validate this instrument in large populations and to improve the correction of scores according to age and education. A recent study demonstrates that classification accuracy of the MoCA was superior to the MMSE (0.80 vs 0.70) in differentiating healthy controls from MCI [78]. Other studies suggest that fewer than 10 patients are needed to be evaluated with MoCA to identify 1 additional true positive cognitively impaired patient compared to using the MMSE, concordant with the high sensitivity of this test [80]. Presently no studies have been conducted to support the hypothesis that MoCA-identification of aMCI+ can improve conversion prediction from MCI to AD when compared to MMSE or reduce required sample sizes in clinical trials. These studies will be essential to understand whether MoCA might be adopted as a new psychometric tool in future clinical trials with disease-modifying drugs. We are currently recruiting aMCI+ in a three-year longitudinal study to

examine whether MoCA can be adopted as a new tool to improve conversion prediction from MCI to AD.

It is important to underline that these cognitive screening tests (MMSE or MoCA) must be administered together with other neuropsychological tests able to detect specific deficits in other cognitive domains; these include executive function (Trail Making Test part B, symbol-digit substitution), delayed episodic verbal and logical recall (Rey Auditory Verbal Learning Test, Hopkins Verbal Learning Test), verbal category and semantic fluency (animals, words beginning with F-A-S). attention (digit span, forward and backward), working memory [Wechsler's Working Memory Index tests (Digit Span, Letter-Number Sequencing and Arithmetic)], processing speed (Trail Making Test part A), visuoconstructional function (clock drawing test, Rey-Osterrieth Complex Figure Test) [81, 82]. This battery of neuropsychological instruments seems to be more sensitive than cognitive screening tests and can provide a more thorough profile of deficits, allowing for distinctions between pure amnestic-MCI (a-MCI), single domain nonamnestic-MCI (na-MCI), multiple-domain nonamnestic -MCI (na-MCI) and the subgroup of multiple-domain amnestic-MCI (a-MCI+) which presents the higher risk of progression to AD [83, 84]. Pure a-MCI should be distinguished from the subgroup a-MCI+, which show an increased risk of conversion to AD in 3 years (50% versus 10%) [83]. A recent longitudinal study has also demonstrated that a-MCI+ subjects are more likely to progress to AD in comparison to patients with na-MCI [85]. Amnestic MCI is characterized by a selective deficit of episodic memory which can be assessed by using the Free and Cued Selective Reminding Test, the Rey Auditory Verbal Learning Test, the California Verbal Learning Test. Scores obtained in these cognitive tests for individuals with MCI should be typically 1 to 1.5 standard deviations below the mean for their age and education matched peers on culturally appropriate normative data (i.e., for the impaired domain(s), when available) [86]. Different studies have demonstrated that pure aMCI is a rare and unstable classification with a low predictive value compared with a-MCI+ [84, 87-89]. Recently a 20-month longitudinal study has explored the trajectory of neuropsychological function in a sample of 81 older adults meeting the criteria for a- and na-MCI [84]. Interestingly, the authors of this study found that all the participants who

had developed AD (12% of the sample) met criteria for a-MCI+ at initial assessment, whereas no participants developed AD directly from single-domain na-MCI or a-MCI subtypes within 20 months. Furthermore Saunders and Summers [76] identified a specific pattern of cognitive impairment including visual episodic memory, verbal episodic memory, short term memory with difficulties in performing tasks involving executive functions such as attention control and working memory. This pattern accurately classified outcome at 20 months in 86.3% of cases thus differentiating stable MCI patients from MCI subjects that progressed to AD.

Recent studies suggest that specific measures of executive function (Color-Word Interference Test, Verbal Fluency) differentiated between MCI participants who displayed cognitive decline over 12 months from those who did not decline [90]. Novel instruments are available to assess executive dysfunction such as Frontal Assessment Battery (FAB) [91, 92]. The FAB consists of six subsets of items exploring different functions related to the frontal lobes and it was originally designed for validating the frontal lobe function in neurodegenerative diseases [91]. Recent studies suggest that FAB total score and subtest scores reflecting interference performances significantly declined in patients with early AD independently from disorientation and memory disorder [92]; further studies are needed to better understand the value of FAB as a psychometric tool able to predict the conversion from MCI to AD. The combination of cognitive screening tests (i.e. MMSE and MoCA) with specific neuropsychological tools represents a new strategy to identify the subgroup of multiple-domain MCI with amnesia (a-MCI+), which seems to present the higher risk of short-term decline and should be therefore considered for inclusion in future disease-modifying therapy trials (Table 1). When considering the progression from MCI to AD we should also consider other neuropsychological variables such as neuropsychiatric symptoms (particularly depression and apathy) which are more prevalent in MCI patients than in cognitively intact older adults [93] strongly influencing the rate of conversion of MCI into AD. Neuropsychiatric symptoms, such as depression and apathy, can be evaluated and quantified in a prodromal phase of AD through validated and reliable psychometric tools, such as NPIQ and Geriatric Depression

Scale (GDS). A recent longitudinal cohort study conducted in a large sample of 1821 MCI patients has demonstrated that neuropsychiatric symptoms in MCI patients (Baseline NPIQ > 0) are associated with an increased risk (40%) of all-cause dementia and in particular of AD. This study has also shown that depressive symptoms in MCI (Baseline GDS > 1) are associated with an increased risk (30%) of AD independent of baseline cognitive or functional status [94]. This study suggest that "mild behavioral impairment" should be considered in addition to cognitive complaints in defining risk factors for progression of MCI to AD. Neuropsychiatric symptoms, and in particular depressive symptoms, may be among the earliest symptoms of preclinical stages of AD and targeting them therapeutically might delay transition to dementia. Depression is known to be a risk factor both for the development of MCI and AD [95, 96]. The precise relationship between depressive symptoms and the risk to develop AD is still uncertain. Some studies suggest that depression is a prodromal feature of dementia [97], whereas other studies have shown a monotonic increase in the risk to develop dementia as a function of the number of depressive episodes [98, 99]. A neurobiological and clinical continuum has been suggested between late-life depression, MCI, and AD [100]. Common genetic factors have been found between AD and depression such as the choline acetyltransferase 4G to A polymorphism [101], the brain-derived neurotrophic factor (BDNF) Val66Met functional polymorphism [102] and the Transforming-Growth-factor (TGF-ß1) +10 C/C functional polymorphism [103]. The presence of depressive symptoms promotes the conversion of MCI into AD [104, 105]. Houde et al. [104] have found that the persistence of depression over two to three years in MCI patients significantly predicts cognitive deterioration to AD. Modrego and Ferràndez [105] have demonstrated that patients with MCI and depression are at more than twice the risk of developing AD than those without depression and, more important, patients with a poor response to antidepressants are at an increased risk of developing AD. Depressive symptoms in MCI subjects are also associated with greater atrophy in AD-affected regions, increased cognitive decline, and higher rates of conversion to AD [106]. Furthermore, depression may occur in 30-40% of the AD patients [107] and it strongly affects clinical evolution of AD [108]. AD patients with major depression show a greater and faster

cognitive impairment than non-depressed patients [108-110]. Evaluation of depressive symptoms as an early manifestation of AD in MCI patients combined with positive biomarkers for both Aβ and neuronal injury, might become important in the near future to identify patients at high risk of conversion into AD (Table 1). These patients might be eligible for neuroprotective strategies aimed at preventing the progression into AD. Past trials have not included MCI subjects with a history of major depression and/or clinically relevant depressive symptoms (as observed in ADNI project) thus reducing sensitivity and also excluding some true cases preclinical AD which progress to AD in less than five years [111]. According to this evidence we believe that the diagnostic accuracy of predicting pre-AD MCI can be actually increased by excluding patients with an history of depression. On the other hand it should be underlined that the development of depressive symptoms in MCI patients, independently from an history of depression, should be monitored in longitudinal studies, because depressive symptoms can be prodromal symptoms of AD and can also increase the risk of conversion from MCI into AD as recently observed in different clinical trials [104-106, 112].
On the basis of this evidence we believe that future clinical trials designed to assess the clinical efficacy of disease-modifying drugs in MCI due to AD should include a thorough evaluation of depressive symptoms at baseline by using different psychometric tools, such as GDS, Hamilton Rating Scale for Depression (HAM-D) and also Beck Depression Inventory 2nd edition (BDI-II) which is a 21-item questionnaire designed for a self-report measure of depressive symptoms [113] [112, 114] in order to identify MCI patients who are most likely to progress to AD [106].

Combination of biological and neuropsychological markers: which evidence ?

In the last five years different studies have been conducted to assess whether or not a combination of different biological markers, such as PET amyloid imaging, MRI-based assessment of hippocampus volume and/or CSF derived biomarkers, may enhance prediction accuracy in MCI patients at risk to develop AD or at least predict future neuropsychological changes [115-117] (Table 2). Combining baseline

CSF p-tau181 and MRI changes (medial temporal lobe atrophy) significantly increases the overall prediction accuracy in the diagnosis of AD from 74% to 84% [116]. These studies (in particular the results from the ADNI project) have demonstrated that MRI is a better predictor of conversion from MCI to AD as compared to CSF biomarkers, although MRI and CSF provide complimentary predictive information on future clinical change [115].

According to the recent revised clinical criteria [16] of the NIA-AA work group a different approach has emerged in the last two years with a few studies now analyzing the advantages of combining biological markers with neuropsychological tests to improve diagnostic accuracy both in AD and MCI patients [118-124] (Table 2). Zhang et al. [119] demonstrated that a combination of MRI, CSF and FDG-PET was able to differentiate AD from healthy controls, achieving a classification accuracy of 93.2% (with a sensitivity of 93% and a specificity of 93.3%) when combining imaging biomarkers, CSF-biomarkers and neuropsychological tests, and only 86.5% when using even the best individual modality of biomarkers. Most importantly this combination method predicted MCI converters within 18 months with a sensitivity of 91.5% and a specificity of 73.4% [119] (Table 2). Davatzikos and colleagues examined MRI and CSF biomarkers together with MMSE scores in ADNI participants both with MCI converted to AD (MCI-c) and not converted to AD (MCI-nc), using SPARE-AD index (Spatial Pattern of Abnormalities for Recognition of Early AD) that summarizes brain atrophy patterns [120]. In this study MCI-nc with most negative baseline SPARE-AD scores (normal brain structure) had significantly higher baseline MMSE scores and relatively low annual rate of MMSE decrease compared to converted MCI. Heister et al. [121] found that the degree of learning impairment in the Rey Auditory Verbal Learning Test combined with medial temporal atrophy and CSF biomarker levels substantially improves risk prediction to convert to AD as compared to other assessments [118, 122, 125]. In this study, combination of greater learning impairment and increased atrophy was associated with the highest risk of conversion to AD [Hazard ratios (HR): 29.0)], where 85% of patients with both risk factors converted to AD within 3 years with a rapid rate (i.e. 15 months) vs 5% of those with neither risk factor [121] (Table 2). The effective gain in predictive

accuracy by combining different biomarkers or neuropsychological variables for the prediction of AD in MCI has been recently examined in a subsample of 81 MCI patients from ADNI database [122]. Combination of the biomarkers (CSF t-tau/Aβ1-42 ratio) with the neuropsychological variables (performances on test of immediate, delayed free recall, executive function) was the best model to predict the conversion from MCI to AD; however, it did not significantly increase the overall classification accuracy when compared with the best single-predictor models. The best neuropsychological predictors were both memory measures (free recall) and non-memory measures (Trail Making Test Part B, digit span, and fluency), confirming that a deficit in executive function (in particular a deficit in TMT-B test) has an high predictive value for the development of AD in a-MCI+ subjects [122] (Table 2).. A recent study has demonstrated that cognitive decline in executive function as well as in other cognitive domains such as memory, language, attention, visuospatial abilities is strongly associated with higher ([^{18}F]FDDNP)PET baseline binding values in frontal and parietal regions in MCI patients [27]. [^{18}F]FDDNP is the only currently available radiotracer to image both amyloid aggregates and neurofibrillary tangles and previous cross-sectional studies have demonstrated that [^{18}F]FDDNP brain binding patterns correspond to neuropathological alterations observed in autopsy studies [29]. Frontal and parietal ([^{18}F]FDDNP)PET binding yielded the greatest diagnostic accuracy in identifying MCI converters after 2 years with a sensitivity of 100% and a specificity of 66.7% compared to temporal [^{18}F]FDDNP PET binding (sensitivity of 83.3% and specificity of 60%) [27] (Table 2). These data suggest that [^{18}F]FDDNP PET scanning combined with neuropsychological test battery measuring different cognitive domains (memory, language, attention, executive functioning and visuospatial ability) might be a new strategy to identify MCI with an increased risk of conversion from MCI to AD and also that this combination might be useful to track the efficacy of disease-modifying drugs (Table 2).

We should consider that prediction of conversion of MCI to AD actually represents the major challenge for an optimal design of prevention trials for disease-modifying drugs. The knowledge of disease progression in terms of clinical changes (ADAS-Cog, MMSE, MoCA, CDR) occurring

in a long-term periods (24 months) is a fundamental step to evaluate the ability of a potential disease-modifying drug to slow neurodegenerative phenomena compared to the control (placebo) group. In a recent study Zhang et al. [124] validated a multimodality-based method in 88 MCI subjects from ADNI project by developing a longitudinal feature selection method to jointly select brain regions across multiple time points for each modality and using a combination of MRI, PET, and cognitive tests (MMSE and ADAS-Cog) at 4 different time points including baseline, 6- month, 12-month and 18-month. This multimodality-based method was able to predict future MMSE and ADAS-Cog scores at 24- month time point, with a classification accuracy of 78.4%, a sensitivity of 79.0%, a specificity of 78.0%, and the conversion of MCI to AD at time points which are at least 6-month ahead of the conversion [124] (Table 2). Finally Cui et al. [123] simultaneously analyzed multiple features from different modalities of data for a 24-months follow-up, including structural MRI, CSF-derived biomarkers and neuropsychological measures, in a cohort form ADNI project consisting of 87 MCI-nc, 56 MCI-c, 111 normal controls and 96 AD patients. The authors demonstrated that neuropsychological measures [Logical Memory II (LM) delayed and immediate recall and Auditory Verbal Learning Test (AVLT)] as well as functional measures assessed by the Functional Assessment Questionnaire (FAQ), outperformed CSF and MRI indices. The combination of selected neuropsychological variables, MRI and CSF indices reached an accuracy of 67.13%, a sensitivity of 96.43% and a specificity of 48.28%, [123] (Table 2). This study indicates that biological and neuropsychological markers are mutually complementary and also suggests that a multimodal feature combination achieves an high sensitivity and appears a promising approach to monitor disease progression finally predicting conversion time from MCI to AD (Table 2). Further studies are needed to validate, in large cohorts of MCI patients, the methods developed in these recent studies in order to adopt multimodal approaches for future clinical trials evaluating disease-modifying drugs for MCI due to AD.

Perspectives for secondary prevention trials in AD

In the last five years all candidate disease-modifying drugs for AD have failed in Phase III clinical trials [13, 15]. There is growing concern that the stage of AD patients recruited in these clinical trials was too advanced (mild to moderate AD patients) to observe a neuroprotective effect of "potential" disease-modifying drugs. Other factors that may have contributed to these failures are the inclusion of subjects who did not truly have AD pathology and/or the non-appropriate use of validated biological markers (i.e the use of biological markers for advanced stages of AD when they are uninformative as predictors of change) [126]. Furthermore, all these trials have been conducted in samples of AD patients recruited with the old clinical criteria. A significant advance has been made in the diagnostic research field of AD with the recent proposal of new diagnostic criteria, which specifically incorporate the use of biomarkers as defining criteria for preclinical stages. Nevertheless, these biological markers (imaging markers and CSF-derived markers) should be combined with specific neuropsychological tests assessing both episodic memory and other cognitive domains (i.e. executive dysfunction) in order to significantly increase the chance to predict the conversion into AD within 24 months as discussed above. The improved predictive prognostic information available from combined use of biological with neuropsychological tests argues strongly for their inclusion in future secondary prevention trials in MCI due to AD patients (Table 2). As discussed in the present review prerequisites for successful preventive trials in AD might be not only the use of appropriate biomarkers (PET-amyloid imaging, MRI, and the $A\beta_{1-42}$/p-tau CSF ratio) [127], but also the enrichment of these trials with selected amnestic MCI patients with multiple-domain cognitive deficits (aMCI+ i.e. the subgroup with the highest risk of conversion). These patients might be selected by using current cognitive screening tests (MMSE, ADAS-Cog) or the new validated tests (MoCA) combined with specific neuropsychological instruments for single cognitive function (memory, attention, executive function and visuoconstructional function).

According to the the evidence presented in table 2 we believe that specific neuropsychological tests for episodic memory such as AVLT immediate free recall, AVLT delayed free recall; AVLT trials 1-5 or specific psychometric tools for executive function such TMT-B, are likely to

increase diagnostic accuracy of the current available biological markers (CSF, PET-amyloid imaging, MRI) in predicting the conversion from MCI to AD (see Table 2). Cognitive decline in executive function is an early event in AD pathogenesis inversely related to Aβ deposition and NFT formation in frontal and parietal regions of MCI patients [27]. For this reason we believe that other novel and more comprehensive tests used to assess executive dysfunction such as FAB should be included in future clinical trials to establish whether this tool can increase the diagnostic accuracy of biological markers more than current psychometric tests (e.g.TMT-B).

We believe that the proposed combination of biological and neuropsychological markers will be essential to identify MCI patients at high risk of conversion, which should be included in future secondary prevention trials to analyze the clinical efficacy of potential disease-modifying drugs (Table 1). This approach could enable significant reductions in sample size requirements for clinical trials of investigational AD-modifying therapies, whereas it would be too expensive to design clinical trials with large sample sizes to detect the preclinical stage of the disorder (healthy controls with evidence of AD pathology) [128].

This advancement in research on biological and neuropsychological markers will be important to evaluate current disease-modifying in AD, among which immunotherapy against Aβ is considered one of the most promising approaches, because it can potentially affect production, aggregation, and deposition of Aβ [15, 129]. Active immunization by vaccination promotes formation of antibodies against pathogenic forms of Aβ, by stimulating an immune response, whereas passive immunotherapy supplies antibodies from an exogenous source which can prevent both Aβ production and deposition (114). Unfortunately, a Phase 2 clinical trial of active immunization using AN1792 (full-length human $A\beta_{42}$ peptide) with QS-21 adjuvant, was stopped prematurely because some patients developed brain inflammation with aseptic meningoencephalitis [130]. Second-generation Aβ vaccines currently in clinical trials have been developed to avoid stimulating adverse immune responses. An Aβ-NH2-immuno-conjugate vaccine, ACC-001 and another second generation Aβ vaccine, CAD-106, comprising multiple copies of $A\beta_{1-6}$associated to Qb

virus-like particles, are currently studied in phase II clinical trials [129, 131].

Passive immunotherapy has been recently proposed as an alternative approach and passive immunotherapy with humanized monoclonal antibodies (i.e. bapineuzumab, solaneuzumab, gantenerumab) is currently studied in different Phase III clinical trials [132]. Given the poor penetration of IgG in CNS, the current view is that passive immunization in AD mainly function from outside CNS, i.e., high titer circulating antibodies would constitute a sink with large capacity and affinity for Aβ, thereby promoting its removal from CNS. Other mechanisms that have been documented include catalytic dissolution of Aβ fibrils and opsonization of Aβ with subsequent phagocytosis by microglia (13, 114)

Among these potential disease-modifying drugs, gantenerumab is the only drug presently studied in prodromal AD (i.e. progressive episodic memory impairment with abnormal biomarkers according to Dubois et al. [133]). In this trial primary outcomes are changes in the Clinical Dementia Rating scale Sum of Boxes (CDR-SOB) and they will be examined in combination with amyloid deposition assessed by PET-amyloid imaging [134]. Previous studies have demonstrated that sample size needed to detect 25% slowing in rate of decline on CDR-SOB can be significantly reduced (-46%) by restricting enrollment to MCI participants testing positive for Aβ and p-tau [128]. It would be interesting to assess whether these enrichment strategies will help to more finely assess response to therapy in this secondary prevention trial. ADAS-Cog will be used as secondary outcome, whereas more sensitive cognitive screening tests (MoCA) or specific neuropsychological tests for single cognitive domain will not be administered to prodromal AD patients. Preliminary results suggest a potential efficacy of solanezumab in mild AD [135]. A first analysis of the solanezumab Phase 3 data from Expedition 1 and Expedition 2 trials suggests a cognitive benefit in both trials overall (1.41 points at ADAS Cog), as well as a functional benefit on IADL [118]. A new phase III trial is ongoing to confirm these cognitive benefits of solaneuzumab [136]. Prevention trials with solaneuzumab, gantenerumab are currently ongoing in subjects with dominantly inherited AD such as the Dominantly Inherited Alzheimer Network (DIAN). Solanezumab will be also the first therapeutic drug to

be evaluated in the Anti-amyloid Treatment in Asymptomatic Alzheimer's Disease (A4) prevention clinical trial, which has been designed to study the efficacy of disease-modifying drugs in LOAD patients, after three-years, by recruiting 1000 clinically normal participants between the ages of 70–85 years whose brain scans show abnormal levels of Aβ deposition by PET-amyloid imaging [137]. Interestingly the A4 trial is the first example of clinical trial planned to assess the efficacy of disease-modifying drugs in AD where biological markers such as PET- amyloid imaging, CSF markers (P-tau and tau), volumetric and functional MRI will be combined with specific neuropsychological tests for single cognitive domain, used as primary clinical outcomes, such as the Free and Cued Selective Reminding delayed recall and LM paragraph recall (episodic memory) and Digit Symbol (executive function) [138]. Both DIAN and A4 trials will be important to assess whether anti-amyloid drugs can prevent or not AD when administered in a presymptomatic stage of the disease [139]. These prevention trials might help to validate the hypothesis presented in this paper that combination of biological and specific neuropsychological markers is a valuable approach to develop disease-modifying drugs for AD.

On the basis of this evidence discussed in the present review we believe that future clinical trials designed to assess the clinical efficacy of disease-modifying drugs in MCI due to AD should include selected amnestic MCI patients with multiple-domain cognitive deficits (aMCI+ i.e. the subgroup with the highest risk of conversion). These patients might be selected by using current cognitive screening tests (MMSE, ADAS-Cog) or the new validated tests (MoCA) combined with specific neuropsychological tests for episodic memory such as AVLT immediate free recall, AVLT delayed free recall; AVLT trials 1-5 or specific psychometric tools for executive function such TMT-B or FAB. In addition depressive symptoms should be thoroughly monitored by using NPI, GDS, HAM-D and BDI-II , because depressive symptoms can be prodromal symptoms of AD and can also increase the risk of conversion from MCI into AD

We believe that the proposed combination of biological and neuropsychological markers will increase the diagnostic accuracy

parameters of the current available biological markers (CSF, PET-amyloid imaging, MRI) in predicting the conversion from MCI to AD and finally that this strategy will be essential to identify MCI patients at high risk of conversion, which should be included in future secondary prevention trials to analyze the clinical efficacy of potential disease-modifying drugs.

Conflict of interest

Declared none

Abbreviations

Aβ= ß-amyloid
AD= Alzheimer's disease
ADAS-Cog= Alzheimer's Disease Assessment Scale-Cognitive Behavior section
ADNI= Alzheimer's Disease Neuroimaging Initiative
a-MCI= amnestic MCI
a-MCI+= multiple-domain amnestic-MCI
APOE= Apolipoprotein E
CDR= Clinical Dementia Rating Scale
CSF= Cerebrospinal fluid
FAB= Frontal Assessment Battery
FDG-PET= Fluorodeoxyglucose positron-emission tomography
fMRI= functional MRI
GDS= Geriatric Depression Scale
IADL= instrumental activities of daily living
MMSE= Mini-Mental State Examination
Montreal Cognitive Assessment= MoCA
MRI= Magnetic resonance imaging
na-MCI= nonamnestic-MCI
NFT= Neurofibrillary tangles

NIA-AA= National Institute of Aging and Alzheimer's Association work group
NMDA= N-Methyl-D-aspartate
NPIQ= Neuropsychiatric Inventory Questionnaire
PiB= Pittsburgh Compound-B
p-tau (181)= phosphorylated-tau(181)

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Table 1. Biological and neuropsychological markers potentially useful to assess the clinical efficacy of disease-modifying drugs in secondary prevention trials

Biomarkers which can predict the conversion from MCI to AD	**Refs**
Aβ accumulation and deposition in the neocortex (PET amyloid imaging)	[27-28]
Hypometabolism in the temporo-parietal region of the cortex as marker of synaptic dysfunction (FDG-PET)	[30]
Selective atrophy in the medial temporal lobe, posterior cingulate and orbitofrontal cortex (MRI)	[34]
Low $A\beta_{1-42}$ in CSF combined with elevated tau and p-tau181 (a marker of neuronal iniury)	[20,43,44]
Reduced performance in both cognitive screening tests (MMSE<26 or MoCA<22) and specific neuropsychological tools detecting multiple-domain MCI (deficit of episodic memory combined with an impairment of executive functions) (a-MCI+)	[71, 108]
Depressive symptoms and apathy (NPIQ, GDS, HAM-D, BDI-II)	[81, 91,92]
Combination of imaging biomarkers (MRI, PET) and/or CSF-derived markers ($A\beta_{1-42}$/p-tau ratio) with selected neuropsychological variables (episodic memory plus executive function)	[105, 109]

Table 2.

Summary of the studies which have examined sensitivity, specificity and classification accuracy of the most promising combination of biological and neuropsychological markers in predicting the conversion from MCI to AD

Biomarkers or relative combination	Sensitivity	Specificity	Classification accuracy	Cut-off	Follow-up	Refs
STAND Scores (MRI)	71%	95%	84%	0.25	24 months	115,118
CSF-P-tau/A β_{42} ratio	87%	72%	79%	0.16	24 months	115,118
CSF-t tau/A β_{42} ratio	87%	75%	81%	0.46	24 months	115,118
CSF-A β_{42}/A β_{40} ratio	100%	53%	70%	---	24 months	116
CSF-P-tau231 levels	75%	73%	74%	---	24 months	116
GMC-MTL(right) assessed by MRI	100%	60%	74%	---	24 months	116
CSF-P-tau231 levels + GMC-MTL(right) assessed by MRI	*100%*	*73%*	*84%*	---	24 months	116
MRI	86%	86.3%	86.2%	---	18 months	119
PET	86.3%	86.6%	86.5%	---	18 months	119
CSF $A\beta_{42}$, t-tau and p-tau	81.9%	82.3%	82.1%	---	18 months	119
MRI+PET+ CSF	93%	93.3 %	93.3 %	---	18 months	119
SPARE-AD (MRI)	94.7%	37.8%	55.8%	0.65	12 months	120
CSF P-tau181/ Aβ42 ratio	89.5%	23.2%	44.2%	---	12 months	120
SPARE-AD (MRI) + CSF P-tau181/Aβ42 *ratio*	*81.6%*	*50%*	*60%*	---	12 months	120
RERC-MRI	53.4%	77%	68.5%	---	24 months	122
LHC-MRI	70.1%	58.7%	63.5%	---	24 months	122
CSF P-tau181	63,9%	58,9%	61.9%	---	24 months	122
TMT-B	49.6%	76.2%	64.6%	---	24 months	122
AVLT–IM REC	74.3%	53%	61.8%	---	24 months	122
AVLT-DEL REC	78.1%	49.4%	61.7	---	24	122

					months	
CSF t-tau/Aβ42 ratio+ AVLT –IM and AVLT-DEL REC + TMT-B	*80.4%*	*51.4%*	*64.1%*	---	36 months	122
TMT-B, RHC and P-tau181/Aβ42 and age	81.8%	73.3%	76.9%	---	36 months	122
MMSE + ADAS-Cog scores + MRI +PET	79%	78%	78.4%	MMSE>27 ADAS-Cog>12	24 months	124
Neuropsychological and functional measures (NM)	91%	48.2%	65%	---	24 months	123
CSF (t-tau/Aβ42 and P-tau181/Aβ42 ratios*)*	80.3%	48.2%	60.8		24 months	123
NM+CSF	94.6%	45.9%	65%		24 months	123
CSF+MRI (7)	71.4%	58.7%	50.5%	---	24 months	123
NM+CSF+MRI	*96.4*	*48.2*	*67.1*	---	24 months	123
	Sensitivity	**Specificity**	**Hazard ratios**	**Cut-off**	**Follow-up**	**Refs**
[^{18}F]FDDNP-PET baseline binding*	100%	66.7%	---	1.07 (frontal and parietal)	24 months	27
[^{18}F]FDDNP-PET baseline binding*	83.3%	60%	---	1.14 (temporal)	24 months	27
AVLT trials 1-5	---	---	---	33 words	29 months	121
HOC SCORE-MRI	---	---	3.9	-1.02 *z* score	29 months	121
CSF P-tau181/A β 42 ratio	---	---	3.8	0.10	29 months	121
Positive hippocampal atrophy (HOC) + positive AVLT trials 1-5	---	---	*29.0*	---	29 months	121
Positive hippocampal atrophy (HOC) + positive CSF	---	---	13.8	---	29 months	121
Positive AVLT trials 1-5+ positive CSF	---	---	13.8	---	29 months	121

Structural Abnormality Index (STAND) scores reflect the degree of AD-like anatomic features on MRI.

MRI: Magnetic resonance Imaging, GMC: gray matter concentration, MTL: Rate of medial temporal lobe atrophy, CSF total and phosphorylated tau (T-tau, P-tau231), SPARE-AD index (Spatial Pattern of Abnormalities for Recognition of Early AD) summarizes brain atrophy patterns.

AVLT: Auditory Rey Verbal Learning Test, TMT-B: trail making test B, AVLT-DEL REC: AVLT delayed free recall; AVLT-IM REC: AVLT immediate free recall; RERC: right entorhinal cortex thickness; LHC: left hippocampus volume assessed by MRI.

MRI (7) represents 7 selected structural features including Left Entorhinal Cortex, Right Middle Temporal Gyrus, Right Hippocampus , Left Hippocampus, Right Inferior Parietal Cortex, Left Retrosplenial Cortex, Left Middle Temporal Gyrus . NM represent 5 selected neuropsychological and functional measures including Functional Assessment Questionnaire (FAQ) , Logical Memory II (LM) delayed recall and LM immediate recall, AVLT delayed recall, AVLT trials 1-5. CSF represents 2 selected CSF features: t-tau/Aβ42 and p-tau181/Aβ42 ratio.

Hippocampal occupancy (HOC) score is an estimate of medial temporal lobe atrophy.

[^{18}F]FDDNP: 2-(1-(6-[(2-[^{18}F]fluoroethyl)(methyl)amino]-2-naphthyl)ethylidene)malononitrile. *In this study [^{18}F]FDDNP PET scanning was combined with a neuropsychological test battery measuring different cognitive domains (memory, language, attention, executive functioning and visuospatial ability).

Chapter V

Selective Serotonin Reuptake Inhibitors and Serotonin and Noradrenaline Reuptake Inhibitors improve cognitive function in partial responders MDD patients: results from a prospective observational cohort study

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Submitted to European Neuropsychopharmacology

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Tables: 3
Keywords: Major Depression, SSRI, SNRI, cognition, psychometric tools

Abstract
Major Depressive disorder (MDD) is often accompanied by cognitive deficits, involving attention, learning, memory and executive functioning. Selective Serotonin Reuptake Inhibitors (SSRIs) and Serotonin and Noradrenaline Reuptake Inhibitors (SNRIs) show efficacy on affective symptoms, but it is unclear whether or not they improve cognitive symptoms. Here we carried out a 12 week-prospective observational study in two cohorts of moderate-severe MDD patients, to test the hypothesis that SSRIs and/or SNRIs may affect cognitive symptoms and assess whether or not such an effect was correlated to their effect on affective symptoms. All patients underwent cognitive and neuropsychiatric assessments at baseline, 4- and 12-week follow-up. Thirty-three patients in the SSRI- and 16 in the SNRI-cohort completed the follow-up. Both SSRIs and SNRIs reduced affective symptoms (HDRS and BDI-II scores) and improved global cognitive function (MMSE and MoCA tests) from baseline to week 12. Moreover, both SSRIs and SNRIs improved executive function (FAB test) and verbal memory (Rey's 15 Words Test). Further analysis showed that global cognitive function, verbal memory and executive function improved both in full responders (HDRS reduction ≥ 50%) and in partial responders patients (HDRS reduction ≥ 25%). Finally, there was not correlation between baseline MMSE, MoCA and FAB scores and the mean change in HDRS or BDI-II after the 12-week treatment. These data show that SSRIs and SNRIs improve cognitive symptoms in MDD independently from their efficacy on affective symptoms; affective and cognitive symptoms may represent distinct psychopathological dimensions of MDD with different response to the pharmacological treatment.

1. Introduction

Major Depressive disorder (MDD) is one of the most prevalent and life-threatening form of mental illnesses and a major cause of morbidity worldwide. It is estimated that 5 to 20% of the general population suffers from it, people with mild depressive episodes included (Kessler et al., 2005).

Depression is associated with significant disability and with excess mortality. In addition to mood and anxious disorders, major depression is often accompanied by cognitive deficits in the domains of attention, learning, memory and executive functioning (Lee et al., 2012). Cognitive deficits may be considered as core symptoms in depression with an impact on functioning, persisting after remission (Baune et al. 2010, Baune and Renger, 2014). Cognitive dysfunction is considered both a state and a trait marker of MDD, but recent studies suggest that it might represent a distinct biological and clinical dimension in (Jaeger et al. 2006; McIntyre et al. 2014). The presence of cognitive symptoms in MDD patients might also predict a low rate of response to antidepressants (Silverstein and Patel, 2011). Currently available antidepressant drugs, such as Selective Serotonin Reuptake Inhibitors (SSRIs) and Serotonin and Noradrenaline Reuptake Inhibitors (SNRIs), provide a useful therapeutic tool, but approximately 30% of MDD patients fail to respond to these drug treatments; furthermore, it is still unclear whether or not these drugs can improve cognitive function in all MDD patients. Some studies suggest that SNRIs have a superior clinical efficacy compared to SSRIs in the treatment of specific cognitive deficits; these studies, however, are only preliminary and will need further validation by multiple and more advanced psychometric tools in larger samples of patients (Herrara-Guzman et al., 2009). Indeed, randomized double-blind clinical trials with antidepressant drugs may fail because of the reduced sensitivity of a single psychometric tool (Santen et al., 2008; Della Pasqua et al. 2010). Therefore, the use of multiple psychometric instruments represent a better strategy in a long-term follow-up to assess the clinical efficacy of second-generation antidepressant drugs (SSRI vs SNRI) on the different biological and clinical dimensions of MDD, such as affective and cognitive symptoms.

Observational studies have not yet been conducted to systematically analyze changes occurring in cognitive function and affective symptoms

during treatment with SSRIs or SNRIs in MDD patients with a recent history of partial response to a previous treatment with an antidepressant drug.

The aim of the present study was to test the hypothesis that SSRIs and/or SNRIs may affect cognitive symptoms in MDD patients and, if so, to evaluate whether or not such an effect is correlated to their effect on affective symptoms. In this respect, we carried out a 12 week-prospective observational study examining the effectiveness of SSRIs vs SNRIs in standard medical practice, in two cohorts of moderate-severe MDD patients, selected for their recent history of partial response to antidepressants.

2. Experimental Procedures

2.1. Ethics statement

The study was approved by the ethical committees of the two coordinating centers,: 1) Azienda Policlinico Catania-Department of Psychiatry (June 27 2012); 2) ASP3 Catania-Villa dei Gerani (July 24 2012).

The study met the ethical administrative Italian legislation in force when the study administrative process started (03.06.2012) according to CM 6 02.09.2002, GU 214 12.09.2002 and D 29.03.2008 of the Italian Medicine Agency (Agenzia Italiana del Farmaco, AIFA) and GU 76 31.03.2008, Art 10 (Procedures for Observational Studies).

The designed study was a prospective, observational (non-interventional), multicenter cohort study conducted in 2 different clinical centers in Sicily (Italy). The study complied with the definition of "observational" (i.e. "non-interventional") study provided in Article 2(c) of Directive 2001/20/EC, meaning that the investigator who carries out the study does not interfere with the physician's decision regarding which drug is clinically pertinent to be prescribed to each individual patient. Therefore, prescription of SSRIs or SNRIs solely resulted from an independent clinical evaluation, according to the physician's clinical judgment, and based on each patient's clinical profile. Moreover, the decision to include a patient in the study, following his/her agreement, was taken independently of the clinical decision to prescribe SSRIs or SNRIs.

Finally, the study did not affect the medical practice of participating physicians and did not trigger additional medical visits.

2.2. Patients

All MDD patients recruited in the study were admitted and hospitalized at the following two psychiatric clinical centers : 1) Azienda Policlinico Catania-Department of Psychiatry (Prof. Eugenio Aguglia), Catania, Italy; 2) Casa di Cura Villa dei Gerani- ASP 3 Catania (Dr. A. Ventimiglia) according to the following inclusion criteria:

1) A diagnosis of acute episode of recurrent (1-4 episodes) Major depressive Disorder (MDD) according to DSM-IV-TR;

2) A recent history (in the last 4 weeks) of partial response to a previous treatment with an antidepressant drug;

3) A prevalence of somatic [Hamilton Depression rating Scale (HDRS) items (10,11,12,13,15,17) > 6] and cognitive symptoms [HDRS items (2,3,9,19,20,21) > 6] (Cleary and Guy 1977);

4) Aged at least 18 years or legal age of majority (without upper limit of age);

5) Initiated into SSRIs or SNRIs for their current depressive episode;

6) Not participating in another study simultaneously;

7) Having signed an informed consent;

8) Accepting to give a personal reference contact.

According to exclusion criteria patients were not able to take part in SSRI or SNRI cohorts if they fulfill at least one of the following criteria: 1) their ability to consent was impaired or questionable (e.g. patients suffering from psychotic depression); 2) they had to stop an ongoing antidepressant that was effective for their depression¸ 3) they were already treated with another antidepressant that they wished to continue in addition to the new prescribed antidepressants drug (SSRI or SNRI); 4) they planned to move during the 12 week the follow-up.

2.3. Study procedures

This prospective observational multi-center cohort study was composed of 2 cohorts. Recruited MDD patients who met the inclusion criteria were subdivided in two different cohorts depending on the class of antidepressant drug prescribed, i.e. SSRI s or SNRIs:

1) SSRI cohort: MDD patients who start the treatment with SSRIs for 12 weeks
2) SNRI cohort: MDD patients who start the treatment with SNRIs for 12 weeks

SSRI cohort: Patients were enrolled and followed in the scope of the observational study until 12 weeks after the SSRI initiation for their current depressive episode. Patients who stop their treatment before 12 weeks, or at 12 weeks, were followed (safety data collected if any) until 2 weeks after the treatment withdrawal.

SNRI cohort: Patients were enrolled and followed in the scope of the observational study until 12 weeks after the SNRI initiation for their current depressive episode. Patients who stop their treatment before 12 weeks, or at 12 weeks, were followed (safety data collected if any) until 2 weeks after the treatment withdrawal.

Contra-indication and precautionary measures mentioned in the Summary of Product Characteristics (SmPC) were respected. Physicians had to refer to local and/or national available prescribing guidelines in MDD. Follow-up of the participating patients were done according to the physician 's standard medical practice and the approved SmPC.

1.4. Psychometric tools and neuropsychological assessment

All included patients underwent cognitive and neuropsychiatric assessments, carried out before the switch (baseline), and at 4 and 12-weeks follow-up. Trained psychologists and neuropsychologists performed all evaluations.

We selected psychometric tools among different typologies of tests, i.e. inventories, projective techniques, self-rating and ratings based on behavioral observation. For each area of evaluation and for each typology of technique, only instruments having Italian translation and adaptation were selected. The selection was based on the reliability and validity shown by these instruments in the assessment of affective and cognitive symptoms.

The revised 4th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM IV-TR, Diagnostic and Statistical Manual of Mental Disorders - Text Revision) published by the American Psychiatric Association (APA, 2000) was used for clinical diagnosis of depression,.

The following psychometric instruments were used to assess both cognitive and affective symptoms in recruited MDD patients:

1) *Tools for the assessment of affective changes*: The 17-item version of the Hamilton Psychiatric Rating scale for Depression (HDRS: Hamilton,1960; 1967) and the Beck Depression Inventory (BDI-II: Beck, 1961);

2) *Tools for the assessment of global cognitive function*: Mini Mental State Examination (MMSE: Folstein e al., 1975) and Montreal Cognitive Assessment (MoCA: Nasreddine e al., 2004). The Mini-Mental State Examination (MMSE) is a brief 30-point questionnaire of a broad array of cognitive functions including orientation, memory and language. MMSE is employed as a screening tool to identify and exclude potential patients with early onset dementia. The Montreal Cognitive Assessment (MoCA) is a brief cognitive screening tool with high sensitivity and specificity for detecting MCI as currently conceptualized in patients performing in the normal range on the MMSE. The main areas of the cognitive domains evaluated in MoCA are executive function, attention, orientation, memory, language and visuoconstructional function

3) *Tools for the assessment of specific cognitive functions*: Rey's 15 Words Test (Rey, 1958); Verbal memory span (Digit-Span: Wechsler, 1981) and Frontal Assessment Battery (FAB: Dubois e al., 2000). The Rey's 15 words test is an explicit verbal memory tests devised by Rey in 1958 with the aim to quantify the ability of immediate and delayed recall. The evaluation of the test of immediate and deferred are considered separately. In fact, the cut-off it is 28.53 for immediate test and 4.69 for the deferred test. This test has been used to evaluate whether different types of dementia show different patterns of immediate and delayed recall and of learning process. Verbal Memory Span: this measurement has been used in several batteries of Wechsler. The Digit Span consists of two different tests: 1) dgits Forward, which consists in the repetition of digits forward; 2) digits backward, which consists in the repetition of digits in reverse. The test provides information about the performance of the auditory short-term memory and attention.

The Frontal Assessment Battery (FAB) consists of six subsets of items exploring different functions related to the frontal lobes, i.e. conceptualization, mental flexibility, motor programming, sensitivity to

interference, inhibitory control, and environmental autonomy. A cut off score of 12 on the FAB has a sensitivity of 77% and specificity of 87% in differentiating between frontal dysexecutive type dementias and Alzheimer's disease.

All clinical and neuropsychological data required by the study protocol were recorded in the Case Report Form (CRF) at the baseline consultation, for both SSRI and SNRI cohorts, and at each subsequent consultation during a maximum follow up period of 14 weeks,

2.5. Statistical analysis

Changes in depressive (HDRS-BDI-II) and cognitive symptoms (MMSE, MoCA, FAB, Rey, Digit Span) between treatment groups and relationship between variables, were analyzed at the end of the treatment (T2-T0) using t-test for repeated measures and t-test for independent samples. We also examined affective and cognitive changes in the different subgroups defined according to the final response to the treatment (responders vs non- responders) (HAMD reduction >50%). Pearson Correlation coefficient was used to analyze the correlation between cognitive score at baseline and the change in depression after the 12 week treatment, as assessed by HDRS and BDI. The level of statistical significance was defined as $p<0.05$.

3. Results

Of the overall 105 subjects screened during the first 18 months of the study, only 52 patients (mean age 54.7± 12.1 SD years; 39 women and 13 men) met the inclusion/exclusion criteria and were then recruited for their inclusion in SSRI or SNRI cohort. All recruited MDD patients were recurrent depressive patients with a current depressive episode at the beginning of the study and a recent history, in the last 4 weeks, of partial response to a previous treatment with an antidepressant drug. All MDD patients were currently treated with an antidepressant drug at the beginning of the study, but were partial responders and then switched to SSRIs (SSRI cohort) or SNRIs (SNRI cohort) for the 12 weeks of treatment.

In the SSRI cohort 33 MDD patients started and completed the treatment for 12 weeks with the following drugs: 14 patients with escitalopram (10 mg/day); 9 patients with paroxetine (20 mg/day), 1 patient with sertraline (100 mg/day), 9 patients with citalopram (20mg/day). 2 patients withdrew from the SSRI cohort before the end of the study (2/3 weeks) for treatment-emergent AEs (nausea) and were therefore excluded from the cohort and the following analyses (see Table 1).

In the SNRI cohort 16 MDD patients started and completed the treatment with the following drugs for 12 weeks: 8 patients with venlafaxine (225 mg/day), 8 patients with duloxetine (60 mg/day). 1 patient withdrew from the SNRI cohort before the end of the study (2/3 weeks) for treatment-emergent AEs (hypertension and headache) and was therefore excluded from the cohort and the following analyses (Table 1).

With a mean baseline HDRS total score of 21.1 ± 6.7 and a mean baseline BDI-II score of 32.4 ± 10.8, 49 MDD patients (mean age 54.3± 12.5 SD years; 37 women and 12 men) were moderately to severely depressed. There were no significant differences between SSRI and SNRI cohorts in the proportion of patients who withdrew from the study. The proportions of patients who withdrew during treatment because of treatment-emergent AEs were 5.7% in the SSRI cohort and 5.8% in the SNRI cohort respectively.

We first examined the clinical efficacy of SSRIs and SNRIs in reducing depressive symptoms as assessed by HDRS and BDI-II. We found that both SSRIs and SNRIs were able to significantly reduce HDRS scores from baseline to week 12. In the SSRI cohort, the mean change in HDRS total score from baseline (22.18 ± 7.38) to week 12 (12.94 ± 7.93) was -9.24, whereas in the SNRI cohort HDRS total score at baseline was 18.94 ± 4.58 and 12.55 ± 8,99 at week 12 (Table2). Furthermore MDD patients in the two cohorts reached comparable HDRS scores at the end of study treatment. When examining the cluster of anxiety symptoms, as measured by HDRS-Factor I (items 10, 11, 12, 13, 15, e 17), we found that only SSRIs decreased significantly HDRS-Factor I scores from baseline values (6.73 ± 2.48) to week 12 (4.39 ± 2.55, $p < 0.05$), as opposed to SNRI cohort where we observed a relevant but not significant reduction in HDRS-Factor I scores from baseline values (6.63 ± 3.1) to week 12 (4.50 ± 3.61). No other significant differences were detected

between the two cohorts in all the other factors of HDRS (data not shown).

At baseline, all depressed patients recruited in the study were partial responders, whereas at the end of the 12 week treatment 40% of the patients became full-responders (HDRS reduction ≥50%), with no significant difference in the number of responders between SSRI cohort (14/33 , 42.4 %) and SNRI cohort (6/16, 37.5%). Among the responders, only 9/49 (18.3%) reached clinical remission (HDRS score < or = 7) at the end of the study with no significant difference between SSRIs (6/33, 18.1%) and SNRIs (3/16, 18.7%). Furthermore, at the end of the 12 week treatment, the remaining 29 MDD patients could be classified in two subgroups: 14 MDD patients as partial responders (HDRS reduction ≥ 25% but <50%); the other 15 MDD patients as non responders (HDRS reduction < 25%) (Table 3).

In the subgroup of full-responders, the mean change in HDRS total score from baseline (21.85 ± 5.98) to week 12 (7.10 ± 3.28) was -14.74 ($p < 0.01$), whereas in all the other MDD patients (partial responders and non-responders) the mean change in HDRS total score from baseline (20.62 ± 7.25) to week 12 (16.86 ± 7.44) was -3.76.

To better assess the effectiveness of second-generation antidepressants we also examined the mean change from baseline in BDI-II total score, which is a self-report symptom inventory and a widely used measure for assessing depressive symptoms. Both SSRIs and SNRIs significantly reduced BDI-II total score from baseline (33.67 ± 11.67) to week 12 (20.06 ± 12.78) in the SSRI cohort and in the SNRI cohort (30.06 ± 8.97 and 20.06 ± 12.39, respectively, Table 2).

We then analyzed the effects of SSRIs and SNRIs on global cognitive function and specific cognitive symptoms from baseline to week 12. Both SSRIs and SNRIs significantly improved global cognitive function in MDD patients as measured by MMSE and MoCA (Table 2). SSRIs and SNRIs significantly ($p <0.05$ for both) increased MMSE scores from baseline to week 12 (26.65 ± 2.78 to 28.15 ± 1.86 in the SSRI cohort and 25.75 ± 3.46 to 27.64 ± 2.82 in the SNRI cohort, respectively, Tab. 3). Similar results were observed with MoCA scores that significantly increased from baseline to week 12 (from 21.88 ± 4.28 to 24.3 ± 3.88 in the SSRI cohort and from 20.69 ± 5.31 to 23.56 ± 4.98 in the SNRI

cohort, Table 2). Moreover, both SSRIs and SNRIs significantly improved executive function as assessed by Frontal Assessment Battery (FAB, Tab. 3). SSRIs and SNRIs significantly increased FAB scores from baseline to week 12 (from 14.69 ± 2.89 to 15.76 ± 2.45 in the SSRI cohort and from 14.44 ± 2,02 to 16.24 ± 2.11 in the SNRI cohort, respectively).

We then examined verbal memory, which is known to be affected by antidepressant drug treatment (Baune and Renger 2014). Both SSRIs and SNRIs significantly improved verbal memory as assessed by Rey's 15 Words Test (Table 2). Immediate Rey test scores significantly increased both in the SSRI cohort [from baseline (31.8 ± 7.14) to week 12 (40.08 ± 10.81)] and in the SNRI cohort [from baseline (30.69 ± 9.04) to week 12 (37.75 ± 10.97)]. Similar results were observed with delayed Rey test scores, which significantly increased both in SSRI cohort [from baseline (5.57 ± 2.16) to week 12 (8.26 ± 2.87)] and in the SNRI cohort [from baseline (5.33 ± 2.36) to week 12 (7.39 ± 3.00)]. On the other hand, Span forward scores increased significantly only in the SNRI cohort [from baseline (5.31 ± 1.30) to week 12 (6.06 ± 0.93)] but not in the SSRI cohort [from baseline (5.33 ± 1.05) to week 12 (5.55 ± 1.23)], while Span backward scores were not significantly affected by antidepressant drug treatment in both groups (SSRI cohort: baseline, 3.33 ± 0.74, week 12, 3.60 ± 0.40; SNRI cohort: baseline, 3.56 ± 1.46, week 12, 3.50 ± 0.90).

To understand whether the clinical efficacy of second-generation antidepressants on cognitive deficits in our sample of MDD patients was independent from the efficacy of these drugs in the treatment of affective symptoms we compared the mean change in all the most relevant cognitive tests from baseline to week 12 in all 49 MDD patients considering the two clinical subgroups identifiable at the end of the study: 1) the 20 full responders MDD patients (HDRS reduction ≥ 50%); 2) the 29 MDD patients including both 14 partial responders (HDRS reduction ≥ 25% but <50%) and 15 non responders MDD patients (HDRS reduction <25%) (Table 3).

Interestingly, global cognitive function significantly improved in both groups as measured by both MMSE and MoCA (Table 3). Furthermore verbal memory and executive function significantly improved both in full responders and partial responders patients (Table 3).

Finally we analyzed correlation between cognitive deficits at baseline and the change in depressive symptoms after the treatment as assessed by HDRS and BDI. Interestingly we did not find a significant correlation between baseline MMSE, MoCA and FAB scores and the mean change detected with HDRS or BDI-II after the 12 week treatment with SSRIs or SNRIs, whereas a significant correlation was found (Pearson Correlation Coefficient R=0.51) between the two psychometric tests, HDRS and BDI-II, used to assess affective symptoms.

4. Discussion

Current antidepressant drugs, which are directed against monoaminergic systems, provide a useful therapeutic tool, but approximately 30% of depressed patients fail to respond to these drugs, while a remission is reached in only 30% of patients (Rush et al. 2006, Caraci et al. 2010). Remission is considered as the main objective in the management of depression, but other relevant unmet needs are include the improvement of cognitive symptoms (Zajecka, 2013; Baune and Renger. 2014). In fact, cognitive deficits are considered as key symptoms of clinical depression (Roiser and Sahakian 2013, McIntyre et al., 2013). Of note, cognitive deficits in depression are also associated both with a suboptimal response to antidepressants and reduced remission rates (Roca et al. 2014). Even in patients who respond to treatment and achieve remission, residual symptoms such as fatigue and cognitive symptoms significantly inhibit functionality and can increase the risk of relapse and recurrence (Zajecka, 2013).

Our data suggest that affective and cognitive symptoms can be considered as different psychopathological dimensions of MDD with different response to the pharmacological treatment with second-generation antidepressants such as SSRIs and SNRIs.

An important question not often addressed in the literature is whether antidepressant drugs with different mechanisms of action such as SSRIs and SNRIs, have different effects on the different biological and clinical dimensions of depression, such as affective and cognitive symptoms.

In the present study we first examined the effects of SSRI and SNRIs on affective symptoms combining both HDRS and BDI-II to measure the response to antidepressant drugs. We found that both SSRIs and SNRIs

reduced depressive symptoms in MDD patients at the end of the 12-week treatment. Due to the reduced sample size and the different HDRS scores at baseline in the two cohorts we cannot conclude that SSRI are superior to SNRI in reducing affective symptoms in MDD patients. The Cleary and Guy factor analysis showed a significant difference at week 12 for anxious symptoms and cognitive disturbance in favour of SSRIs compared with SNRIs., but further observational studies in larger samples are needed to clarify the differential effects of these two drug classes on the different clinical dimensions of MDD.

In the present study we recruited MDD patients with a current history of partial response to a previous treatment with an antidepressant drug. In this sample the switch to SSRI or SNRIs lead to full response only in 40% of the patients with no significant differences in response rate between the two drug classes. The present seem at odd with evidence from meta-analysis of randomized double-blind controlled clinical studies, where response rates are higher in SNRI-treated patients than in SSRI-treated patients (Papakostas et al. 2007). Nevertheless, it should be underlined that our study is a prospective, observational, non-interventional, cohort study designed to assess the effectiveness of SSRI s and SNRIs in the treatment of affective and cognitive symptoms of MDD, whereas randomized double-blind controlled clinical studies are designed to detect clinical efficacy of antidepressants versus placebo.

Interestingly, we found that remission rates in our observational study at the end of the 12 week treatment were low (around 18%) both for SSRIs and SNRIs, but comparable with rates detected in larger and multicenter prospective observational studies (Gaynes et al. 2009, Rush et al. 2009), suggesting that the inclusion criteria adopted in this study were well defined to identify an highly homogeneous group of partial responders MDD patients, similar to MDD patients recruited in other observational studies (Howland 2008, Warden et al. 2009).

Here we combined, for the first time, "standard " psychometric tools, validated for the assessment of affective symptoms in MDD patients (HDRS and BDI-II) with neuropsychological tools such as MMSE, MoCA, FAB, Rey's 15 Words Test, developed to detect cognitive deficits in mild cognitive impairment and/or in early AD (Caraci et al. 2014).

Different studies have demonstrated that some antidepressants can improve not only mood-related symptoms, but also cognitive symptoms in depressive patients (Herrara-Guzman et al. 2009).

A recent systematic review has examined the evidence available on the effects of antidepressants on cognitive dysfunction in MDD, focusing on domains of memory, attention, processing speed and executive function (Baune and Renger. 2014). The authors propose as a new direction in the field the development of a neuropsychological consensus cognitive battery to support the clinical assessment of cognitive symptoms in MDD. According to this new scenario we have adopted in the present study a neuropsychological battery including "old" and "new "tools such MMSE, MoCA, FAB, Rey's 15 Words Test, Digit Span. Interestingly we found that both SSRIs and SNRIs significantly improve global cognitive function as assessed by MMSE and MoCA. Other clinical studies have found that SSRIs such as citalopram and sertraline (Bondareff et al. 2000; Rocca et al. 2005), escitalopram (Savaskan et al. 2008) and fluoxetine (Gallassi et al. 2006) improve MMSE scores, but these studies have been conducted in elderly patients, whereas MDD patients selected in our study were middle-aged patients.

In the present study, MDD patients treated with SNRIs (venlafaxine 150-22 mg/die and duloxetine 60 mg/die) showed improvement in MMSE scores similar to those of the SSRI cohort, whereas in other studies duloxetine was not superior to placebo on the Verbal Learning and Recall Test (Raskin et al. 2007, Robinson et al. 2014). Of note, the improvement in global cognitive function was confirmed in our study also by MoCA, recently proposed as a cognitive screening test for milder forms of cognitive impairment, that has surpassed the well-known limitations of the MMSE in terms of sensitivity (Nasreddine et al. 2004; Larner et al. 2012). The MoCA is a brief cognitive screening tool with high sensitivity and specificity for detecting MCI as currently conceptualized in patients performing in the normal range on the MMSE (Dong et al. 2012). The main areas of the cognitive domains evaluated in MoCA are executive function, attention, orientation, memory, language and visuoconstructional function. In our study we show, for the first time, that MDD had a mean baseline MoCA total score <22, which seems to be low compared to the values found in middle-aged healthy controls

(Santangelo et al. 2014). A MoCA total score <22 can be found in MCI patients and this test has been proposed to select MCI with an high risk to convert into AD (Caraci et al. 2014). We found a clinically relevant increase in MoCA scores (more than 2.5 points) after 12 week treatment in both cohorts. Thus, this tool may deserve a further validatation in larger observational studies as well as in randomized double-blind placebo-controlled trials.

Some studies have suggested a superior clinical efficacy of SNRIs compared to SSRIs in the treatment of specific cognitive deficits such as episodic memory and working memory (Herrera-Guzmán et al. 2009). Here we found that both SSRI s and SNRIs improved verbal memory (Rey test_Immediate and delayed) in MDD patients, but not working memory as assessed by Backward Digit Span. Forward Digit Span scores, an alternative tool to measure verbal memory, significantly improved only in the SNRI cohort, consistent with data by Herrera-Guzmán et al. (2009). Our data on verbal memory as assessed by Rey Verbal Learning and Recall Test are also in line with other studies with SSRIs such as citalopram (Zobel et al. 2004), or SNRIs such as duloxetine (Raskin et al. 2007, Baune and Renger. 2014), but some reports showed no improvement in the efficiency of auditory-verbal declarative or working memory after 8-week therapy with four different SSRIs (fluoxetine, sertraline, citalopram, paroxetine) (Talarowska et al. 2010).

Therefore further studies are needed to assess whether SSRIs and SNRIs can differentially affect verbal and working memory in partial responders MDD patients.

Herrera-Guzmán et al. (2010) conducted a comparison of 36 patients treated with a SSRI and 37 patients treated with an SNRI for 24 weeks. Cognitive performance was compared with 37 healthy subjects. The administration of both treatments improved working memory, as well as attention and all the executive functions, but the cognitive functions of depressed patients did not improve enough to reach the level of performance of the control subjects. Along this line we found in our study a small, but significant improvement in executive function that was detect by using FAB, a tool originally designed for validating the frontal lobe function in neurodegenerative diseases, which consists of six subsets of items exploring different functions related to the frontal lobes (Dubois et

al. 2000). It is known that the severity of depression is associated with impaired performance in executive functioning and episodic memory, but not with impaired semantic memory (McDermott and Ebmeier, 2009). We show for the first time that both SSRIs and SNRIs can rescue deficits in executive function in MDD patients. It will be important to confirm these data in larger studies by combining FAB with Digit Symbol Substitution Test (DSST). DSST is a validated tool to detect executive dysfunction, recently adopted in randomized double-blind placebo-controlled trials where the superiority of a new multimodal antidepressant drug, vortioxetine, has been demonstrated in improving executive function in MDD patients when compared to duloxetine (Katona et al. 2012, McIntyre et al. 2014).

The main finding of our observational study is that the effects of SSRIs and SNRIs on cognitive symptoms are independent from their clinical efficacy on affective symptoms. A new way of analyzing the relationship between change in depressive symptomatology and change in cognitive function can be the comparison between responders and non-responders, at the end of the treatment period. Interestingly we found that global cognitive function (MMSE and MoCA scores), executive function and verbal Memory (FAB scores and Rey test_Immediate and_Delayed) significantly improved in full-responders (HDRS reduction $\geq$ 50%) as well as in the other 29 MDD patients including partial responders (HDRS reduction $\geq$ 25% but <50%) and non responders MDD patients (HDRS reduction <25%). In our sample, global cognitive function and executive function scores at baseline (MMSE, MoCA and FAB scores) did not help to predict the HDRS and BDI-II changes in response to either SSRIs or SNRIs, as observed in previous studies (Talarowska et al. 2010); this suggests that cognitive deficits and affective symptoms should be considered as distinct biological and clinical dimensions of MDD.

Different mechanisms can be hypothesized to explain the clinical efficacy of SSRIs and SNRIs on cognitive symptoms in MDD, among which we should consider an increased expression of BDNF and an enhanced localization of this factor at synaptic level (Calabrese et al. 2009), as well as the rescue of other neurotrophins such as Transforming-Growth-Factor-ß1 (TGF-ß1) (Caraci et al. 2010). New studies should be conducted in recent validated animal models of depression, such as

chronic mild stress (CMS) to understand whether SSRIs and SNRIs can revert cognitive deficits in CMS-treated mice and, if so, to elucidate the underlying molecular mechanisms.

A limitation of our study is the relatively small sample of partial responders MDD patients. However, our study compares favorably with other studies of depressive subtypes, which have tended to feature relatively small samples (Roca et al 2014, Talarowska et al. 2010, Baune and Renger 2014). A major strength of our study is that we recruited for SSRI and SNRI cohorts two highly homogeneous groups of MDD patients with a recent history (in the last 4 weeks) of partial response to a previous treatment with an antidepressant drug, where for the first time multiple psychometric tools were applied to assess both cognitive and affective symptoms were applied for a long-term follow-up (3 months), showing for the first time that second-generation antidepressants such as SSRI and SNRIs can revert cognitive deficits in partial responders MDD patients independently from their clinical efficacy on depressive symptoms.

We believe that our results might be relevant also to plan new studies and replicate these data in elderly MDD patients and/or in MCI patients with a recent history of MDD, which is known to be a risk factor for the development of Alzheimer's disease (AD). Depression is among the earliest symptoms of preclinical stages of AD and the presence of depressive symptoms significantly increases the conversion of Mild Cognitive Impairment (MCI) into AD (Modrego et al. 2004; Houde et al. 2008; Caraci et al.2014). A long-term treatment with antidepressants is known to reduce the risk to develop AD (Kessing et al. 2009) and a prophylactic treatment with SSRIs prevents cognitive deficits in models of AD (Nelson et al. 2007). It might be therefore interesting to assess whether both SSRI s and SNRIs can rescue cognitive deficits also in elderly depressed MCI patients with a high risk to develop AD, thus preventing the conversion from MCI to AD.

Role of founding source

No sponsor acted in any phase of the present study. Funding for this study was provided by the International PhD Program in Neuroscience, School of Medicine, University of Catania, Catania, Italy.

Contributors

Author F Caraci designed the study and wrote the protocol of the observational study. Authors S Castellano and A Ventimiglia (Department of Educational Sciences) rated depressive and cognitive symptoms in MDD patients. Authors A Ventimiglia, (Villa dei Gerani), S De Vivo, E Belelli recruited MDD patients at the first clinical center (Villa dei Gerani), whereas E Aguglia, MS Signorelli and E Fazio recruited MDD patients in the other clinical center (Unit of Psychiatry, A.O.U. Policlinico-V. Emanuele, University of Catania). Author S Di Nuovo undertook the statistical analysis. Authors F Caraci, S Salomone, S Castellano F Drago and S Di Nuovo wrote the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

No conflict of interest exists for any the Authors of the present paper

Acknowledgments

This observational study was supported by the International PhD Program in Neuroscience, School of Medicine, University of Catania, Catania, Italy.

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Table 1. Sociodemographic and clinical characteristics of MDD patients

	Total	SSRI	SNRI
Gender n(%)			
Male	12 (24.5)	9 (27.2)	3 (18.75)
Female	37 (75.5)	24 (72.8)	13 (81.25)
Age	54.37	55.4	56.35
(Mean) SD	(12.59)	(11.55)	(10.39)
Education	9.59	8.72	9.89
	(4.04)	(3.77)	(4.23)
Previous episodes Recurrent MDD n(%)			
1-2 previous episodes	23(46.9)	14 (42.4)	6 (42.8)
≥ 3 episodes N(%)	26 (53.1)	19 (57.6)	8 (57.2)
HDRS	21.1	22.18	18.94
Mean (SD)	(6.7)	(7.38)	(4.58)
BDI-II	32.4	33.67	30.06
Mean (SD)	(10.8)	(11.67)	(8.97)
Antidepressant drug prescribed during the 12 weeks of the study	cscitalopram (14) citalopram (9) paroxetine (9) sertraline(1) venlafaxine(8) duloxetine(8)	cscitalopram(14) citalopram (9) paroxetine (9) sertraline(1)	venlafaxine(8) duloxetine(8)

SSRI= Selective Reuptake inhibitors;
SNRI= Serotonin and Noradrenaline Reuptake Inhibitors;
HDRS=Hamilton depression Rating Scale; BDI-II= Beck Depression Inventory

Table 2. Affective and cognitive changes in the two cohorts of MDD patients during the prospective observational study

	SSRI cohort n=33 Mean (±SD)			SNRI cohort n=16 Mean (±SD)		
	Baseline	**12 weeks**	***t* (*P value*)**	**Baseline**	**12 weeks**	***t* (*P value*)**
HDRS	22.18 (7.38)	12.94 (7.93)	7.06 (**0.002**)	18.94 (4.58)	12.75 (8.99)	3.07 (**0.01**)
HDRS Factor I	6.73 (2.48)	4.39 (2.55)	6.05 (**0.004**)	6.63 (3.10)	4.5 (3.61)	1.93 (0.07)
BDI-II	33.67 (11.67)	20.06 (12.78)	5.83 (**0.004**)	30.06 (8.97)	20.06 (12.39)	5.22 (**0.005**)
MMSE	26.65 (2.78)	28.15 (1.86)	-3.13 (**0.009**)	25.75 (3.46)	27.64 (2.82)	-2.57 (**0.02**)
MoCA	21.88 (4.28)	24.95 (3.47)	-4.17 (**0.005**)	20.69 (5.31)	23.45 (4.64)	-4.10 (**0.005**)
FAB	14.69 (2.89)	15.76 (2.45)	-3.06 (**0.009**)	14.44 (2.02)	16.24 (2.11)	-3.78 (**0.008**)
Immediate Rey test	31.80 (7.14)	40.08 (10.81)	-6.27 (**0.003**)	30.69 (9.04)	37.75 (10.97)	-3.77 (**0.008**)
Delayed Rey test	5.57 (2.16)	8.26 (2.87)	-7.60 (**0.002**)	5.33 (2.36)	7.39 (3.0)	-4.16 (**0.005**)
Span forward	5.33 (1.05)	5.55 (1.23)	-1.19 (0.24)	5.31 (1.30)	6.06 (0.93)	-2.67 (**0.02**)
Span backward	3.33 (0.74)	3.61 (0.93)	-1.79 (0.08)	3.56 (1.46)	3.50 (0.82)	0.25 (0.81)

t= Two-sample t-test

Table 3. Cognitive assessment in MDD patients classified as: 1) full responders (HDRS reduction ≥50%) or 2) partial responders (HDRS

reduction ≥ 25% but <50%)/ non responders (HDRS reduction <25%).

	Responders n=20 Mean (±SD)			Partial/Non-responders n=29 Mean (±SD)		
	Baseline	**12 weeks**	***T* (*P value*)**	**Baseline**	**12 weeks**	***t* (*P value*)**
MMSE	26.44 (2.61)	28.55 (1.73)	-3.88 (**0.01**)	26.30 (3.30)	27.60 (2.43)	-2.32 (**0.03**)
MoCA	22.30 (4.51)	24.95 (3.47)	-5.0 (**0.006**)	20.93 (4.71)	23.45 (4.64)	-3.73 (**0.01**)
FAB	14.37 (3.06)	16.12 (2.17)	-4.8 (**0.007**)	14.77 (2.31)	15.77 (2.47)	-2.49 (**0.02**)
Immediate Rey test	30.42 (7.7)	38.22 (11.33)	-4.79 (**0.007**)	32.14 (7.81)	40.08 (10.56)	-5.50 (**0.005**)
Delayed Rey test	5.44 (1.55)	7.43 (2.35)	-6.50 (**0.003**)	5.53 (2.59)	8.35 (3.22)	-6.53 (**0.003**)
Span forward	5.80 (1.06)	6.15 (0.88)	-1.44 (0.17)	5.00 (1.07)	5.41 (1.24)	-2.05 (0.06)
Span backward	3.80 (1,15)	3.85 (0.99)	-0.21 (0.83)	3.14 (0.83)	3.38 (0.78)	-1.57 (0.13)

t= Two-sample t-test

General Discussion

Psychometrics and evaluation of omega-3 fatty acid clinical efficacy in MDD

Omega-3 fatty acids are polyunsaturated fatty acids (PUFA) with a double bond at the third carbon atom from the end of the carbon chain (Scorletti and Byrne, 2013). PUFA can be classified in various groups by their chemical structure in omega-3 and omega-6 (plus omega-9 and conjugated) fatty acids: the omega-3 PUFA (also called ω−3 fatty acids or n−3 fatty acids) refers to a group of PUFA in which the first double bond is 3 carbons from theend (omega) carbon atom of the molecule; the omega-6 (also referred to as ω−6 fatty acids or n−6 fatty acids) are a family of PUFA that have in common a final carbon–carbon double bond in the n−6 position, that is, the sixth bond, counting from the methyl end (Grosso et al., 2014; Simopoulos, 2002).

The three types of omega-3 fatty acids involved in human physiology are ALA (found in plant oils), EPA, and DHA (both commonly found in marine oils).

Omega-3 PUFA have been long investigated for their anti-inflammatory effects in inflammatory-related diseases (Din et al., 2004) and have been considered of particular interest for the treatment of certain forms of chronic diseases related with their anti-inflammatory effects (Simopoulos, 2006). Data from cross-sectional studies exploring the association between omega-3 dietary intake and the prevalence of depression are various and contrasting. Some studies reported a significant association between omega-3 fatty acids intake and depressive symptoms.

However, some studies suggest that the relation between depressed mood and omega-3 fatty acids intake may reflect a wider association between depressed mood and lifestyle (Grosso et al., 2014).

According to some studies omega-3 have a protective role in the onset of depression (Sontrop et al., 2008), other studies have found instead that their consumption does not decrease the risk of depression (Lucas et al., 2011) . Probably, this contrasted data depend from several methodological issues.

Previous meta-analytic studies reported a general positive effects of omega-3 PUFA intake in ameliorating symptoms of depression.

In major depression, risk of illness may be due to abnormality in phospholipid metabolism, namely alteration of omega-3 PUFA uptake mechanism with specific impairment of the enzyme Type IV cytosolic phospholipase A2 and the fatty acid CoA ligase 4, although this genetic alteration was not found in bipolar mood disorder. These recent observations at molecular level could explain the low omega-3 PUFA levels in patients with depression, accompanying (or enhancing) the decreased dietary intake (Grosso et al., 2014).

An effect of omega-3 intake suggested to positively influence the depressive status, is the potential interaction with the serotonergic and dopaminergic transmission, including metabolism, release, uptake, and receptor function (Grosso et al., 2014). However, since omega-3 PUFA play a fundamental role in the structural development and maintenance of a normal brain, it has been hypothesized that chronic supplementation with PUFA may induce antidepressant-like effects in parallel to brain structural changes. Morphological changes, such as reduction in the gray matter volume within the prefrontal cortex, the hippocampus, and the striatum, have been repeatedly reported in the brain organization of depressed patients (Grosso et al., 2014).

The current therapeutic strategies against depression include drugs which enhance either serotonergic neurotransmission, noradrenergic neurotransmission, or both. However, in 30% of the cases, there is little or no response to the medication, and almost half of patients treated with tricyclic anti-depressants do not show significant clinical improvements (Massart et al., 2012).

The role of omega-3 on preventing psychiatric diseases, if acting through short-term anti-inflammatory effects or on the cerebral parenchyma itself through a long-term structural or functional action, remain to be clarified. It can be speculated that all types of action can occur simultaneously: on one hand, by maintaining and increasing the brain structures, and preserving their function by interacting with phospholipid metabolism and, hence, the modulation of signal transduction; on the other hand, preventing or decreasing the inflammatory status occurring during depression (Grosso et al., 2014).

The present study demonstrated the efficacy of omega-3 in depressed patients, but inconclusive results were found for patients with other pathological conditions (namely schizophrenia and AD) as well as in healthy subjects or with perinatal depression. Omega-3 PUFA were also effective on bipolar disorder, although the evidence was weakened by the exclusion of three studies conducted on patients with MDD or on patients with depressive symptoms in which a lack of rigor in patients' selection may lead to the inclusion of both normal emotional states and "subthreshold" depressed subjects, eventually affecting the results.
The study, in addition, highlights a number of methodological criticisms that mainly concern the inclusion criteria. The inclusion criteria, in fact, should be more rigid and should be based on the score to psychometric tests rather than solely on clinical diagnosis. Psychometric instruments, in fact, represent an essential tool not only for a better diagnostic or for evaluating the efficacy of a treatment, but also for use inclusion criteria more rigid in order to make better the methodology in efficacy studies.

The combination of neuropsychological and biological markers in MCI and early AD: relevance for the diagnosis and implications for the pharmacological treatment

Recently, the criteria for the clinical diagnosis of AD have been revised by the National Institute on Aging and the Alzheimer's Association workgroup (Mangialasche et al., 2010) and new criteria focus on the presymptomatic stage of AD and incorporate biomarkers to identify early stages of AD, susceptible of being treated with disease-modifying drugs (Salomone et al., 2012; Vellas et al., 2011).
Clinical trials on disease-modifying drugs will be therefore focused, in the next years, on prevention rather than treatment of AD. Neuropsychological tools combined with validated biological markers might be essential to detect the earliest clinical manifestations of AD and might be also particularly useful in monitoring the response to disease-modifying therapies in amnestic MCI patients who are at high risk to develop AD. Presently, there are few biological markers for the early identification of MCI which progresses to AD and MCI which does not progress, among which we should consider the association of elevated tau with low levels of $A\beta_{42}$ in CSF, hippocampal atrophy assessed with

magnetic resonance imaging (MRI), and positron-emission tomography (PET) evidence of Aβ deposition (Cummings, 2011; Drago et al., 2011)

According to this scenario genetic studies might be also important for the identification of new biological markers which can predict the progression from MCI to AD, in particular in amnestic MCI patients which have the highest risk to develop AD. Furthermore these genetic studies should be conducted also considering the presence or the absence of other recognized risk factors for AD such as depression, diabetes, hyperhomocysteinemia (Ownby et al., 2006; Zemva and Schubert, 2011; Zhuo et al., 2011).

APOE4 allele is the major susceptibility factor for late-onset forms of AD, but recent studies, as discussed in this review, suggest that other genetic determinants might also studied to determine specific risk profiles of LOAD patients.

Neuropsychiatric symptoms may be among the earliest symptoms of preclinical stages of AD and targeting them therapeutically might delay the transition to dementia. Genetic studies of neuropsychiatric symptoms, in particular depression, are therefore important both for the diagnosis and treatment of LOAD. Genetic variations of neurotrophic factors, such as BDNF and TGF- β1, are associated with the risk to develop depression in AD patients and, most importantly, increase the conversion of MCI patients into AD. Longitudinal GWAS in amnestic MCI patients with or without depressive symptoms should be therefore conducted in the next years to examine whether genetic variations of neurotrophins of BDNF and TGF-β1 can affect the rate of conversion into AD by increasing the risk to develop depressive symptoms. However these studies should also consider the contribute of genetic variations in other neurotrophins genes, such as the Nerve Growth Factor (NGF) locus, which can influence the occurrence of LOAD (Di Maria et al., 2012). Genetic studies will be also important for the selection of patients eligible for neuroprotective strategies aimed at rescuing neurotrophin signaling and preventing the progression of AD.

Rescue of TGF-β1 signaling might represent a new strategy to promote neuroprotection in amnestic MCI patients at high risk to convert into AD (Caraci et al., 2012). Different psychotropic drugs are known to increase

TGF-β1 signaling such as lithium, agonists of group II metabotropic glutamate receptors, and antidepressants (Caraci et al., 2011).

Lithium has neuroprotective action in animal models of AD, not only via the inhibition of GSK-3β, but also through other mechanisms, including reduction of Aβ production and the release of TGF-β1 (Caraci et al., 2011). In patients treated with lithium for psychiatric disorders, the risk of developing AD is reduced (Nunes et al., 2008; Yeh and Tsai, 2008). A recent placebo-controlled clinical trial in patients with amnestic MCI showed that long-term lithium treatment slow the progression of cognitive decline and also reduces CSF concentration of P-tau (Forlenza et al., 2011), but presently we do not know whether these disease-modifying properties of lithium might be related to TGF-β1 gene variants and/or to a rescue of TGF-β1 signaling.

Agonists of group II metabotropic glutamate receptors protect cortical neurons against ß-amyloid toxicity through the release of TGF-β1 from glial cells (Caraci et al., 2011). Orthosteric mGlu2/3 receptor agonists are under development for the treatment of schizophrenia (Patil et al., 2007; Kinon et al., 2011). Therefore, these drugs might be helpful for the treatment of psychotic symptoms which are common in the early stage of AD. However, the neuroprotective properties of orthosteric mGlu2/3 receptor agonists might be also evaluated in amnestic MCI patients at high risk to convert into AD.

Finally, different second-generation antidepressant drugs, including venlafaxine, paroxetine and sertraline, significantly increase circulating TGF-β1 levels in patients with major depression (Sutcigil et al., 2007; Lee and Kim, 2006). It is known that a long-term treatment with antidepressants can reduce the risk to develop AD. On the other hand, studies of antidepressants for depression in AD show conflicting results, with several negative findings reported in recent large trials (Enache et al., 2011); therefore, the efficacy of antidepressants as first-line treatment of depression in AD has been recently reconsidered because of the absence of benefit compared with placebo and increased risk of adverse events (Banerjee et al., 2011). Furthermore, depressed MCI patients with a poor response to antidepressants are at an especially increased risk of developing dementia. It will be interesting to examine whether genetic variations of TGF-β1 (i.e. the +10 CC genotype) can influence the rate of

LOAD in long-term treated depressed patients and/or the response to antidepressants in MCI or AD patients.

Perspectives for secondary prevention trials in early AD

In the last five years all candidate disease-modifying drugs for AD have failed in Phase III clinical trials (Mangialasche et al., 2010; Salomone et al., 2012). There is growing concern that the stage of AD patients recruited in these clinical trials was too advanced (mild to moderate AD patients) to observe a neuroprotective effect of "potential" disease-modifying drugs. Other factors that may have contributed to these failures are the inclusion of subjects who did not truly have AD pathology and/or the non-appropriate use of validated biological and neuropsychological markers. Furthermore, all these trials have been conducted in samples of AD patients recruited with the old clinical criteria. A significant advance has been made in the diagnostic research field of AD with the recent proposal of new diagnostic criteria, which specifically incorporate the use of biomarkers as defining criteria for preclinical stages. Nevertheless, these biological markers (imaging markers and CSF-derived markers) should be combined with neuropsychological tests assessing both episodic memory and other cognitive domains (i.e. executive dysfunction) in order to significantly increase the chance to predict the conversion into AD within 24 months as discussed above. The improved predictive prognostic information available from combined use of biological with neuropsychological tests argues strongly for their inclusion in future secondary prevention trials in MCI due to AD patients. According to most studies discussed in this thesis, prerequisites for successful preventive trials in AD might be not only the use of appropriate biomarkers (PET-amyloid imaging, MRI, and the $A\beta_{1\text{-}42}$/p-tau CSF ratio) (Vellas et al., 2011), but also the enrichment of these trials with selected amnestic MCI patients with multiple-domain cognitive deficits (the subgroup with the highest risk of conversion). These patients might be selected by using new cognitive screening tests (MoCA) combined with specific neuropsychological instruments for single cognitive function (memory, attention, executive function and visuoconstructionalfunction).

We believe that combination of biological and neuropsychological markers will be essential to identify MCI patients at high risk of

conversion, which should be included in future secondary prevention trials to analyze the clinical efficacy of potential disease-modifying drugs. This approach could enable significant reductions in sample size requirements for clinical trials of investigational AD-modifying therapies, whereas it would be too expensive to design clinical trials with large sample sizes to detect the preclinical stage of the disorder (healthy controls with evidence of AD pathology) (Holland et al., 2012). The most promising disease-modifying drugs in AD field, which are currently studied in Phase II-III clinical trials, are active immunotherapy (ACC-01, CAD-106) and passive immunotherapywith humanized monoclonal antibodies (i.e. bapineuzumab, solaneuzumab, gantenerumab) (Delrieu et al., 2012). Among these potential disease-modifying drugs,gantenerumab is the only drug presently studied in prodromal AD (i.e. progressive episodicmemory impairment with abnormal biomarkers according to Dubois et al. (2010). In this trial primary outcomes are changes in the Clinical Dementia Rating scale Sum of Boxes (CDR-SOB) and amyloid deposition assessed by PET-amyloid imaging. ADAS-Cog will be used as secondary outcome, but specific neuropsychological tests for single cognitive domain have not been planned. Preliminary results suggest a potential efficacy of solanezumab in mild AD. A first analysis of the solanezumab Phase 3 data from Expedition 1 and Expedition2 trials suggests a cognitive benefit in both trials overall (1.41 points at ADAS Cog), as well as a functional benefit on IADL. A new phase III trial is ongoing to confirm these cognitive benefits of solaneuzumab (Di Maria et al., 2012). Prevention trials with solaneuzumab, gantenerumabare currently ongoing in subjects with dominantly inherited AD such as the Dominantly Inherited Alzheimer Network (DIAN)to assesswhether these drugs can prevent AD when administered in a presymptomatic stage of the disease (Wada et al., 2005).

These prevention trials may validate the hypothesis presented in this paper that combination of biological and neuropsychological markers is a valuable approach to develop disease-modifying drugs for AD.

Effects of second-generation antidepressants on cognitive symptoms in MDD: the role of psychometric tools

Different studies have demonstrated that major depression is often accompanied by cognitive deficits that are regarded as consistent, replicable,clinically significant,and of small to medium effect size (McIntyre et al., 2013).

Cognitive impairments in clinical depression have most often been identified in the domains of attention, learning and memory, and executive functioning (Beblo and Lautenbacher, 2006; Lee et al., 2012). Cognitive deficits such as immediate memory and attention (Baune et al., 2010) last even when depressive severity decreases and/or depression is remitted (HDRS <7) in both medicated and unmedicated MDD patients (Baune et al., 2012).

Cognitive dysfunction might therefore be considered as both, a state-and a trait-marker of major depression. Recent studies have investigated the effects of second-generation antidepressants on cognitive function (Baune et al., 2014). It has been suggested that some SSRIs and SNRIs can decrease the resting-state functional connectivity independent of mood change and in areas known to mediate reward and emotional processing in the brain (McCabe and Mishor, 2011), antidepressant treatment may also decrease cognitive function. According to this scenario it is clinically relevant to examine the cognitive consequences of MDD from those of SSRI treatment and from cognitive evaluation of MDD subjects. A medication-naïve state before starting the administration of antidepressants has been recently highlighted in a study by Herzallah et al. (2013). More- over, additional factors such as demographic, psychological, and biological factors have been suggested to influence the profile and severity of neuropsychological deficits (Beblo et al., 2011).

Even if the relationship between cognitive deficits and general functioning is controversial, there is evidence that cognitive deficits are core symptoms with an impact on functioning in depression which can persist also after remission. It is known that deficits in executive functioning have a mediating effect on the relationship between depression and impaired activities of daily living (Kiosses and Alexopoulos, 2005). Moreover, MDD patients with cognitive deficits are less compliant to antidepressant treatment and show also an increase risk for suicide (Martinez-Aran et al., 2009; Westheide et al., 2008).

All this evidence suggests that cognitive symptoms represent a major clinical dimension in MDD and a new target in the pharmacological treatment of depression.

Some antidepressants have been reported to improve not only mood-related symptoms, but also cognitive function in depressive patients. Research has primarily SSRIs and SNRIs indicating that SNRIs are superior to SSRIs at improving certain cognitive functions (Herrara-Guzman et al., 2009). In contrast, SSRI antidepressants such as citalopram have been suggested to diminish neural processing of aversive and rewarding stimuli in healthy individuals (McCabe et al., 2010).

On the other hand different antidepressants have been examined and seem to improve cognitive function including selective serotonin reuptake enhancer (SSREs), tricyclic antidepressants (TCAs), and other specific drugs (Allain et al.,1992; Nickel et al., 2003; Gallassi et al., 2006; Holtzheimer et al., 2008; Katona et al., 2012; Levkovitz et al., 2012);

SSRIs and the SSRE tianeptine are able to improve certain cognitive functions, whereas limited evidence was found for effects of the SNRI duloxetine on cognition, but it possibly improves social functioning. A preliminary study tested the effects of four different SSRIs (fluoxetine, sertraline, citalopram, paroxetine) on auditory–verbal declarative and working memory performance in a case-control design comparing the results of patients with remission to those without remission (Talarowska et al., 2010). As a result, patients with and without remission made significantly less errors in the Stroop Test showing no significant difference to the control group anymore but no improvements on the other psychometric tests (Stroop Test time of reading and of color naming, Rey test). However, another clinical trial among depressed adults showed that all measured cognitive functions (WAIS-R, Rey test) improved as well as depressive symptoms (Zobel et al., 2004). A randomized case-control study among elderly patients also found that treatment with escitalopram significantly improved affective and cognitive symptoms (GDS, MMSE) as well as facial identity recognition memory for angry faces (Savaskan et al., 2008). Furthermore, in a 1- year follow-up among elderly patients with minor or subsyndromal depressive symptomatology the effects of sertraline and citalopram were compared suggesting that both antidepressants equally improved depressive and cognitive symptoms and

social functioning (Rocca et al., 2005). It is interesting to underline that in two studies the effect of the antidepressant treatment with sertraline was accompanied by a beneficial effect on psychomotor slowing and executive functions (Constant et al., 2005; Schrijvers et al., 2009). A 3-months open multicentre study among elderly depressed patients indicated that tianeptine is able to improve both depressive and cognitive symptoms (Saiz-Ruiz et al.,1998).

To assess functional ability after treatment with duloxetine two double-blind, randomized, placebo-controlled trials were conducted under the same protocol (Oakes et al., 2012). The two trials did not yield consistent findings on all measures but both the trials demonstrated significant improvements with duloxetine treatment in depressive symptoms and in social functioning in the beginning as early as week4 and continuing to the end of 12 weeks. In one study among elderly patients it was shown that duloxetine did not exceed placebo on any cognitive measure (Robinson et al., 2014), whereas in another study duloxetine was superior to placebo on the Verbal Learning Rey Test but not on other psychometric test assessing cognitive function [MMSE, Digit Symbol Substituion Test (DSST)] (Raskin et al., 2007).

In a prospective cohort study with database linkage 281 elderly medical in patients were diagnosed with major, minor or no depression and followed up with the MMSE and the HDRS over 1 year to examine the relationship between antidepressant medication and long-term cognitive effects (Han et al., 2011). The authors found an association between antidepressant medication and cognitive improvement in minor depression, but not in major or no depression. A case-control study examined the cognitive response to antidepressant treatment (nortriptyline or paroxetine) for patients with late-life depression and compared patients to those without concomitant cognitive impairment (Butters et al., 2000). It was found that as a group, the elderly depressed patients showed a small improvement in overall cognitive and social functioning and that patients with cognitive impairment improved in specific domains ,but they were unable to reach normal levels of performance. In a study among young depressed adults the neuropsychological effects of the dopamine modulator bupropion and the cognitive predictors of treatment response to the drug were examined. The overall results suggest that memory and mental processing speed

performance could be predictors of response to bupropion, and they are also the functions that are improved after bupropion treatment (Herrera-Guzman et al., 2008).
In the third study presented in this doctorate thesis we have adopted a new strategy of psychometric evaluation combining , for the first time, "standard " psychometric tools validated for the assessment of affective symptoms in MDD patients (HDRS and BDI-II) with neuropsychological tools such as MMSE, MoCA, FAB, Rey's 15 Words Test developed to detect cognitive deficits in mild cognitive impairment or in early AD (Caraci et al., 2014).
This new psychometric protocol help us to identify new clinical effects of second-generation antidepressants. The main finding of our observational study is that both SSRIs and SNRIs can improve cognitive symptoms independently from their clinical efficacy on affective symptoms.
A neurobiological and clinical continuum exists between major depression, MCI, and dementia. According to this clinical continuum we believe that our strategy of neuropsychological assessments, which includes HDRS, BDI-II, MMSE, MoCA, Rey's test, FAB, can be applied to a wide range of patients from MDD to MCI/AD. This neuropsychological battery might represent a new tool to detect in future clinical studies affective symptoms and early cognitive deficits not only in MDD patients, but also in MCI patients with an high risk to convert into AD.

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