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Clinical Studies of Biomarkers in Suicide Prediction

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ABSTRACT

Suicide is a major clinical problem in psychiatry and suicidal behaviours can be seen as a nosological entity per se. Predicting suicide is difficult due to its low base-rate and the limited specificity of clinical predictors. Prospective biological studies suggest that dysfunctions in the hypothalamo–pituitary–adrenal (HPA) axis and the serotonergic system have predictive power for suicide in mood disorders. Suicide attempt is the most robust clinical predictor making suicide attempters a clinical high-risk group. A prediction model that incorporates biological testing to increase specificity and sensitivity of prediction of suicide risk is of potential clinical value.

The aim of these studies was to investigate the predictive potential of two biomarkers: the nonsuppression in the dexamethasone suppression test (DST) and low 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid (CSF) in suicide prediction in clinical high-risk groups and to investigate relationships of the two neurobiologic correlates and some psychological components of the vulnerability to suicide.

DST and CSF monoamine metabolite data from two cohorts of suicide attempters and mood disorder inpatients (nearly 400 patients) were analysed in relation to subsequent death by suicide and to suicide intent and hopelessness. Optimal threshold of the DST in suicide prediction was analysed by Receiver Operating Characteristic (ROC). Interrelationship of two potential biomarkers was analysed in a subgroup of patients stratified by suicide attempt as a clinical predictor.

Suicide mortality rates were 9.4% for unselected mood disorder inpatients and 22% for those hospitalised after a suicide attempt. DST nonsuppression was found to be a biologic predictor of suicide in mood disorder inpatients with index suicide attempt yielding a risk ratio of 2.8; the optimal threshold for DST nonsuppressor status in suicide prediction was different for males and females. CSF 5-HIAA predicted suicide in short term while DST non-suppression seemed to be a long-term predictor of suicide risk for male suicide attempters. The interrelationship of two biomarkers was different in suicide attempters compared to mood disorder inpatients without suicide attempt and in male suicide victims compared to survivors indicating that the two biomarkers can be seen as independent biologic risk factors for suicide. Suicide intent measured by Beck Suicide Intent Scale is correlated to CSF HVA/5-HIAA ratio but failed to predict suicide in the clinical high-risk group.

DST non-suppression and low CSF 5-HIAA were independent biomarkers of suicide risk in suicide attempters. Support was lent to reintroduction of the DST as a complementary measurement of biological vulnerability in the clinical high-risk group of hospitalised male suicide attempters with mood disorder.

Keywords: suicide, suicide attempt, HPA axis, dexamethasone suppression test (DST), serum cortisol, Receiver Operating Characteristic (ROC), serotonin, CSF 5-HIAA, depression, prediction, suicide intent, hopelessness.

LIST OF PUBLICATIONS

This thesis is based on the following original articles and manuscripts referred to in the text by Roman numerals:

I
Samuelsson M, **Jokinen J**, Nordström A-L, Nordström P.
CSF 5-HIAA, Suicide Intent and Hopelessness in the Prediction of Early Suicide in Male High Risk Suicide Attempters.
Acta Psychiatrica Scandinavica 2006; 113: 44-47.

II
Jokinen J, Carlborg A, Mårtensson B, Forslund K, Nordström A-L, Nordström P.
DST non-suppression predicts suicide after attempted suicide.
Psychiatry Research 2007; 150(3): 297-303.

III
Jokinen J, Nordström A-L, Nordström P.
The Relationship Between CSF HVA/5-HIAA ratio and Suicide Intent in Suicide Attempters.
Archives of Suicide Research 2007; 11(2): 187 – 192.

IV
Jokinen J, Mårtensson B, Nordström A-L, Nordström P.
CSF 5-HIAA and DST Non-suppression – independent biomarkers in suicide attempters?
Journal of Affective Disorders E-Pub May 2007.

V
Jokinen J, Nordström A-L, Nordström P.
ROC Analysis of Dexamethasone Suppression Test Threshold in Suicide Prediction after Attempted Suicide.
Journal of Affective Disorders E-Pub July 2007.

VI
Jokinen J, Nordström A-L, Nordström P.
CSF 5-HIAA and DST nonsuppression - Orthogonal Biologic Risk Factors for Suicide in Male Mood Disorder Inpatients.
Manuscript

Contents

ABBREVIATIONS

ACTH adrenocorticotrophic hormone
APA American Psychiatric Association
AUC Area under the curve
BBB Blood brain barrier
CRHR1 corticotropinreleasing hormone receptor 1
BHS Beck Hopelessness Scale
CI Confidence Interval
GR glucocorticoid receptor
CRH corticotropin releasing hormone
CSF Cerebrospinal fluid
DA Dopamine
DEX/CRH test the combined dexamethasone/corticotropin releasing hormone test
DSM-IV Diagnostic and Statistical Manual of Mental Disorders, 4th edition
DST Dexamethasone Suppression Test
ECT Electroconvulsive therapy
fMRI Functional Magnetic Resonance Imaging
5-HIAA 5-hydroxyindoleacetic acid
5-HT serotonin
5HTT Serotonin transporter
HPA axis Hypothalamic-Pituitary-Adrenal axis
HVA Homovanillinic acid
ICD-10 International Classification of Diseases, 10th edition
MAO Monoamine Oxidase
MDD Major Depressive Disorder
MR mineralocorticoid receptor
NA Noradrenaline
NS Non significant
PET Positron Emission Tomography
PVN Paraventricular Nucleus
ROC receiver operating characteristics
RR Relative Risk
SCID-I Structured Clinical Interview for DSM-IV Axis I disorders
SCID-II Structured Clinical Interview for DSM-III-R personality disorders
SD Standard Deviation
SMR Standardized Mortality Ratio
SIS Scale for Suicidal Intent
SLE stressful life event
TPH Tryptophan Hydroxylase
WHO World Health Organization

1 INTRODUCTION

1.1 The scope of the suicide problem

In the year 2000, approximately one million people died from suicide: one death every 40 seconds. In the last 45 years suicide rates have increased by 60% worldwide. Suicide is now among the three leading causes of death among those aged 15-44 years (both sexes); these figures do not include suicide attempts up to 20 times more frequent than completed suicide (see http://www.who.int/whosis, WHO). Every suicide has serious impact on at least six other people and the psychological and social aftermaths of suicide on the family and community is difficult to estimate. The cost of suicide due to depression was estimated at €232 million in 2003 (in 2005 prices) in Sweden (Sobocki et al., 2007). In the field of psychiatry, suicide risk is a major concern in everyday clinical praxis.

1.2 Classification of suicidal behaviour

Suicidal behaviour as a concept includes a range of behaviours from suicidal ideation to suicide attempts and completed suicide (Table 1). Suicidal behaviour varies with respect to manifestation, performance, seriousness and lethality and it is characterized by a variety of terminology in the psychiatric literature. The American Psychiatric Association's (APA) definitions of terms are used in this thesis (APA, 2003).
Suicide is defined as a self-inflicted death with evidence (either explicit or implicit) that the person intended to die (APA, 2003). This term should be utilized only in the case of death.
Suicide attempt is defined as self-injurious behaviour with a non-fatal outcome accompanied by evidence (either explicit or implicit) that the person intented to die (APA, 2003).
Deliberate self-harm is defined as willful self-inflicting of painful, destructive, or injurious acts, but without intent to die. Deliberate self-harm is used especially in the UK (Skegg, 2005).
Suicidal ideation is defined as thoughts serving the agent of one's own death. It may vary in seriousness depending on the specificity of suicide plans and the degree of suicidal intent (APA, 2003).
In this thesis the focus is on three clinical groups of patients: mood disorder inpatients with and without suicide attempt and those who later committed suicide. There has been debate whether those attempting suicide and those committing suicide, present a single or two separate populations during the past decades (Linehan, 1986; Beautrais, 2001). It seems that they are distinct but overlapping populations (Oquendo, 2006).

Table 1. Classification of suicidal behaviour by Beck (1986).
I. Suicide Ideation
Thinking, planning
Impulse or desire
A. Intent to die (High, medium, low, none)
II. Suicide attempt
A. Intent
B. Lethality
C. Method
III. Completed suicide
A. Intent
B. Method

1.3 Epidemiology of suicide and attempted suicide

Suicide is a major public health problem around the world. It is among the leading causes of death, and suicide accounts for more deaths than homicide and war combined. In Sweden the suicide rate was 13.4 /100 000 in 2001, the rate for males was 18.9/100 000 and 8.1/100 000 for females. There has been a 30% decline during the past 15 years. In USA, for example, the suicide rate is 13.9/100 000 (2002). The highest annual suicide rates are in the Baltic countries and former Soviet republics (> 27/100 000) and the lowest in Latin American and Islamic countries (< 6.5/100 000). Men have a higher rate of suicide than women, usually the male to female ratio is approximately 3-4:1 (World Health Organization, 2005). Men commit suicide 2.5 times more frequently than women in Sweden; the ratio was 5:1 in 1970. The suicide rates vary significantly among the various age groups, gender and different countries.
Most psychological autopsy studies report that over 90% of the suicide completers had a diagnosable psychiatric disorder at the time of death, and approximately 60% of all suicides are due to mood disorder (Lönnqvist et al., 1995; Mann, 2003). The estimate of the lifetime prevalence of suicide in those ever hospitalised for suicidality was 8.6%, for affective disorder patients hospitalized without specification of suicidality, the lifetime risk of suicide was 4.0% (Bostwick and Pankratz, 2000). In a large population-based Swedish register-study of mortality of mood disorder patients followed up from the onset of the illness, the standardized mortality ratios (SMRs) for suicide were 20.9 for males and 27 for females with unipolar disorder, and 15 and 22.4, respectively, for bipolar disorder (Ösby et al., 2001).
Official statistics on attempted suicide are not usually collected annually, as is the case for completed suicides. Several epidemiologic surveys have reported population-based estimates of lifetime prevalence of a suicide attempt ranging from 0.7% to 5.9% (Kessler et al., 1999; Weissman et al., 1999; Norlev et al., 2005).
Suicide attempts are more common among young people than the elderly, whereas suicide is more common among the elderly. Between 2% to 12% (median 6%) of young people reported a lifetime history of suicide attempt (Beautrais, 2002). A suicide attempt is one of the strongest predictors of the subsequent suicide. The suicide risk after an attempt is up to 40 times the expected rate (Harris and Barraclough, 1997; Suominen et al., 2004a). In a recent Finnish study 8% of suicide attempters commited suicide during the follow-up of 12 years (Suominen et al., 2004b), a higher long-range suicide risk after a current suicide attempt in depression of 15 % was reported earlier (Nordström et al., 1995). The risk of suicide was highest during the first year following the index attempt (Nordström et al., 1995; Suominen et al., 2004b). Suicide is the main cause of excess deaths, but the increased risk of deaths from other unnatural and natural causes is also of major public health concern causing about half the excess deaths (Ostamo and Lönnqvist, 2001, Ösby et al., 2001).
Repetition is one of the core characteristics of suicidal behaviour. Among those who commit suicide, up to 37% have attempted suicide previously (Harris and Barraclough, 1997). A suicide attempt is the strongest known clinical predictor for suicide (Nordström et al., 1995; Harris and Barraclough, 1997; Möller, 2003; Owens et al., 2005). Owens et al. (2002) reported suicide risk among self-harm patients is hundreds of times higher than in the general population. Isometsä and Lönnqvist (1998) reported that 56% of suicide victims were found to have died at their first suicide attempt.

1.4. Familial and genetic factors in suicidal behaviour

Family, twin and adoption studies have been concordant in proposing the involvement of genetic factors in the predisposition to suicidal behaviour (Bondy et al., 2006). Family studies of suicide and suicide attempts show a several fold increased rate of suicidal behavior in the relatives of either suicide completers or attempters, compared to relatives of controls (Brent et al., 2002; Johnson et al., 1998). Adoption studies have shown concordance for suicide among biological, but not adoptive relatives (Schulsinger, 1979). Twin studies show a much greater concordance for completed and attempted suicide among monozygotic than among dizygotic twins, with heritability estimates of 45–55% (Roy et al., 2001). The heritability of suicidal behaviour, especially suicide, is comparable to the heritability of other major psychiatric disorders, such as schizophrenia and bipolar disorder. Suicide and psychiatric illness in relatives are risk factors for suicide, and the effect of family suicide history is independent of the familial cluster of mental disorders (Qin et al., 2002; Runeson and Åsberg, 2003). It is estimated that 43% of the variability in suicidal behaviour may be explained by genetics, while the remaining 57% may be explained by environmental factors (Roy, 1993; Roy et al., 1995; McGuffin et al., 2001).

1.5 Stress-diathesis model and risk factors of suicidal behaviour

Suicidal behaviour has multiple causes, in which according to a stress-diathesis model, both genetic constitution and acquired susceptibility contribute to a person's predisposition to suicidal acts in stressful situations (Mann, 2003). Although the presence of a psychopathology is a strong predictor for suicide, even in the psychiatric groups at the highest risk, only a minority of patients with these diagnoses commit suicide, indicating the importance of a predisposition to suicidal behaviour that is independent of the main psychiatric disorders (Mann, 2003; Turecki, 2005). Mann and his co-authors (1999) proposed a stress-diathesis model in which the risk for suicidal acts is not only determined by a psychiatric illness (the stressor) but also by a diathesis. The diathesis may be resumed in as a tendency to experience more suicidal ideation and to be more impulsive and thus being more prone to act on suicidal ideas and impulses. A trait factor, such as aggression/impulsivity, was significant in distinguishing past suicide attempters from non-attempters. Their model showed that subjective depression, hopelessness and suicidal ideation were greater in suicide attempters than in non-attempters despite comparable rates of objective severity for depression or psychosis (Mann et al., 1999). The onset or aggravation of a psychiatric disorder is always a stressor, but other types of stressors, such as a psychosocial crisis, can also contribute. The diathesis for suicidal behaviour includes a combination of factors such as familial and genetic components, childhood experiences, gender, psychological support system, religion, availability of highly lethal suicide methods and various other factors (Mann, 2002). The model helps to distinguish those remaining vulnerable, despite seeming to have recovered, and how this underlying vulnerability relates to the acute suicidal state. The components of the diathesis are potential therapeutical targets.
The genetic factors, which may interact with stressful life events (SLEs) (Caspi et al., 2003), are often instrumental to the presence of mood disorders and other psychiatric conditions, which are common in suicidality (Bondy et al., 2006). The hypothesis of genetic moderation implies that differences between individuals, originating in the DNA sequence, bring about differences between individuals in their vulnerability to the environmental causes of many pathological conditions of the mind and body. Nongenetic familial factors as insufficient parenting, sexual and physical abuse may contribute to risk of suicidal behaviour.

Environmental risk factors for suicidal behaviour include maternal stress during pregnancy, restricted fetal growth, birth complications, deprivation of normal parental care during infancy, childhood physical maltreatment and neglect, exposure to violence, substance abuse, toxic exposures and head injury (Mann, 2003; Mittedorfer-Rutz et al., 2004).
These environmental causes are considered to be only contributory because exposure to them does not always generate disorder. Both human and animal studies consistently reveal variability in individuals' behavioural responses to environmental pathogens. Such response heterogeneity is associated with individual differences in temperament, personality, cognition and autonomic physiology, all of which are known to be under genetic influence (Caspi and Moffitt, 2006). New findings concerning the role of genetics and personality traits and neural conduction in suicidal behaviour have recently been published (Wasserman et al., 2007a); identifying and resolving complex patterns and mechanisms of neurobiological gene–environment interactions, which may contribute to suicide, is an important future research focus.

There is clear evidence that the activity of three neurobiological systems play a role in the pathophysiology of suicidal behaviour. This includes hyperactivity of the hypothalamo-pituitary-adrenal (HPA) axis, dysfunction of the serotonergic (5-HTTergic) system and excessive activity of the noradrenergic system (Mann, 2003). Hyperactivity of the HPA axis and high activity of the noradrenergic system appear to be involved in the response to stressful events (Mann, 2003). Dysfunction of the serotonergic system is thought to be trait like and associated with disturbances in the regulation of anxiety, impulsivity and aggression (Mann and Currier, 2007).
A number of psychological variables are associated with suicidal behaviour: impulsivity, dichotomous thinking, cognitive rigidity, hopelessness, problem-solving deficits, over-general autobiographic memory (tendency to remember events in a summarized and over-general way) and biases in future judgement (Beck, 1986; Williams and Pollock, 2000).
Hopelessness is a key psychological variable of suicidal behaviour (Beck, 1986). Hopelessness can be a state or trait-related variable (Mann et al., 1999). Hopelessness has proven to be a better predictor of suicide than depression, and it may mediate the association between depression and suicidal behaviour (Beck, 1986). Hopelessness, or the absence of rescue factors such as positive expectancies, thereby may also occur independent of depression, or apparent excess of degree of depression. Whether hopelessness leads to suicidal behaviour depends upon the presence or absence of risk and protective factors. How patients react to suicidal thoughts and plans creates the conditions in which they persist and escalate. Both efforts to suppress these feelings or to ruminate about them strengthen hopelessness and decrease problem-solving capacity (Williams et al., 2007). The combination of a poor problem-solving capacity and hopelessness has become the main domain of research of psychological vulnerability to suicide.
The level of suicidal intent defined as the degree to which the individual wished to die at the time of attempted suicide and measured by the Beck Suicide Intent Scale (Beck et al., 1974), predicts suicide and can be easily assessed in clinical settings (Harriss et al., 2005; Suominen et al., 2004b). The high intent suicide attempters have been reported to have higher scores in depression measured by MADRS (Kumar et al., 2006). High-lethality suicide attempts are associated with more suicide intent (Placidi et al., 2001) and a history of major depressive disorder has been reported to influence intent to die in violent suicide attempters (Astruc et al., 2004).

1.6 Neurobiology of suicidal behaviour

1.6.1. Hypothalamic-Pituitary-Adrenal (HPA) Axis

The HPA system is the final common pathway in the mediation of the stress response. Briefly, the hypothalamus releases corticotropin-releasing hormone (CRH) in response to a stressor, CRH acts on the pituitary gland, triggering the release of adrenocorticotropin (ACTH) into the bloodstream, which subsequently causes the hormonal endproduct of the HPA-axis, corticosteroid release from the adrenal cortex (mainly cortisol in humans). Cortisol normally exerts a negative feedback effect to shut down the stress response after the threat has passed, acting upon the levels of the pituitary and hypothalamus. Cortisol is a major stress hormone with effect on many organs and brain areas through two types of receptors, i.e. the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR), which have a specific and selective distribution in the brain (Reul and de Kloet, 1985). GRs have been found in multiple brain regions such as the hippocampus, amygdala and prefrontal cortex, which are relevant to cognition. The hippocampus and prefrontal cortex are largely (but not exclusively) inhibitory to HPA axis secretion, whereas the amygdala is implicated in activation of glucocorticoid secretion (for review see Herman et al., 2005).
CRH-expressing neurons in the hypothalamic paraventricular nucleus (PVN) project not only to the median eminence but also to other brain areas, where they regulate the adrenal innervation and thus the sensitivity of the adrenal for ACTH via the autonomic system (Buijs and Kalsbeek, 2001). CRH also shows central effects, including cardiovascular regulation, respiration, appetite control, stress-related behavior and mood, cerebral blood flow regulation and stress-induced analgesia (for review see Swaab, 2005).
Importantly, multiple neuropsychiatric diseases are accompanied by alterations in glucocorticoid secretion, suggesting that dysfunction of the HPA axis may be involved in the deleterious effects of stress on affective state. For example, resistance to glucocorticoid feedback is observed in a substantial proportion of individuals suffering from melancholic depression (Kathol et al., 1989), implying episodic hyper-secretion and attendant consequences on somatic and cognitive function. In contrast, PTSD patients exhibit decreased basal corticosteroid levels (Yehuda et al., 1991; de Kloet et al., 2006) and decreased responsiveness to stress (Heim et al., 2000). Taken together, the research data indicate the importance of maintaining an optimal level of HPA responsiveness, in that mental illness may be associated with either hyper- or hypo-secretion of glucocorticoids.
The postmortem studies point to excessive activation of the HPA axis in depressed suicide victims showing increased numbers of CRH neurons in the paraventricular nucleus of the hypothalamus (Raadsheer et al., 1994) and increased CRH immunoreactivity in monoamine-containing pontine nuclei in depressed suicide men (Austin et al., 2003), increased CRH in the cerebrospinal fluid (Arato et al., 1989), decreased CRH receptors in the frontal cortex (Nemeroff et al., 1988) and a shift in the ratio of CRH-R1/R2 in the pituitaries of suicide victims (Hiroi et al., 2001).

1.6.1.1. Dexamethasone suppression test (DST) and suicidal behaviour

Interest in cortisol functioning in psychiatric patients led to the development of the dexamethasone suppression test (DST) as a formal test of HPA function (Carroll et al., 1968). The DST, frequently abnormal in mood disorder patients, is considered to measure glucocorticoid receptor-mediated negative feedback (Pariante and Miller, 2001).

The synthetic glucocorticoid dexamethasone is administered with the purpose to inhibit cortisol release into to the blood circulation. Individuals who are not capable to suppress serum cortisol concentrations to a certain limit are considered to have a dysfunctional HPA axis and are denoted nonsuppressors of cortisol.
The original method, designed as a test on melancholic depression, included oral intake of 1 mg dexamethasone at 11 p.m., followed by plasma sampling the following day at 8 a.m., 4 p.m. and 11 p.m. (Carroll, 1981a). Among psychiatric patients, a cut-off limit for nonsuppression of cortisol, defined as 5 microgram/dL is regarded to give sensitivity of 50-60% and a specificity of 80% for melancholic depression. The ROC analysis was performed to characterize the discriminative properties of the DST in depression diagnostics as demonstrated by Mossman and Somoza (1989a, 1989b). Beginning with Bunney and Fawcett who suggested a possible association between HPA disturbance and suicide in 1965, a body of research has focused on such associations. The most robust finding using the DST is that suicide but not suicide attempt is associated with non-suppression on the DST (Lester, 1992). Eight prospective reports on the DST in studies of mood disorder patients, which included both previous suicide attempters and non-attempters, reported that the majority of subjects who committed suicide were DST nonsuppressors (Boza et al., 1988; Carroll et al., 1981b; Coryell, 1990; Coryell and Schlesser, 1981; Norman et al., 1990; Roy et al., 1986a; Yerevanian et al., 1983; Yerevanian et al., 2004) while two studies found no relation (Black et al., 2002; Träskman- Bendz et al., 1992). Coryell and Schlesser (2001) estimated that DST non-suppressors had a 14 fold higher risk of suicide compared to suppressors, over a 15-year follow-up period. They note that the next most powerful predictor, a prior serious suicide attempt, indicated only a threefold increase in risk.
A recent meta-analysis (Mann et al., 2006) concluded that non-suppressors have more than 4.5 -fold increased risk of suicide compared with suppressors. There is though some evidence that DST results may not be a useful predictor for mood disorder outpatients or for those with no clinical evidence of suicidality (Coryell et al., 2006).

Concerning suicide attempts, consistent with retrospective studies (Brown et al., 1986; Modestin and Ruef, 1987; Secunda et al., 1986), prospective studies have found that DST suppression status and level of post dexamethasone plasma cortisol did not predict reattempts (Black et al., 2002; Norman et al., 1990; Roy, 1992; Roy et al., 1986b).
Two studies found an association between DST non-suppression and seriousness of suicide attempts at baseline (Norman et al., 1990; Targum 1983), and one study over the follow-up period (Coryell, 1990). The definition of a serious attempt varied though across the studies from high medical damage (Norman et al., 1990) to necessitating hospitalization (Targum et al., 1983). In the third study, DST non-suppressors were more likely to make a psychologically, rather than medically, serious attempt during the follow-up (Coryell, 1990). Roy (1992) found that previous violent attempters had higher maximum post DST plasma cortisol levels than previous nonviolent attempters at baseline, but no significant differences were observed between attempters, violent or nonviolent, and non-attempters during a 5-year follow-up.
One prospective study of DST and suicide attempts found surprisingly that suppressors had a higher rate of suicide attempts both prior to and after entry into the study (Black, 2002). This large study (N=432) included the early sample of Coryell and Schlesser (2001) that found more suicides in DST non-suppressors consistent with that group having made more serious suicide attempts. The reason for the different findings between these two studies is unclear. Non-suppression on the DST may be associated with suicide because it predicts a failure to respond to antidepressant treatment or a tendency for early relapse such as shortly after discharge (Appelhof et al., 2006; Brouwer et al., 2006).

Two prospective studies (Targum, 1984; Yerevanian et al., 1983) have reported that non-suppressors, particularly those who fail to normalize during the inpatient treatment, have worse outcome in terms of remission and relapse, conditions which clinical follow-up studies have suggested elevate risk for future suicidal behaviour (Oquendo et al., 2002).
The pharmacokinetics of dexamethasone varies significantly among severely depressed patients and this appears to contribute in part to the lack of specificity of the DST (Mossman and Somoza, 1990). Besides the dexamethason pharmacokinetics there are some other important confounding factors to consider when perfoming and interpreting the DST. The DST was introduced with the purpose to detect Cushing's disease (Liddle, 1960) and this disease has to be taken into consideration. Other confounding factors are major physical illness, acute weight loss, hypoglycaemia, acute alcohol withdrawal, and pharmacological agents (Holsboer, 1983). Recently results from animal studies have shown that blood–brain barrier (BBB) function influences neuroendocrine regulation (Muller et al., 2003).

1.6.1.3 Genes involved in the HPA axis and suicidal behaviour

There are relatively few studies in humans of genetic liability for HPA axis dysfunction. A small number of clinical studies in depressed patients have explored the genetic influence on HPA axis function using various candidate genes and methods. Binder et al. (2004) found associations of response to antidepressants and recurrence of depressive episodes with polymorphisms in the FKBP5 gene—a glucocorticoid receptor-regulating co-chaperone. A recent report of alterations of the corticotropin-releasing hormone receptor 1 (CRHR1) gene as marker for suicidality in depressed males exposed to low levels of stressful life events (Wasserman et al., 2007b) and a previous report on the relation between the CRH-regulated TBX19 gene and certain neurotic personality traits among suicide attempters (Wasserman et al. 2007c) provide new evidence on the role of genetics of HPA axis dysregulation and suicidal behaviour. One study has reported that haplotype variation at the CRHR2 locus is associated with suicidal behaviour in bipolar disorder (deLuca et al., 2007).

1.6.2 The Serotonin system

Phylogenetically, the serotonergic system (5-HT) is one of the oldest transmitter systems in the brain. Serotonin is a monoamine widely distributed in the brain and involved in mood and impulse control. The serotonergic system plays a role in the regulation of a range of biological functions including sleep, circadian rhythm, appetite and cognition, Combining a complex innervation of most cortical and subcortical structures, with multiple receptor subtypes, there is a diversity of signaling pathways and functional roles that explain the association of serotonin with many different types of psychopathological conditions (Mann, 1990).
The serotonergic system plays a major role in depression and additional associations with suicidal behavior, impulsive aggression, anxiety disorders, alcoholism and eating disorders have been reported. Postmortem studies localize serotonergic abnormality to the ventromedial prefrontal cortex (Arango et al., 1995; Mann, 2000).
The 5-hydroxyindoleacetic acid (5-HIAA) concentration in the cerebrospinal fluid (CSF) is thought to reflect events in the brain. Although this has been contested, mainly because of the contribution to CSF 5-HIAA from the spinal cord, indirect evidence suggests that CSF measures are valid markers of brain events. Thus, 5-HIAA in the CSF and in the cerebral cortex are correlated and CSF 5-HIAA concentrations increase or decrease as predicted by pharmacological principles, when drugs known to affect the serotonin system are given to patients (Åsberg, 1997).

5-HIAA concentrations depend on factors such as age and sex, body height, body posture and movement before and during lumbar puncture and amount of CSF taken during the tap. The reason why the amount drawn is important is the steep concentration gradient of 5-HIAA in the CSF. A small sample of CSF taken from the first portion of fluid drawn at a spinal puncture thus contains a much lower concentration of 5-HIAA than a larger sample drawn from the same patient. If unequal amounts are drawn from patients and controls, differences can thus very easily be created (Åsberg, 1997).
Most important in clinical studies, the CSF monoamine metabolite concentrations are altered by treatment with psychotropic drugs such as classical tricyclic antidepressants. Serotonin uptake inhibitors also reduce CSF concentrations of 5-HIAA and the interference with serotonin turnover appears to affect homovanillic acid (HVA) concentrations as well. Lithium treatment increases CSF 5-HIAA. CSF 5-HIAA is lower in abstinent alcoholics (Borg et al., 1985) suggesting subnormal serotonergic activity in alcoholics during abstinence from alcohol.

1.6.2.1. CSF 5-HIAA as a biomarker of suicidal behaviour

Over 30 years ago, Åsberg (1976a) observed a bimodal distribution of CSF (5-HIAA) in depressed individuals. The low CSF 5-HIAA group had a higher proportion of individuals who either eventually committed suicide or had previously attempted suicide by violent methods. Since this initial observation more than 20, mostly retrospective, studies have examined the relationship between CSF 5-HIAA and suicidal behavior in mood disorders (Asberg, 1997). Fewer prospective studies have examined the relation between serotonergic function and future suicidal behavior in mood disorders. Prospective studies of suicide completion and the serotonergic system uniformly report that low CSF 5-HIAA levels and a history of attempting suicide predict suicide (Mann and Currier, 2007). The first prospective study of CSF 5-HIAA and suicidal behavior reported that, in a sample of 68 depressed subjects, the two who committed suicide shortly after discharge had lower levels of CSF-5HIAA and had made previous suicide attempts (Åsberg et al., 1976b). Subsequent studies also observed a higher incidence of suicide during the follow-up period amongst those with low CSF 5-HIAA at index admission, compared with the above median CSF 5-HIAA group (Träskman et al., 1981; Nordström et al., 1994). The risk of suicide was highest in the first year following discharge from index hospitalization, reporting suicide rates of 17%–21% in the low CSF 5-HIAA group compared with 2–7% in the high CSF 5-HIAA group in the first year of follow-up. Other studies also report that lower baseline CSF 5-HIAA in mood disorder subjects with a history of attempting suicide predict those who later commit suicide (Roy et al., 1989; Träskman-Bendz et al., 1992).
The association between CSF 5-HIAA and suicide attempts is less clear (Mann et al. 1996). Roy et al. (1986a) despite finding no difference in baseline CSF 5-HIAA between depressed patients who had previously attempted suicide, depressed patients without previous attempt, and controls, found lower CSF 5-HIAA in subjects who reattempted suicide over the four year follow-up period (Roy et al., 1989).
Two studies (Åsberg et al., 1976b; Träskman et al., 1981), reported that baseline CSF 5-HIAA of suicide completers was in the same range as those who had previously attempted suicide with violent methods, or went on to make violent attempts in the follow-up period. They suggest that the association between the serotonergic system and suicidal behavior might be related to the association of low CSF 5-HIAA and poor impulse control and hence to self-directed aggression (Åsberg and Träskman, 1981). This is consistent with reports that low CSF 5-HIAA predicts recidivism in arsonists and murderers (Virkkunen et al., 1996).

However, a more recent prospective study (Engstrom et al., 1999) could not replicate these results, finding no differences in CSF 5-HIAA levels between violent or non-violent suicide attempters, or between suicide attempters and completers (Engstrom et al., 1999) In this sample only 26 of 120 suicide attempters used violent methods, which might not have provided enough statistical power.

Greater planning and lethality of the suicide attempt correlate with lower CSF 5-HIAA (Mann and Malone, 1997; Oquendo et al., 2003; Placidi et al., 2001). Suicidal acts are associated with aggressive and impulsive traits that are also associated with serotonergic dysfunction (Oquendo and Mann, 2000; Oquendo et al., 2003; Placidi et al., 2001). Lower levels of CSF 5-HIAA predict future aggression against property or homicide in prospective studies of alcoholic fire setters and violent offenders (Virkkunen et al., 1996).

A 14- year follow-up study found that impulsivity, aggression, or anxiety did not distinguish future suicide attempters or suicides and did not correlate with CSF 5-HIAA. In the short term (3 years following index evaluation), survival time correlated negatively with anxiety and impulsivity and positively with socialization and CSF 5-HIAA, suggesting that greater anxiety and impulsivity, and low socialization and low CSF 5-HIAA, predict higher short-term suicide risk (Nordström et al., 1996).

The relationships between impulsivity, aggression, the serotonergic system and suicidal behavior are complex. In non-prospective studies lower CSF 5-HIAA and more highly lethal suicidal behavior associated with greater planning and less impulsivity (Placidi et al., 2001), while low lethality attempts involve less planning and CSF 5-HIAA levels comparable to nonattempters with major depression (Mann et al., 1996). Lower CSF 5-HIAA was also associated with severity of lifetime aggressivity and a history of higher lethality suicide attempt (Mann et al., 1996; Placidi et al., 2001; Träskman- Benz et al., 1992). CSF 5-HIAA levels were lower in high-lethality attempters compared to low-lethality attempters in depressed patients with alcohol dependence (Sher et al., 2007). The causal relationship between lower serotonin function and aggressive or impulsive behaviors is demonstrated by the increase in aggressiveness and impulsiveness following lowering serotonin function transiently by acute tryptophan depletion in healthy male volunteers (Cleare and Bond, 1995).

It is important to note that the abnormalities in serotonergic function associated with suicidal behavior can be distinguished from those of a major depressive disorder (Mann et al., 2000), mood disorder patients having a widespread abnormality in serotonin function affecting most of the prefrontal cortex and many other cortical and subcortical areas (Milak et al., 2005). Fewer serotonin transporters and more 5-HT1A binding to the ventral prefrontal cortex characterizes suicide and indicates less serotonin input, which might underlie reduced behavioural inhibition and a greater probability of acting on suicidal feelings (Arango et al., 1995). In contrast, the abnormality in post-mortem brain tissue from suicides, or in depressed suicide attempters identified by PET, is localized to ventrolateral parts of the prefrontal cortex, perhaps reflecting those brain regions involved in suicide intent, decision-making, and impulse regulation (Oquendo et al., 2003).

Low CSF 5-HIAA has also been reported in suicide attempters with schizophrenia or personality disorders compared to psychiatric controls (Cooper et al., 1992; Gardner et al., 1990).

1.6.2.2. Genes of the Serotonin system and suicidal behaviour

The enzyme tryptophan hydroxylase (TPH) is involved in the biosynthesis of serotonin, converting L-tryptophan in a rate-limiting step into 5-hydroxytryptophan. The latest meta-analysis included nine studies and confirmed the association between the A218C polymorphism and suicidal behavior using both the fixed effect method and the random effect method (Bellivier et al., 2004; Courtet et al., 2005).
The identification of the brain-specific, second isoform TPH2 gene, promised to be a step forward in investigating the genetic contribution to suicidality, as this isoform apparently plays a more important role in the brain. The first investigation of 10 SNPs in the TPH2 gene in a sample of 263 suicide victims and 266 ethnically matched healthy controls showed an association of one SNP with completed suicide (Zill et al., 2004). Subsequent haplotype analysis provided evidence for the association of several haplotypes with completed suicide. A comprehensive investigation performed a linkage analysis in 1798 subjects from four different populations and detected significant haplotype linkage of TPH2 to suicide attempt and major depression (Zhou et al., 2005). Another single marker and haplotype analysis detected significant association of a haplotype with both suicide attempts and bipolar affective disorder (Lopez et al., 2007). The TPH2 gene and its 5' upstream region variants may be involved in the predisposition to suicide in MDD (Lopez de Lara et al., 2007). Negative findings have also been published (De Luca et al., 2005; Mann et al., 2007) In summary, the results on TPH2 are promising but somewhat inconsistent and further studies are needed to clarify the role of this gene in several facets of suicidal behaviour.

The gene coding for the monoamine oxidase A (MAOA), contains a variable number of tandem repeats (VNTR) polymorphism in the promoter region. The 30bp repeated sequence is present in 3, 3.5, 4 or 5 copies, and alleles with 3.5 or 4 copies are transcribed 2-10 times more efficiently than those with 3 or 5 copies (Sabol et al., 1998). MAOA is one of the key enzymes in the metabolism of serotonin as well as noradrenalin. Studies investigating the possible association of this MAOA-VNTR polymorphism and suicidal behavior have yielded inconsistent results (Rujescu et al., 2007).

The serotonin transporter (5-HTT) is located on the presynaptic membrane of serotonergic neurons and is responsible for the re-uptake of released serotonin from the synaptic cleft. The 5'-promotor region of the 5-HTT gene contains a functional insertion/deletion variant (5-HTTLPR) with two common alleles that were designated as 'short' (s) and 'long' (l).
A meta-analysis conducted by Anguelova et al. (2003) found a significant association of the s-allele with suicidal behavior. A second meta-analysis, including 18 studies with 1521 suicide attempters or completers and 2429 controls, found no overall association of 5-HTTLPR alleles with suicidal behavior (Lin and Tsai, 2004). The same authors furthermore observed a significant association of the s-allele with violent suicidal behavior, but not with nonviolent suicide. Lin and Tsai (2004) concluded that violent suicidal subjects might be a relatively homogenous group and that patients carrying the s-allele are likely to act more impulsive and aggressive. S-allele may predispose for suicidal behavior characterized by high determination and medical damage (Wasserman et al., 2007d). In conclusion, there is evidence that the 5-HTTLPR polymorphism is involved in the predisposition to suicidal behavior. In a famous longitudinal genetic study, Caspi et al. (2003) found a functional polymorphism in the promoter region of the serotonin transporter (5-HTT) was associated with the likelihood of developing major depression and suicidality in relation to stressful life events. Individuals with one or two copies of the short allele of the 5-HTT promoter polymorphism had a higher incidence of depression and suicidality in relation to recent stressful life events than

individuals homozygous for the long allele. Moreover, childhood maltreatment predicted adult depression only among those carrying the short allele. Thus, the role of the serotonergic system in suicidal behavior as well as mood disorders may be mediated by genetic and environmental factors.

1.6.3. The Dopamine System

1.6.3.1. CSF HVA and suicidal behaviour

Deregulation of the dopaminergic system has been documented in major depression (Dailly et al., 2004), however post-mortem and retrospective studies of dopaminergic function and suicidal behavior are few and inconclusive (Mann, 2003). Prospective studies of dopaminergic system function and the prediction of suicidal behavior have reported divergent findings. Roy and colleagues, in two studies, found that those with a history of suicide attempt had lower CSF homovanillic acid (HVA), a dopamine metabolite, (Roy al., 1986a) compared with non-attempters and controls. On follow-up 5 years later, those with prior attempts who reattempted or committed suicide, had lower baseline CSF HVA (Roy et al., 1989) compared to those who did not reattempt, had never attempted, and controls. Other prospective studies did not find CSF HVA predicts suicide or correlates with clinical factors related to suicide such as depression, aggression or impulsivity (Engstrom et al., 1999; Nordström et al., 1994). Low HVA was associated with past potential lethality of suicidal acts (Ågren, 1983). Recently CSF 5-HIAA and HVA were reported to predict lethality of future suicide attempts in patients with bipolar disorder (Sher et al., 2006a) and depressed suicide attempters had lower CSF HVA levels compared to depressed non-attempters (Sher et al., 2006b). Repeating lumbar punctures to record monoamine levels across the course of illness, Traskman-Bendz et al. (1984) found no significant difference in CSF HVA within subjects between depressed and recovered states suggesting that CSF HVA is a mood state independent biochemical trait.

1.6.3.2. CSF HVA/5-HIAA ratio

Monoamine studies have found, in some cases, that the ratio between metabolites shows a stronger association with suicidal behavior, although the predictive value of these ratios is unclear. Such a ratio factors out common variance due to characteristics such as the shared CSF transport system and effects on monoamine metabolites levels related to CSF gradient due to variation in length of the spinal canal.
Engstrom et al. (1999) reported lower HVA/5-HIAA ratios in suicide attempters compared with surgical controls at baseline, although there was no differences between attempters and those who completed suicide during the follow-up. Roy et al. (1986a) also found lower baseline CSF HVA/5-HIAA ratio in depressed subjects compared to controls.
In this study, among depressed patients with prior suicide attempts, DST non-suppressors had a significantly lower mean CSF HVA/5-HIAA ratio than suppressors. Roy and his coauthors suggest that depressed patients who attempt suicide may have a more marked imbalance between the turnover of dopamine and serotonin in terms of relatively lower dopaminergic activity and turnover. Non-prospective studies of violent offenders have reported significant correlations between CSF HVA/5-HIAA ratio and psychopathic traits of aggression and violence suggesting dysfunction in the relative activity of the two systems (Söderström et al., 2003).

1.6.4. Interrelationships of HPA axis and serotonin system

Dysregulation of the HPA axis and 5-HT system has been implicated in the pathophysiology of disease states such as affective disorders, anxiety disorders, and obesity (Porter et al., 2004). Enhanced 5-HT neurotransmission increases plasma concentrations of cortisol, whereas depletion of the 5-HT precursor or 5-HT transporter reduces cortisol (Fuller and Snoddy, 1980 and 1990; Vielhaber et al., 2005).
A reciprocal functional interaction between the central 5-HT system and HPA axis has been shown to exist under normal physiological conditions and has been postulated to be of particular relevance in pathological states. During stress, activity of the brain 5-HT system and HPA axis rises. Given that high cortisol levels initially cause higher CNS 5-HT turnover, hypothetically, during continuous or frequent exposure to stress, availability of brain serotonin may diminish and vulnerability to pathology may increase. Stress-induced depletion of brain serotonin has been shown in animal research (Jans et al., 2007).

1.7. Clinical Prediction of Suicide Risk

Clinical prediction of suicidal behavior in mood disorders is difficult because individual risk factors account for a small proportion of the variance in risk and lack sufficient specificity. Efforts to identify clinical risk factors for suicide through the follow-up of depressed patients have yielded relatively few robust predictors. Those identified by at least three studies are (in order of decreasing frequency) suicide attempts, male sex, being single or living alone, inpatient status, and hopelessness (Oquendo et al., 2006).
A history of attempted suicide has consistently been documented to increase risk of eventual suicide. A 1967 meta-analysis of 15 prospective studies documented a 10–20% increase in the risk of suicide among previous attempters (Dorpat and Ripley, 1967) and subsequent prospective studies have reported that a history of attempting suicide significantly increases the risk of suicide in depressed individuals (Angst et al., 2002). Of interest, one 10-year follow-up study reported that subjects who committed suicide had significantly fewer past attempts than surviving attempters (Gladstone et al., 2001). This is consistent with the understanding that suicide completers and attempters are distinct, although overlapping, cohorts, and that high-lethality suicide attempts are less common. Thus, a history of prior attempts may have a different predictive capacity for low-lethality suicide attempts vs. high-lethality attempts or suicides.
Epidemiologic studies report that 15% of individuals in the community with a lifetime diagnosis of MDD and 29% of those with bipolar disorder acknowledge a suicide attempt at some point in their lifetime (Chen and Dilsaver, 1996). In a reccent cross-sectional study, it was shown that 51% of cohort of patients with bipolar disorder had attempted suicide either before or during the index episode (Valtonen et al., 2005). Risks of suicide is also elevated in these disorders and between 2% and 12% of those with Major Depressive Episode (MDE) end their lives by committing suicide (Bostwick and Pankratz, 2000).
Prospective studies suggest that the period immediately following discharge from hospital is the time of highest risk. Follow-up studies have reported that the 6- to 12-month period immediately following hospitalization carries the highest risk of suicide for mood disorder inpatients (Gladstone et al., 2001). However, risk of suicidal behavior remains elevated. Angst et al. 2002 documents a persistent risk of suicide over 25 years of follow-up. A history of past suicidal behavior, recurrent or refractory depression, alcoholism comorbid with major mood disorders all appear to increase risk. Development and refinement of predictive models that

use multiple domains, and which may assist clinicians in identifying individuals at high risk of suicidal behavior is a major challenge.

1.8. Meta-analyses of biomarkers in the prediction of suicide in mood disorders

Predicting suicide is difficult due to its low base-rate and the limited specificity of clinical predictors. Prospective biological studies suggest that dysfunctions in the serotonergic system and HPA axis have some predictive power for suicide in mood disorders. A prediction model that incorporates biological testing to increase specificity and sensitivity of prediction of suicide is of potential clinical value. Meta-analyses of prospective biological studies of suicide and cerebrospinal fluid 5-hydroxyindoleacetic acid (CSF 5-HIAA) and suicide and the dexamethasone suppression test (DST) in mood disorders using the penalized quasi-likelihood (PQL) and bootstrap method yield odds ratios for prediction of suicide of 4.48 and 4.65 respectively (Mann et al., 2006). Two combinatory prediction models, the first requiring positive results on more than one test, and the second requiring a positive result on either one of two tests, were tested to assess their sensitivity, specificity, and predictive power using biological data from published and unpublished studies. The prediction model that requires both DST and CSF 5-HIAA tests to be positive results in 37.5% sensitivity, 88% specificity, and has a positive predictive value of 23%. The prediction model that requires either DST or CSF 5-HIAA tests to be positive results in 87.5% sensitivity, 28% specificity, and has a positive predictive value of 10%. Thus, models attempting to predict a lethal outcome that is uncommon perform very differently making model choice of major importance (Mann et al., 2006).

2 AIMS

The aim of these studies was to investigate the predictive potential of two biological markers: CSF 5-HIAA and the DST nonsuppression in suicide prediction in clinical high-risk groups and to investigate relationships of neurobiologic correlates and psychological components of the vulnerability to suicide. To achieve this objective more specific aims were formulated.

The aim of studies I and III was to assess the predictive value of CSF 5-HIAA, the Suicide Intent Scale (SIS) and the Beck Hopelessness Scale (BHS) in a group of patients with a high suicide risk, *i.e* men admitted to a psychiatric clinic after a suicide attempt and to investigate relationships between the CSF 5-HIAA, HVA and the HVA/5-HIAA ratio and SIS, the Montgomery Asberg Depression Rating Scale (MADRS) and the Chapman Anhedonia Scale.
The aim of study II was to assess the predictive value of non-suppressor status in the dexamethasone suppression test for suicide in a historical cohort of depressed inpatients with and without history of a suicide attempt using the suicide mortality as the outcome criterion.
The aim of study V was to assess the optimal predictive threshold level of post DST plasma cortisol at 4:00 p.m. for suicide with ROC analysis in depressed inpatients with an index suicide attempt.
The aim of studies IV and VI was to investigate the interrelationship of two biomarkers: CSF 5-HIAA and the cortisol response in the DST in mood disorder inpatients with and without an index suicide attempt and in those who committed suicide during the follow up.

3 MATERIAL AND METHODS

3.1 Ethical aspects

The ethics committee of the Karolinska University Hospital approved the study in Papers I and III on the first of April 1985, Dnr 85:62. The Regional Ethical Review Board in Stockholm approved the study protocol for Papers II, IV, V and VI on the 2 of November 2005, Dnr 2005/1152-31/1.

3.2. Patients

Patients in studies I and III
Fifteen male patients (age range: 20-49; mean 33 years) not receiving any antidepressant treatment admitted to the department of Psychiatry at the Karolinska Hospital after a suicide attempt were included after informed consent was obtained. Patients in this high suicide risk sample were diagnosed according to DSM III; 13 fulfilled criteria for mood disorders and two for psychotic disorder. DSM III diagnoses were converted to DSM IV system in paper III. Seven patients had comorbidity for alcohol abuse and eight patients fulfilled even criteria for a personality disorder. Seven patients had a family history for suicide. Index episode suicide attempt were five cases of self-poisoning by drug overdose, five cases of asphyxiation (car exhaust), two cases of cuts and stabs, one case of incineration, one hanging and one attempt with electricity in the bath.
Five early suicides (within 2 years), one later suicide and one fatal accident were identified. The suicide method was intoxication in three cases, one patient jumped and one died with electrocution. Two of completers had made a previous suicide attempt before the index attempt.

Patients in study II
This is a cohort study involving 382 psychiatric inpatients (126 men and 256 women, mean age 52 years, S.D.=16,4) admitted to the psychiatric clinic at the Karolinska University Hospital between 1980–2000 with a DSM diagnosis of mood disorder: unipolar, major depressive disorder, single episode or recurrent, bipolar disorder, depressed or dysthymic disorder. Patients with substance abuse or psychotic disorder (schizophrenia spectrum) were excluded. Information about the index suicide attempt was registered. One hundred fourteen patients had attempted suicide just before admission. Patients with a medical condition (or taking medication) known to interfere with the results at the time of the DST were excluded. Of the 382 patients with DST results, 167 (44%) had an 8:00 a.m., 4:00 p.m., and/or 11 p.m. postdexamethasone cortisol level greater then 5 μg/dl and were considered non-suppressors, 215 (56%) patients were suppressors. The DST non-suppressor and DST suppressor subjects did not differ in gender distribution (68% respective 66% females). The DST non-suppressor (n=167) group was followed for a mean of 18.7 years (S.D.=6) with a median of 20.1 years, range=5–25. The DST suppressor (n=215) group was followed for a mean of 17.2 years (S.D.=6) with a median of 18.4 years and a range of 5–25. Two groups differed in mean age. The DST nonsuppressors had a mean age of 55 years (S.D. 15.5); the DST suppressor group had a mean age of 50 years (S.D. 16.8), p=0.0014. 114 patients (30%) had made a suicide attempt preceding admission, they had a mean age of 47 years (S.D.=17) compared with patients without index suicide attempt, mean age 54 (S.D.=16), p=0.0005.

Patients in study V

Of the 114 suicide attempters in the study II, those who had complete information of postDST s-cortisol at 4 p.m. were included in the cohort of 106 psychiatric inpatients (35 men and 71 women, mean age for men 41.5 years, S.D. = 14.1, mean age for women 50.6, S.D. = 18). 28 patients had used a violent suicide attempt method (definition according to Träskman 1981). All patients were followed up for the cause of death. The patients who died within the follow-up period were identified and the causes of death were obtained from Statistics Sweden which keeps the National Swedish Cause of death register for a National Board of Health and Welfare. During a mean follow-up time of 17 years, 50 patients had died, 25 suicides were identified from the death certificates. The subsequent analysis concerns the patients who committed suicide.

Of the 106 patients with DST results, 44 (41.5 %) had an 8:00 a.m., 4:00 p.m. and/or 11 p.m. postdexamethasone cortisol level greater then 5 μg/dl and were initially considered non-suppressors, 62 (58.5 %) patients were suppressors.

The DST non-suppressor and DST suppressor subjects did not differ in gender distribution. There was no significant difference in follow-up time between men or women or between the non-suppressors and the suppressors. The DST non-suppressor (N=44) group was followed for a mean of 17,7 years (S.D.=6.4) with a median of 19 years, range=7–25. The DST suppressor (N=62) group was followed for a mean of 16.4 years (S.D.=6) with a median of 15,7 years and a range of 6–25.

The DST non-suppressors had a mean age of 46.5 years (S.D. =17.6); the DST suppressor group had a mean age of 49 years (S.D.=17) (NS). Men were younger with mean age 41.5 (S.D.=14) than women, mean age 50.6, (S.D.=18), $p < 0.05$, (student's t-test).

Patients in studies IV and VI

58 psychiatric inpatients (23 men and 35 women, mean age 47 years, S.D. = 14) Twenty-five patients had attempted suicide just before admission. Patients with a medical condition (or taking medication) known to interfere with the results at the time of the DST and lumbar punctures were not included.

A cohort of 58 psychiatric inpatients (23 men and 35 women, mean age 47 years, S.D. = 14) from the cohort of 382 (study II) who had also CSF monoamine metabolite data was formed. Patients admitted to the psychiatric clinic at the Karolinska University Hospital fullfilled a diagnosis of mood disorder: unipolar, major depressive disorder, single episode or recurrent, bipolar disorder, depressed or dysthymic disorder. Patients with substance abuse or psychotic disorder (schizophrenia spectrum) were excluded. Information about the index suicide attempt was registered. Twenty-five patients had attempted suicide just before admission.

In study VI all patients were followed up for cause of death. The patients who died within the follow-up period were identified and the causes of deaths were obtained from Statistics Sweden, which keeps the National Swedish Cause of death register for the National Board of Health and Welfare. During a mean follow-up time of 21 years, 30 patients had died, 11 suicides (6 men and 5 women) were identified from the death certificates. 19 patients (6 men and 13 women) had died of natural causes or in accidents.

3.3 Lumbar puncture and CSF monoamine metabolites

Lumbar punctures were performed in a standardized manner between 8 and 9 am after fasting in bed since midnight. 12 ml of CSF was drawn with the patient in a sitting position, the needle being inserted between lumbar vertebrae IV and V. The CSF was immediately centrifuged and stored at –80 C. The CSF monoamine metabolites HVA and 5-HIAA were analysed by using mass fragmentography (GC-MS) according to methods developed by Bertilsson (1981). All CSF samples were analysed with the same technique and the same laboratory at the Department of Clinical Pharmacology, Huddinge University Hospital.The coefficient of variation of the analytical method is less than 5%.

3.4 The Dexamethasone test

One mg of dexamethasone was given orally at 11:00 p.m., and plasma cortisol levels were determined from blood samples drawn the following day at 8:00 a.m., 4:00 p.m. and 11:00 p.m. using a commercial radioimmunoassay. The non-suppressor status (cortisol level 5µg/dl= 138 nmol/l or above in any sample following day) was analysed.

3.5 Clinical Assessments

The Suicide Intent Scale is a 15-item instrument designed to measure the factual aspects of a suicide attempt: the circumstances surrounding it and the patient's thoughts and feelings at the time of the attempt. Individual responses are coded on a 0 to 2 scale and the total SIS can range between 0 and 30. (Beck et al., 1974a)
The Beck Hopelessness Scale is a 20-item true/false instrument with statements of pessimistic beliefs about oneself and the future. One half of the items are reverse-scored. Total score can range from 0 to 20 (Beck et al., 1974b).
The Montgomery Asberg Depression Rating Scale
Depression was rated using the Montgomery Asberg Depression Rating Scale (Montgomery and Asberg, 1979), which has been widely used to measure depression severity. The MADRS consists of 10 items and each item is rated on a scale of 0 (no abnormality) to 6 (severe).
The Chapman Scale of Anhedonia
The anhedonia scale supposedly measures a life-long characterological defect in the ability to experience pleasure (Chapman et al., 1976).

3.6 Follow-up procedure regarding suicidal behaviour

All patients were followed up for cause of death. The patients who died within the follow-up period were identified and the causes of deaths were obtained from Statistics Sweden which keeps the National Swedish Cause of death register for the National Board of Health and Welfare. Suicide was defined as judged and reported by the forensic medical pathologist to the Statistics Sweden in all cases.

3.7 Statistical analyses

The chi-square test and Fisher's exact test were used to evaluate categorical and non-parametric data and the two-sample t-test and One-Way Analysis of Variance (ANOVA) for continuous variables normally distributed. Logistic regression models were used to adjust for confounding factors.

In study II DST result and two other potential predictors of suicide: the presence of a suicide attempt during the index illness episode before admission and male sex were selected on the basis of the previously described literature review. Each potential predictor was dichotomized, and the two resulting groups were compared by using survival analysis.
In studies IV and VI post dexamethasone plasma cortisol levels at 8:00 a.m., 4:00 p.m. and 11:00 p.m. were analysed in relation to the CSF 5-HIAA levels separately. Median split subgrouping for further analysis of CSF 5-HIAA levels in suicide victims and survivors was applied. Statistical analysis (with JMP V software, SAS Institute inc., Cary, NC, USA) was conducted with regression analysis (Pearson product moment) and Pearson Chi-square and Fisher's exact test were used for crosstabulations. The p value was set at <.05.
In study V the receiver operating characteristics (ROC) analysis was performed.
The ROC analysis provides a comprehensive picture of the ability of a test to make the distinction being examined over all decision thresholds. ROC curve is a plot of specificity versus sensitivity for all confidence thresholds. The area under a ROC curve is the most widely used index for summarizing information contained in a ROC curve. The area under a ROC curve has been shown to be equal to the probability of correctly ranking a (diseased, nondiseased) pair, and can be estimated either by the Wilcoxon statistics or by the parametric binormal model method.

4 RESULTS

Predictive values of CSF 5-HIAA, Suicide Intent and Hopelessness (Study I)
The major finding was that mean CSF 5-HIAA distinguished between suicides (Mean + SD) (60 ± 19 nm) and survivors (106 ± 38 nm) (unpaired two-tailed t-test, p value <0.05). Low CSF 5-HIAA identified all the men who committed early suicide. The predictive value of CSF 5-HIAA for early suicide was 5/8 = 62.5%. The suicide intent score for the total sample ranged from 10 to 27 and did not distinguish the suicides (18 ± 7) from the survivors (18 ± 5). The hopelessness scores (range 3–20) were similar between the groups (14 ± 5 vs. 13 ± 6, ns). Low CSF 5-HIAA concentration predicted suicide with a sensitivity of 100% and a specificity of 70%.

Suicide risk and the dexamethasone suppression test (Study II)
Thirty-six suicides (9.4%) occurred during the follow-up time: 22 women (8.6%) and 14 men (11 %). Twenty of the suicide victims were nonsuppressors (12 women and 8 men) and 16 suppressors (10 women and 6 men) in DST status. The overall suicide risk among depressed inpatients with non-suppression was 12% and for suppressor groups 7.4% (N.S). One major finding was that DST non-suppression did not distinguish between suicides and survivors in the group of depressed inpatients (N.S). The result remained non-significant even when adjusted for gender, for age and for violent versus non-violent method for completed suicide. When survival time and cumulative suicide risk after DST are taken into account, the result still remains nonsignificant concerning the potential of DST to predict short- or long-term suicide risk in a group of psychiatric inpatients with mood disorder. In this cohort 114 patients had made a recent suicide attempt. The index episode suicide attempt before admission to the wards predicted suicide in this population ($p<0.0001$). This variable was entered together with DST suppressor status as an independent variable in a regression analysis of the likelihood of suicide. In this model, non-suppression did not increase the likelihood of future suicide (risk ratio=1.6, p=0.64). A suicide attempt in the index episode generated a risk ratio of 5.3 ($p< 0.0001$).

Suicide risk and the dexamethasone suppression test in suicide attempters (Study II)
When we only included suicide attempters with mood disorder, the DST non-suppressor status predicted suicide (Fisher's exact test, one-tail p=0.0261) in this group. The five, ten and 20-year survival (Fisher's exact test, one-tail p=0.0773, p=0.0457, p=0.0175, respectively). Gender specific analysis showed that the finding is significant for men (Fisher's exact test, one-tail p=0.0168) but not for women (Fisher's exact test, one-tail p=0.1973).

Overall mortality and dexamethasone suppression test (Study II)
In this material non-suppressors had a higher total mortality compared with suppressors. Nonsuppressor status predicted death from causes other than suicide (Fishers Exact Test one-tail p=0.0088) in the group in general. When analyzing further, men and women separately, the finding remained significant only for men (Fishers Exact Test, one-tail p=0.0147) but not for women (Fishers Exact Test, one-tail p=0.1060). Most natural causes were found, including respiratory disease, circulatory, neurological, endocrine, digestive disorders, and symptoms, signs, and ill-defined conditions. There was a predominance of circulatory and respiratory disorders as other causes of death.

Correlations between between monoamine measures and ratings of Suicide Intent, MADRS and Chapman Anhedonia(Study III)
Within the suicide attempter group, the HVA/5-HIAA ratio showed a significant negative correlation to the scores in the Suicide Intent Scale (SIS) ($r=-0.57$, $p<0.05$).
The HVA/5-HIAA ratio did not correlate either to MADRS scores (mean 21, SD 10.6), or to the scores in Chapman Scale of Anhedonia (mean 16.4, SD 8.7). CSF HVA and CSF 5-

HIAA levels did not correlate to SIS or MADRS or Chapman Anhedonia scores in a significant way.
There was an intercorrelation between HVA and 5-HIAA (r= 0.56; p=0.0063). The HVA/5-HIAA ratio did not differ between patients and controls (p=0.61). There was no significant intercorrelation between MADRS scores and the scores in the Chapman Scale of Anhedonia (p=0.72).

Interrelation of CSF 5-HIAA and DST non-suppression(Study IV)

In the sample as a whole, the serum cortisol level at 4:00 p.m showed a positive correlation to CSF 5-HIAA (r = 0.3, p<0.02). In the patients with an index suicide attempt, serum cortisol level at 4:00 p.m correlated positively with CSF 5-HIAA (r=0.65, p<0.0006), but not in the non-attempters (NS). Table 1 summarizes results of biomarker analysis and shows the distribution of patients in relation to median split CSF 5-HIAA and cortisol nonsuppression. Fisher's exact test revealed that the finding is significant only in suicide attempters p=0.011 (two-sided), indicating that these two biomarkers may be seen as independent biomarkers in this group.
For suicide attempters, there was a trend for correlation between CSF 5-HIAA and the serum cortisol level at 8:00 a.m. (r=0.4, p<0.0553) and 11:00 p.m.(r=0.36, p<0.0892). For non-attempters the correlations at 8:00 a.m. and 11:00 p.m. were non-significant.

Suicide risk and the dexamethasone suppression test (Study V)

Twenty-five suicides (23.6 %) occurred during the follow-up time: 16 women (22.5%) and 9 men (25.7%). Sixteen of the suicide victims were non-suppressors and 9 suppressors in the DST status, p< 0.047 (Pearson, chi-square test). DST non-suppression distinguished between suicides and survivors in the group of depressed inpatients with an index suicide attempt (Jokinen et al., 2007). Gender specific analysis showed that the finding is significant for men (Fisher's exact test, 1-tail, p=0.0435), but not for women (NS).

Suicide risk and post DST serum cortisol levels at 4:00 p.m. (Study V)

In the next step we analysed post DST serum cortisol levels at 4:00 p.m., which correlated significantly with suicide (p< 0.0192). Suicide victims had a significantly higher serum cortisol concentrations (mean 7.7 μg/dl, S.D.=6.6, median 7.7 μg/dl) compared with survivors (mean 4.6 μg/dl, S.D.= 5.1 median 1.9 μg/dl), p<0.014 at 4:00 p.m. Gender specific analysis revealed that the finding remained significant for male suicide victims, p<0.0275, but not for female suicide victims, (NS). There was no significant difference in the mean post DST serum cortisol levels between men and women or between male and female suicide victims. Cortisol concentrations did increase significantly with age in suicide victims (r=0.61, p<0.0012) but not in survivors (p< 0.74). When age was entered as a covariate in a Cox regression analysis, the relationship between post DST serum cortisol concentration at 4:00 p.m. and eventual suicide remained significant (Wald X2 =5.4, df=1, p=0.0198).

Optimising the threshold for non-suppressor status in the the DST using the Receiver Operating Characteristic analysis (Study V)

To estimate which threshold level of post DST serum cortisol concentration at 4:00 p.m. optimally predicts suicide we analysed the ROC curves and ROC tables. In the whole group (n=106) the ROC analysis revealed that a lower threshold of 3.3 μg/dl for the nonsuppressor status predicted 17 of 25 suicides in suicide attempters (sensitivity of 0.68 compared with 0.60 i.e. 15 of 25 suicides with a threshold of 5 μg/dl at 4:00 p.m.). The Area Under Curve for all suicide attempters was 0.636.
In male suicide attempters the lower threshold of 3.3 μg/dl changed sensitivity from 67% to 89% and the Odds ratio from 6.7 to 18. The Area Under Curve (AUC) was 0.763 for DST in male suicide attempters. Even though the DST non-suppression or post DST serum cortisol at 4:00 p.m. did not predict suicide for women, we performed ROC analysis. For female suicide attempters with mood disorder the threshold of 7.3 μg/dl maximized the diagnostic

information of DST as a biological test for suicide prediction. The AUC for women was 0.572.

CSF 5-HIAA and DST nonsuppression - Orthogonal Biologic Risk Factors for Suicide in Male Mood Disorder Inpatients (Study VI)

Eleven suicides (19%) occurred during the follow-up: five women (5/35,14 %) and 6 men (6/23, 26 %). The mean follow-up time was 21 years (S.D.= 5 years).

In the sample as a whole, the serum cortisol level at 4:00 p.m. showed a positive correlation with CSF 5-HIAA ($r = 0.3$, $p<0.02$). In the male suicide victims, the serum cortisol level at 4:00 p.m. correlated positively with CSF 5-HIAA ($r=0.84$, $p<0.036$), but not in the male survivors (NS). For male suicide victims, there was a trend for correlation between CSF 5-HIAA and the serum cortisol level at 8:00 a.m. ($p<0.062$).

CSF 5-HIAA below the median predicted all early male suicides (within one year, range= 5-282 days) whereas all male DST nonsuppressors committed suicide after more than one year (range= 520-930 days). In female suicide victims (n=5), the serum cortisol level at 4:00 p.m. did not correlate with CSF 5-HIAA (NS).

Table 1 summarizes the results of the biomarker analysis and shows the distribution of patients in relation to median split CSF 5-HIAA and DST nonsuppression. When broken down by gender, the Fisher's exact test revealed that the finding remains significant only in male suicide victims $p=0.05$, indicating that these two biomarkers may be seen as orthogonal biologic risk factors for suicide in this group.

We also analysed biomarker interrelation in 6 males who had died of natural causes during follow-up. In these patients the post DST serum cortisol level at 4:00 p.m showed a negative correlation with CSF 5-HIAA ($r = -0.89$, $p<0.0164$).

5 DISCUSSION

5.1 Main findings

Mortality in our studies

In study II the suicide mortality rate for hospitalised mood disorder inpatients was 9.4%, which is in line with the literature: in 1970, Guze and Robins reviewed 17 studies of suicide in patients with primary affective disorder and concluded that 15% of depressed patients would die by suicide. In 1990, Goodwin and Jamison reviewed 13 additional studies to replicate the results of Guze and Robins and concluded that 19% of depressed patients would die by suicide. Both reviews considered studies that consisted almost exclusively of hospitalized patients and calculated proportionate mortality (the percentage of the dead who died by suicide) rather than case fatality (the percentage of the original sample who died by suicide). In the latest meta analyses the estimate of the lifetime prevalence of suicide in those ever hospitalised for suicidality was 8.6%, for affective disorder patients hospitalized without specification of suicidality, the lifetime risk of suicide was 4 % (Bostwick and Pankratz, 2000). In study II the suicide mortality rate for hospitalised mood disorder inpatients without index suicide attempt was 4.2%. For patients with suicide attempt the suicide mortality rate was 22 % during the follow-up. In a recent Finnish study, 13% of suicide attempters (26% of men and 8% of women) commited suicide during the follow-up of 37 years (Suominen et al., 2004c), a high long-range suicide risk after a current suicide attempt of 15 % was reported earlier (Nordström et al., 1995). In our cohort of suicide attempters there was no gender difference in long-term follow-up suicide mortality rates. In study I of hospitalised male suicide attempters, five patients of fifteen (33%) committed suicide within 2 years after their index suicide attempt, confirming the suggestion that they constituted a group with a very high suicide risk.

CSF 5-HIAA as a biologic predictor of suicide risk

The major finding in study I was that mean CSF 5-HIAA distinguished between suicides and survivors. Low CSF 5-HIAA identified all the men who committed early suicide. The predictive value of CSF 5-HIAA for early suicide was 62.5%. This is higher than values in the literature (Mann et al., 2006).

Low CSF 5-HIAA predicts future suicide, which is consistent with low post-mortem brain serotonin findings of suicide completers (Arnago et al., 1995; Mann, 2003). This is in line with previous studies of suicide mortality (Mann and Currier 2007) among patients who have attempted suicide and supports the possible association between low CSF 5-HIAA and future suicide.

Suicide Intent and Hopelessness scores did not differ between the suicide completers and the survivors. One possibility is that the scales did not differ because of the high mean i.e. a roof effect. The Suicide Intent scores in this group were almost as high as the break point of 19 or above significantly predicting suicide (Niméus et al., 2002). Even the Hopelessness scores were much higher than those that indicate suicide risk (Beck et al., 1985). These results indicate that scales such as the SIS and the BHS may be of limited value in the prediction of suicide in a high-risk group of hospitalised suicide attempters.

This study had the following limitations: sample size was small and it included only male patients with high level of comorbidity.

DST nonsuppression as a biologic predictor of suicide risk

According to several studies and meta-analyses depressed patients with abnormal DST have a higher risk for suicide (Coryell and Schlesser, 1981; Lester, 1992; Coryell and Schlesser, 2001; Mann et al., 2006). The identification of interactions between DST results and other clinical predictors would enhance risk assessment, but modest sample sizes have limited such analyses in earlier cohorts. In this large historical cohort of 382 mood disorder inpatients non-suppression in the dexamethasone suppression test did not increase suicide risk. In a recent meta-analysis by Mann et al. (2006), the odds ratio of suicide is estimated to be 4.5-fold greater among non-suppressors compared with suppressors. For inclusion in this DST meta-analysis, reports had to include both suppressors and non-suppressors, and to report DST results for both suicides and non-suicides. Seven of the twelve studies met these criteria (Mann et al., 2006). The total number of suicides included in this meta analysis was 28 non suppressors and 12 suppressors (Mann et al., 2006). In our study we analysed 36 suicides. Including our sample in the meta-analysis of Mann, the odds ratio of suicide would be 2.2-fold greater among non-suppressors in unselected mood disorder inpatients.

When we only included suicide attempters with mood disorder, the DST non-suppressor status predicted suicide. 36.4% of DST non-suppressors committed suicide while the rate was 12.9% for suppressor, risk ratio 2.8. The figures broken down by gender showed that 46.2% of men hospitalised for suicide attempt and being DST non-suppressor died by suicide during the follow up compared with 12.5% for suppressors yielding a risk ratio of 3.7.

In this study the depression severity and Axis II diagnoses were not assessed which is a limitation.

In the study V we analysed the optimal cut-off of post-dexamethasone cortisol at 4:00 p.m. for suicide prediction in 106 hospitalised suicide attempters with mood disorder using ROC analysis.

We found that both DST nonsuppression and high post DST serum cortisol at 4:00 p.m. predicted suicide in hospitalised suicide attempters with mood disorder. In this study we analysed the DST in suicide prediction with different thresholds for nonsuppression. The reference threshold of 5 μg/dl for non-suppressor status in the DST has been used to try to differentiate depression from most pertinent comorbid psychiatric conditions and especially a melancholic depression (American Psychiatric Association, 1987). We hypothesized that a different threshold might be optimal to identify the patients who later committed suicide. For any test in which the distributions of results from the two categories of subjects overlap, there are inevitable trade-offs between sensitivity and specificity. As the decision threshold, used to classify the subjects as positive or negative based on test results, is varied over the spectrum of possible results, the sensitivity and specificity will move in opposite directions. For each decision threshold, there is a combination of sensitivity and specificity. Only the entire spectrum of sensitivity/specificity pairs provides a complete picture of test accuracy. Given that the concern is greater over missing a potential suicide, one would seek to maximize sensitivity and thus identify most of the potential suicides. When we chose a lower threshold of 3.3 μg/dl for post DST serum cortisol at 4:00 p.m., we had a better sensitivity in this material predicting two more suicides. In our material DST as test had a sensitivity of 60% with a conservative threshold and 68% with a lower one obtained from the ROC curve. In the meta-analysis of Mann (2006) DST alone had a sensitivity of 50%, specificity of 56% and positive predictive value of 9.5%, which are lower than the values in our material. In our analysis the lower threshold obtained from the ROC curve for males changed the odds ratios from 6.7 to 18 for the test. The optimal threshold levels were different for men and women (3,3 μg/dl for men, 7,3 μg/dl for women). The higher cut-off for women in this material

offered a better specificity but did not change the sensitivity of the test. This is to our knowledge the first study to use ROC to evaluate DST cut-off in suicide prediction. The DST was initially suggested as a diagnostic test to validate major depressive episode diagnoses by an objective laboratory parameter. At least the last ten years the DST is more a tool in preclinical and clinical research. Analysing DST results in suicide prediction in the clinical high-risk group showed higher sensitivity, specificity and positive predictive values in all three subgroups than in earlier studies where the test was performed in unselected mood disorder patients. Still the positive predictive value of the test remains low and in the general clinical praxis it could be regarded as a potential complement to careful clinical evaluation of suicide risk. This data seem to offer some evidence for the reintroduction of the DST as a tool in decision-making in psychiatric suicide prevention in clinical high-risk groups as depressed inpatients with suicide attempt. The pathophysiological mechanisms of the suggested gender specific thresholds of DST in suicide prediction are uncertain and require further investigation.

Sex-related differences in the stress response are well-known from the animal experimental literature, but in humans the findings seemed inconsistent, probably, at least partly, due to the different methods used to stimulate the HPA-axis and to the age of the subjects. Gender-related differences in sex hormone levels are a confounder in HPA-axis responsivity. Although there are exceptions, in general between puberty and menopause, the HPA-axis and autonomic responses tend to be lower in women compared to men of the same age (Kajantie and Phillips, 2006). It has also been found that elderly men activate the HPA-axis to a greater extent than women in response to psychological stress (Uhart et al., 2006). There are gender differences in the number of CRH expressing neurons in the human hypothalamic PVN: a significant age-related increase of CRH neurons in men, but not in women; men have significant more CRH neurons than women from the age of 24 years onward (Bao et al., 2007). Moreover, during aging, cortisol levels in the CSF and in plasma are found to increase progressively between the ages of 20 and 80 years (Laughlin and Barrett-Connor, 2000). Age-related activation of CRH neurons could be due to a series of factors, such as a decreased function of the hippocampus, which suppresses the activity of the HPA-axis and which is more sensitive to the process of aging than the PVN (Bao et al., 2007). In this respect, it is of interest that a sex difference has also been reported in hippocampal aging, e.g., a significant age-related decline of hippocampal volume was found in men but not in women (Bouix et al., 2005). Increasing insensitivity of the HPA-axis to the feedback of cortisol may be another factor involved in the activation of the HPA-axis during aging.

The increase in HPA-axis activity in depressed patients raises the question of the possible underlying pathogenic mechanisms. So far, an imbalance in the ratio between MR and GR has been shown in depressed patients, but it is not clear whether this should be considered cause or effect. Impaired negative feedback control of the HPA-axis and adrenal hypertrophy are commonly found in a subgroup of depressed patients. They coincide with episodes of depression and reverse, at least partially, after recovery (Bao et al., 2007). Some observations suggest that the impaired negative corticosteroid feedback on the HPA-axis in a number of healthy probands at risk for affective disorder is caused by a disturbed corticosteroid receptor function, indicating a genetically transmitted risk factor (Holsboer, 2000). Genetic variations of the GR may explain why only some 50% of the depressed patients show hypercortisolaemia and why considerable variation in their symptoms occurs (Holsboer, 2000).

An alternative possibility involves changes during early development that can induce altered feedback control of the HPA-axis that may persist into adulthood, and could lead to acquired GR resistance in some specific feedback areas and GR hypersensitivity in other brain regions (Bao et al., 2007). Depression and anxiety have been found to be more frequent in children of

mothers who were pregnant during the 'hunger winter' in the Netherlands during the Second World War (Brown et al., 1995).

Interrelationships of CSF 5-HIAA and DST non-suppression as biomarkers in suicide prediction.

In studies IV and VI we wanted to test the hypothesis that HPA-axis hyperactivity and serotonergic deficits are orthogonal risk factors for suicidal behaviour as proposed in the literature (Fawcett et al., 1997, Westrin et al., 2003, Mann et al. 2006, Coryell et al., 2007). The HPA-axis hyperactivity is thought to result in psychic pain and agitation and the serotonergic hypofunction in impulsivity. If these processes are independent of each other, their biological measures should be additive in their implication of suicide risk.

Our finding of different interrelationship between CSF 5-HIAA and serum cortisol after DST in suicide attempters compared to depressed inpatients without suicide attempt indicates that the link between the two systems might be altered in suicide attempters. A significant correlation normally supports a link between two different observations. However in this case most suicide attempters seemed to be either non-suppressors of cortisol in the DST or have CSF 5-HIAA below median, indicating that the two phenomena are independently associated with suicidality. However, this does not mean that DST is not related with serotonergic function in suicide attempters.

In study VI when analysing suicide victims separately the correlation was strong for males and Fisher's exact test revealed that the finding is significant only in this group indicating that these two biomarkers may be seen as orthogonal biologic risk factors for suicide in male mood disorder inpatients. The interrelationship between the serum cortisol and CSF 5-HIAA was significantly different in male suicide victims compared with other groups: male survivors, female suicide victims and survivors. This is in line with the hypothesis on the DST and CSF-5- HIAA in relation to suicidality and depression in suicide attempters. To our knowledge this is the first study to verify the hypothesis of HPA-axis hyperactivity and serotonergic hypofunction as orthogonal risk factors for completed suicide. Interestingly this was true only in male suicide victims indicating a gender difference in biologic vulnerability for suicide. The analysis of contribution of the clinical risk factor suicide attempt would have needed a larger sample size.

CSF 5-HIAA is relatively stable and under substantial genetic control (Rogers et al., 2004) and is considered to be a trait. The relationships between impulsivity, aggression, suicidal behavior and the serotonergic system are complex as reviewed by Mann and Currier, 2007. Since the original finding of low CSF 5-HIAA and suicidality (Åsberg et al., 1976), several prospective studies of the serotonergic system, using measures of CSF 5- HIAA, report that the majority of subjects who reattempt or commit suicide during the follow-up period have CSF 5-HIAA levels below the median (Roy et al., 1989; Träskman-Bendz et al., 1992; Nordström et al., 1994; Nordström et al., 1995).

Interestingly CSF 5-HIAA below the median predicted early suicide (within 1 year) in males in this study indicating that it may be a short-term predictor.

In male suicide victims the DST nonsuppression was independent of CSF 5-HIAA as a biologic risk factor. All males who committed suicide after one year were nonsuppressors indicating that the DST nonsuppression may be seen rather as a long-term biologic predictor of suicide risk. The findings of HPA dysregulation and poor treatment response and higher relapse risk may partly explain why nonsuppression in the DST is associated with suicide. Time spent at highrisk illness phases may be one major determinant of overall risk for suicide. DST nonsuppression may be interpreted as state related risk. However, hypersecretion of cortisol can be detected in asymptomatic individuals at genetic risk of depression and may represent an illness endophenotype (Mannie et al., 2007).

A recent report of alterations of the corticotropin-releasing hormone receptor 1 (CRHR1) gene as marker for suicidality in depressed males exposed to low levels of stressful life events (Wasserman et al., 2007) highlights the importance of HPA axis dysregulation in suicidal behaviour. Suicidal depressed patients may be genetically predisposed to react with high levels of HPA activity upon a low threshold of stress, through some alterations of the CRHR1 gene expression or functionality (Merali et al. 2004), possibly experiencing chronic state of HPA activation and/or a reduced feedback mechanism.
Given that high cortisol levels initially cause higher CNS 5-HT turnover, hypothetically, during continuous or frequent exposure to stress, availability of brain serotonin may diminish and vulnerability to pathology may increase. Stress-induced depletion of brain serotonin has been shown in animal research (Jans et al., 2007). Serotonergic neurotransmission is impaired and the depression can be seen as a condition of combined hypercortisolism and an apparent hypoactivity of serotonergic transmission, there is, however, no consistent evidence of a simple relationship between HPA axis function and 5-HT function in depression. The putative reduction in central 5-HT function has not been shown to be a direct consequence of hypercortisolaemia indicating rather that the 5-HT system and HPA axis have complex inter-relationships (Porter et al. 2004). Serotonin reuptake-inhibiting antidepressants such as citalopram acutely stimulate cortisol and ACTH secretion in healthy volunteers, whereas mirtazapine acutely inhibits the ACTH and cortisol release, probably due to its antagonism at central 5-HT (2) and/or H (1) receptors. These differential effects of antidepressants on cortisol and ACTH secretion in healthy subjects after single administration are also reflected by their different time course in the down-regulation of hypothalamic-pituitary-adrenocortical (HPA) axis hyperactivity in depressed patients as assessed by serial dexamethasone (DEX)/corticotrophin-releasing hormone (CRH) tests (Schule, 2007). Reuptake-inhibiting antidepressants gradually normalise HPA axis hyperactivity in depressed patients during several weeks of treatment via up-regulation of mineralocorticoid and glucocorticoid receptor function. A reduction in HPA axis activity is not necessarily followed by a favourable clinical response and some depressed patients keep on showing nonsuppression in the DEX/CRH test despite clinical improvement. However, there are convincing data suggesting that persisting nonsuppression in the DEX/CRH test despite clinical remission predicts an enhanced risk for relapse of depressive symptomatology with respect to the long-term outcome.

Our finding of different inter-relationship between CSF 5-HIAA and serum cortisol after DST in male suicide victims compared to depressed male inpatients who did not commit suicide indicates that the link between the two systems is also altered in suicide completers. These findings are consistent with the hypothesis that HPA-axis hyperactivity and serotonergic deficits comprise orthogonal risk factors for suicide.
Given the multi-causal nature of suicidal behaviour no one biological index will be adequate to predict suicide, however multiple biological tests could be included in a prediction model, for example CSF 5-HIAA and the dexamethasone response, in order to assess both trait and state related risks. A combinatory or sequential screening method using largely orthogonal variables that addresses both traits and state-dependent risk is optimal for predicting risk (Mann et al., 2006). These two biological measures seem to be additive in their implication of risk in male mood disorder inpatients. Prospective studies of these associations would be instructive.

6 CONCLUSIONS

6.1 Conclusions and clinical implications

Suicidal behaviour was markedly prevalent among mood disorder inpatients and suicide risk was high, about 10%. When analysing the clinical high-risk group of suicide attempters separately the risk was five times higher and DST nonsuppression is a biologic risk factor for suicide. The optimal threshold of DST non-suppression for suicide prediction may be different for men and women. Low CSF 5-HIAA was a short-term biologic predictor of suicide risk for male suicide attempters, whereas DST non-suppression was a long-term predictor reflecting the importance of time spent at high-risk illness phases and may represent an illness endophenotype. The interrelationship of the two biomarkers was different in suicide attempters compared to mood disorder inpatients without suicide attempt and in male suicide victims compared to survivors indicating that the two biomarkers can be seen as independent biologic risk factors for suicide.
The DST was initially suggested as a diagnostic test to validate major depressive episode diagnoses by an objective laboratory parameter. At least the last ten years the DST is more a tool in preclinical and clinical research. Analysing DST results in suicide prediction in the clinical high-risk group of suicide attempters showed higher sensitivity, specificity and positive predictive values in all three subgroups than in earlier studies where the test was performed in unselected mood disorder patients. Still the positive predictive value of the test remains low and in the clinical praxis it could be regarded as a potential complement to careful clinical evaluation of suicide risk. This thesis offers evidence for the reintroduction of the DST as a tool in decision-making in psychiatric suicide prevention in clinical high-risk groups as depressed inpatients with suicide attempt.

6.2 Implications for future research

Future directions of biologic suicide research include neurochemical and genetic studies in combination with clearly determined phenotypes and intermediate phenotypes in order to determine components of biologic vulnerability of suicidal behaviour. The potentional significance of finding underlying biological differencies in the HPA axis regulation in the molecular level in suicidal patients could represent a valuable step towards developing tools in suicide risk assessments and treatment. Combining imaging and neuropsychological tests of decision-making to measure risk of attempting suicide when depressed are other important research tools. Novel pharmacological agents acting on different targets of the biological vulnerability for suicidal behaviour, like HPA axis dysregulation, are needed.

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