The Loss when Losing a Loved One.

Epidemiological studies of prolonged grief disorder.

Heidi Saavedra Pérez

The work described in this thesis was performed within the framework of the Rotterdam Study at the Department of Epidemiology of the Erasmus Medical Center, Rotterdam, the Netherlands. The contribution of the study participants, the staff from the Rotterdam Study and all general practitioners and pharmacists is gratefully acknowledged.

The Rotterdam Study is funded by Erasmus MC and Erasmus University Rotterdam; the Netherlands Organization for Health Research and Development, the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam Financial support for the publication was kindly provided by the Department of Epidemiology, Erasmus Medical Center and Erasmus University Rotterdam.

Cover image: Eileen Campuzano

Layout: Conejo de Colores

Printing:

ISBN:

The loss when Losing a Loved one.

Epidemiological studies of prolonged grief disorder.

Het verlies bij het verliezen van een geliefde.
Epidemiologische studies van langdurige rouwstoornis.

Proefschrift
ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. R.C.M.E Engels

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
dinsdag 12 februari 2019 om 15.30 uur

Heidi Saavedra Pérez
geboren te Panamá, Panamá.

Erasmus University Rotterdam

PROMOTIECOMMISSIE:

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CHAPTER 1

Introduction

INTRODUCTION

The most common serious adverse life event is the experience of the death of a significant person. Grief is the most usual reaction to such a loss, and is considered a normal and natural, albeit difficult, transition (1).

Grief as a topic of study emerged in 20th century. Freud (1917), in his paper on mourning and melancholia attempted to differentiate the normal process of mourning from melancholia. Despite their similarities, Freud states, there are some fundamental differences: mourning is recognized as a healthy and normal process that is necessary for the recovery of the loss and would not be seen as pathology nor a need for medical intervention. However, melancholia is pathological condition, and a dangerous illness due to its poor prognosis and the common suicidal tendency (2).

Though loss is an event that most people will encounter multiple times in their lives, it also can be a severely stressing experience. Although grief usually resolves within a delimited period, it may still have negative consequences for the health of a person: the exacerbation of preexisting levels of depression (3), increased risk of suicide (4), anxiety disorders (5), and decline in cognitive functioning (6-9), physical health problems, and higher rates of disability, use of medication and hospitalization (10) have all been associated with grief.

Moreover, about 9-20% of the persons cannot deal with the loss and show symptoms of complicated and unresolved grief, termed prolonged grief disorder (PGD) (11). The prevalence varies with age, social, cultural and clinical background (12, 13). We can remember the famous queen of Castile, named Joanna the Mad, who could not cope with the death of her husband Philip the

Handsome, and during 8 months, she remained with her husband's coffin in a funeral procession that awoke awe and fear in the population.

PGD includes a set of symptoms of separation distress, such as yearning for the deceased or intense feelings of loneliness; and traumatic distress, such as feelings of disbelief or that life is empty, being emotionally numb or troubled accepting the death, or bitterness. The symptoms are prominent, remain elevated at 6 months and beyond after the loss to the point of functional impairment, and are often resistant to antidepressant treatment (14, 15). PGD strongly affects the wellbeing of the person and has a great impact on the quality of life (16, 17). PGD has been associated with sleep disturbances (18), depression and a higher risk of suicide (19), and poor health (20). The impact of PGD on the cognitive functioning of adults and elderly persons is less known.

This PhD thesis is divided into 8 chapters: Chapter 2 explores PGD, cognition and brain volumes. Chapter 3 focuses on PGD and cognitive decline. Chapter 4 explores PGD and sleep quality. Chapter 5 focuses on PGD and cortisol levels. Chapter 6 presents the research on the relation of silent brain infarcts with depression in the elderly. Chapter 7 presents markers of cerebral small vessel disease with severity of depression in the general population. Finally, in chapter 8, I discuss the main findings of this thesis.

Cognition and Prolonged grief disorder

From the earliest clinical descriptions of grief, researchers have noted that individuals with PGD have greater neurocognitive deficits compared to persons with normal grief and persons without grief (21). Several studies investigated the relation of (acute) grief with cognitive impairment, showing memory decline (immediate and delayed recall) in participants with grief (22,23); and worse performance in tests of attention (24).

Recently, a descriptive study examined global and domain-specific cognitive functioning in individuals with PGD using the Montreal test. They found that participants had lower total Montreal scores, and visuospatial and attention scores relative to control participants (25). Yet, the relation between PGD remains a gap in the literature.

Despite the absence of longitudinal studies of PGD and cognition, several explanations of the potential impact on cognitive decline have been put forward: Persons, who lose a loved one, show

sensory-perceptual alterations that have been associated with hallucinations and delusions. These deficits are more common in persons with PGD than in those without PGD (25% and 2%, respectively) (26). Equally, the attention of persons with PGD is usually directed toward aspects of the environment associated with the deceased (27). These findings suggest that persons with PGD may exhibit attentional bias or impairment in their interpretation of information of their external environment (28). They also exhibit a grief-related avoidance behavior (29), which is positively associated with overall PGD symptom severity (30).

However, the temporal relation between PGD and cognition has not been determined; also, whether cognitive function in persons with PGD declines over the time has not been studied. We cannot rule out reverse causality; that is that cognitive problems precede the onset of grief and are related to its persistence. The underlying hypothesis for my thesis is that poor cognitive performance in persons with PGD relates with brain structural changes and the cognitive decline could be a symptom of the onset of a mild cognitive impairment.

The aim of this thesis was to examine the relationship of PGD with different domains of cognition, and with brain volumes assessed by MRI in the general population. We also compared the cognitive decline prospectively through 7 years.

Sleep and Prolonged grief disorder

When a loved one dies, the majority of bereaved persons develops sleep problems, and this has been a frequent topic of study (31). Being widowed or without a partner has been associated with lower sleep quality (32). Few studies have study the prospective association between PGD and quality sleep, showing an overall poor sleep quality (33)

The aim of this chapter was to examine the relationship between the sleep qualities and sleep duration in persons with normal grief and PGD, and a non-grieving reference group.

The hypothalamic-pituitary-adrenal axis and Prolonged grief disorder

Under conditions of stress, the hypothalamic-pituitary-adrenocortical (HPA) axis is stimulated and activates the secretion of cortisol into the bloodstream. An acute psychosocial stress like losing a loved one is typically accompanied by increased secretion of cortisol, as an adaptation to the stressor and then a return to normal levels; but it is the chronic dysregulation of cortisol that is

implicated in a host of psychological and physical health conditions (34).

Previous studies of grief and cortisol showed more dysregulation on cortisol patterns and an increased mortality risk of the bereaved person (35,36). These few previous studies showed conflicting and controversial results, in one study a flatter slope across the day was observed in persons with PGD, but in another study the occurrence of a flatter slope in persons with grief was independent of the symptoms of PGD (37,38). In this chapter, we aimed to examine the association of morning cortisol and summary cortisol measures, e.g. the slope and the morning rise, with grief and PGD.

Silent brain infarcts and depression

Alexopoulus introduced the term of vascular depression in 1997, and presenting this hypothesis he postulated that cerebral small vessel diseases can cause or exacerbate depression in elderly people (39). Krishnan proposed the same concept focusing on vascular lesions such as white matter lesions (WMLs) using magneting resonance imaging (MRI) methods (40). In this chapter, we aimed to test the association of non-clinical cerebral small vessel disease with depression longitudinally in general population.

Study Setting

The analyses performed in this thesis were based on data from Rotterdam Study, a large population based prospective cohort among adults aged 55 or over living in the Ommoord district in the city of Rotterdam in The Netherlands (78 % of 10,215 invitees) (21). In the Rotterdam Study, the measurement of PGD is through the Inventory of Complicated Grief (ICG), which was introduced in the fourth follow-up examination (2002-2004) of the original cohort and in the second follow-up examination of the additional cohort (2004-2005). All data for this study were collected during an interview at the participant's home. (41).

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CHAPTER 2

Cognition, structural brain changes and complicated grief. A population-based study

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Psychol Med. 2015 May;45(7):1389-99. doi: 10.1017/S0033291714002499. Epub 2014 Nov 3.

Abstract

Background. Several psychosocial risk factors for complicated grief have been described. However, the association of complicated grief with cognitive and biological risk factors is unclear. The present study examined whether complicated grief and normal grief are related to cognitive performance or structural brain volumes in a large population-based study.

Method. The present research comprised cross-sectional analyses embedded in the Rotterdam Study. The study included 5501 non-demented persons. Participants were classified as experiencing no grief (n = 4731), normal grief (n = 615) or complicated grief (n = 155) as assessed with the Inventory of Complicated Grief. All persons underwent cognitive testing (Mini-Mental State Examination, Letter–Digit Substitution Test, Stroop Test, Word Fluency Task, word learning test – immediate and delayed recall), and magnetic resonance imaging to measure general brain parameters (white matter, gray matter), and white matter lesions.

Total brain volume was defined as the sum of gray matter plus normal white matter and white matter lesion volume. Persons with depressive disorders were excluded and analyses were adjusted for depressive symptoms.

Results. Compared with no-grief participants, participants with complicated grief had lower scores for the Letter–Digit Substitution Test [Z-score −0.16 v. 0.04, 95% confidence interval (CI) −0.36 to −0.04, p = 0.01] and Word Fluency Task (Z-score −0.15 v. 0.03, 95% CI −0.35 to −0.02, p = 0.02) and smaller total volumes of brain matter (933.53 ml v. 952.42 ml, 95% CI −37.6 to −0.10, p = 0.04).

Conclusions. Participants with complicated grief performed poorly in cognitive tests and had a smaller total brain volume. Although the effect sizes were small, these findings suggest that there may be a neurological correlate of complicated grief, but not of normal grief, in the general population.

Key words: Brain lesions, cognitive performance, cognitive tests, complicated grief, normal grief, structural brain volumes.

Introduction

The human reaction to bereavement is characterized by a variety of feelings, thoughts and behaviors, of which grief is often regarded as the most common reaction (Rozenzweig et al. 1997). One possible consequence of bereavement is an unresolved and prolonged grief, termed complicated grief. Complicated grief includes a set of symptoms such as persistent intense yearning, and longing for and disruptive preoccupation with thoughts of the deceased. These symptoms are prominent, elevated at 6 months and beyond after the loss, and are often resistant to antidepressant treatment (Pasternak et al. 1991; Horowitz et al. 1997).

Complicated grief has been referred to as 'traumatic grief', 'complicated grief disorder' and as 'prolonged grief' (Shear et al. 2011) and is distinctly different from depression 'accounted for by bereavement', as mentioned in the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV). Recently, complicated grief has been included in the DSM-5 under the name 'persistent complex bereavement disorder' (Boelen & Prigerson, 2012).

Of the population experiencing bereavement, complicated grief affects between 9% and 20% (Newson et al. 2011), with variations based upon social, cultural and clinical background as well as age. Complicated grief strongly affects the well-being of the bereaved (Lannen et al. 2008) and it is associated with sleep disturbances (Hardison et al. 2005), depression and a higher risk of suicide (Szanto et al. 1997), abuse of alcohol (Hardison et al. 2005) and poor health (Lannen et al. 2008).

In addition, psychosocial risk factors have been described, such as a loss, unexpected death or suicide (Ginzburg et al. 2002; Mitchell et al. 2004), lack of social support or inability to adapt to the resulting changes (Ott, 2003). However, the etiology of complicated grief is not well established.

Recently, a cross-sectional study of 211 older adults reported that bereavement was associated with poorer memory performance, especially in men (Rosnick et al. 2010).

Another study of 50 elderly people observed that bereaved persons performed worse in tests of attention, information processing speed, and verbal

fluency (Ward et al. 2007) when compared with non-bereaved persons. Furthermore, whether such associations also exist for complicated grief remains to be studied.

In the elderly, accumulating pathology in the brain can lead to structural changes visible on magnetic resonance imaging (MRI). These structural changes include cortical atrophy and white matter lesions, which have been associated with cognitive decline (Vernooij et al. 2009). Such changes could increase the vulnerability to complicated grief. However, studies exploring complicated grief and structural brain changes have not been performed.

The aim of the current study was to examine the relationship of complicated grief with different domains of cognition and with brain volumes assessed by MRI in the general population. We tested two hypotheses. First, we postulated that persons with complicated grief symptoms perform worse in cognitive tests than those without grief or with normal grief. Second, we postulated that persons with symptoms of complicated grief have less brain volume than persons without grief or with normal grief.

Method

Study participants

The study utilizes data from the Rotterdam Study, a large population-based cohort designed to examine the occurrence of chronic diseases. The study has been described in detail elsewhere (Hofman et al. 2011) and was approved by the medical ethical committee of the Erasmus Medical Center. Participants gave written informed consent.

From July 2004 to September 2009, 6321 persons were interviewed at home; this interview included the Inventory of Complicated Grief (ICG). Individuals with Mini-Mental State Examination (MMSE) score <23 (n = 346), major depression (n = 112), and with an ICG score >22 but with less than 6 months since the loss of a loved one (n = 29), missing data on the MMSE (n = 163) or on the question 'Are you currently experiencing grief?' (n = 170) were excluded. Therefore 5501 eligible persons aged over 45 years, with complete data on complicated grief symptoms and cognitive functioning were available.

From August 2005 onwards, participants from the Rotterdam Study were invited for brain MRI. Individuals with dementia, claustrophobia or MRI contraindications were excluded. of the 4566 persons that

were approached for imaging, 3759 participated (84%). After the same exclusion criteria as above were applied, 3607 persons with data on complicated grief for the structural MRI study were available.

Assessment of complicated grief

All participants were asked if they were currently grieving. If the answer was positive we asked formal follow-up questions 'When did this person die?', and 'Who was this person?' Participants who were mourning over someone with severe disease or a pet were not eligible for follow-up questions and classified as controls. The participants who answered the first question affirmatively were assessed for complicated grief with the Dutch version of the ICG (Prigerson et al. 1995). The ICG is considered the 'gold standard' for measurement of complicated grief in older adults because it has high internal consistency, and good convergent and criterion validity. A total of 17 questions were asked and responses were provided on a five-point scale to reflect an increase in severity (never, seldom, some-times, often, always) (Newson et al. 2011). One item from the original English inventory, 'I feel bitter over this person's death', was removed as a pilot study revealed that this sentiment had a very similar meaning within the Dutch language as the included item: 'I feel anger over this person's death'. Two further items (relating to seeing and hearing the deceased) were combined into one due to their similarity.

A summary score for the ICG was calculated by totaling each individual item score (responses from 0 = never to 4 = always) across the 17 items providing a potential score range of 0 to 68. Participants with a score of less than 22 were considered as participants with grief symptoms. Participants with a score of 22 or greater and with symptoms reported for at least 6 months were considered to have complicated grief. This cut-off was based on the cut-off in the original version of the ICG (original cut-off of 25 from 19 items).

We classified participants into three groups: no grief (control group); persons with 'normal' grief (experiencing non-complicated grief as shown by an ICG score < 22); and those with complicated grief (ICG score >=22). The non-grieving control group included persons who had experienced bereavement in the past but were not grieving at the time of interview. Likewise, persons grieving for a pet or a loved one with a severe disease were included in the control group.

We also performed additional analyses, using a cut-off of 30, to define participants with complicated grief (Shear et al. 2005;

Zuckoff et al. 2006). This resulted in 703 persons with 'normal' grief (experiencing non-complicated grief as shown by an ICG score <30) and 67 persons with complicated grief (ICG score >=30). An additional short assessment instrument with 13 items (including two severity questions) has been introduced to establish prolonged grief disorder. Seven of the eleven items correspond to items in the ICG. The mean score on these items for persons with complicated grief in our study was 14.13 v. 5.38 for persons with normal grief (p <=0.001).

Assessment of cognitive functions

All participants underwent the MMSE, the Stroop Test, the Letter–Digit Substitution Test (LDST), the Word Fluency Task, and a 15-word verbal learning test.

The MMSE is a widely used test for screening dementia and provides a reliable measure of global cognitive functions.

The LDST, a modified version of the Symbol–Digit Modalities Test, was used to measure processing speed. Substitution tests are essentially speed dependent tasks that require the subject to match particular signs symbols, digits or letters to other signs within a specified time period. Participants make as many letter digit combinations as possible within 60 seconds, following an example that shows the correct combinations.

The LDST has the advantage of using letters and digits, signs that are well known to those taking the test. Substitution tasks involve visual scanning, mental flexibility, sustained attention, psychomotor speed, and speed of information processing (van Hoof & Lezak, 1995; Natu & Agarwal, 2002; Vander et al. 2006).

The Stroop Test consists of three standard trials. Trials 1 and 2 measure attention and concentration. In trial 1, the cards show color names printed in black and participants are asked to name the printed word. In trial 2, the cards show colored blocks and participants are asked to name the printed color. Trial 3 is an interference trial considered an effective measure of an executive function. The cards show color names printed in a different color from the color name and participants are asked to name the color of the ink. The outcome variable is the time needed to finish trial 3 (Reeve & Schnadler, 2001).

The Word Fluency Task was used to test verbal fluency. Participants are asked to name as many animals as possible within 60 s. The 15-word verbal learning test tests memory functions with immediate recall and delayed recall components. Participants were given a list of 15 unrelated words repeated over five

different trials and were asked to repeat.

Another list of 15 unrelated words was given and the client was asked again to repeat the original list of 15 words and again after 30 min. For each participant, we calculated Z-scores for each test separately except for MMSE.

We constructed a compound score for global cognitive function with the average of all individual tests except the MMSE (Prins et al. 2005).

Assessment of general cerebral parameters and white matter lesions

Brain MRI was performed on a 1.5 Tesla scanner (USA) with an eight-channel head coil and included T1-weighted, proton-density-weighted and fluid-attenuated inversion recovery sequences (Ikram et al. 2011). Post-processing steps have been described elsewhere and include a conventional k-nearest-neighbor brain tissue classifier extended with white matter lesion segmentation (de Boer et al. 2009), obtaining quantitative measures of white matter volume, gray matter volume and white matter lesion volume. Total brain volume was defined as the sum of gray matter plus normal white matter and white matter lesions.

Assessment of covariates

Potential confounders were selected based on previous publications and included determinants of grief or brain atrophy (Ikram et al. 2008; Rosnick et al. 2010). Information was collected in home interviews and physical examination. The following variables were tested as possible confounders: age (continuously per year), sex, level of education (low, medium, high), systolic blood pressure (mmHg), diabetes mellitus, history of stroke, history of depression, history of anxiety, current depressive symptoms, and alcohol consumption. Diabetes mellitus was defined as a fasting serum glucose level of 57.0 mmol/l and/or the use of blood glucose-lowering drugs. Stroke was defined according to World Health Organization criteria as a syndrome of rapidly developing clinical signs of focal or global disturbance of cerebral function, with symptoms lasting 24 h or longer or leading to death. History of depression was defined by self-reported history of depression with treatment by a psychiatrist or psychologist or use of antidepressant medication as measured by pharmacy records as described previously (Luijendijk et al. 2008). History of anxiety was assessed by the Composite International Diagnostic Interview (Hek et al. 2011) according to DSM-IV criteria. Current depressive symptoms were assessed with a validated Dutch version of the Center for Epidemiologic Studies Depression scale (range 0–60) (Beekman

et al. 1997), with a score of 16 or above suggesting clinically relevant depressive symptoms. All participants with clinically relevant depressive symptoms were interviewed by one of two clinicians using the Present State Examination, a semi-structured psychiatric interview (Wing et al. 1990). Participants with diagnosis of major depression, as classified according to the DSM-IV, were excluded.Alcohol consumption was classified as low if the participant drank zero to two glasses per day, moderate if he/she drank three to four glasses per day, and high if he/she drank five or more glasses per day.

Statistical analysis

Information on demographic characteristics was compared among the groups using a $\chi 2$ test for categorical data and an analysis of variance for continuous variables.

First, we investigated the association of complicated grief with measures of cognitive performance. We tested the differences between participants with normal grief and controls. Next we compared participants with complicated grief with controls as well as with those participants with normal grief (the latter two groups each used as reference) with analyses of covariance (ANCOVA).

Second, we explored the association of complicated grief with total brain volume and subsequently with gray matter, and white matter separately. Also, we investigated white matter lesions. Again, we compared the three groups using ANCOVA. We mutually adjusted brain volume parameters and cognitive function when testing the association with complicated grief.

All analyses were adjusted for age and sex, level of education, systolic blood pressure, diabetes, and history of stroke, history of depression and anxiety, current depressive symptoms, and alcohol consumption.

The ICG is designed as a screening measure and not to assess severity of grief. However, we performed continuous analyses in persons grieving using the scores on the ICG to test whether there is a dose response relationship between the grief symptoms score and cognition function or MRI brain measures independently of the predefined cut-off.

Results

Of the 5501 eligible participants, 4731 were classified as experiencing 'no grief', 615 as experiencing 'normal grief', and 155 as experiencing 'complicated grief'.

Table 1 presents the characteristics of the study population. The main causes for grief were death of a partner (complicated grief, 26%; normal grief, 20%) or parent (complicated grief, 23%; normal grief, 29%). When compared with persons without grief or with normal grief, participants with complicated grief were more likely to be female, older, have current depressive symptoms and a lower MMSE, consume less alcohol and have a history of depression, stroke and diabetes.

Table 2 shows the cognitive test scores across the three groups. Participants with complicated grief had lower scores in the LDST [Z-score −0.16 v. 0.04, 95% confidence interval (CI) −0.36, −0.04, p = 0.01] and the Word Fluency Task (Z-score −0.15 v. 0.03, 95% CI −0.35 to −0.02, p = 0.02) compared with no-grief participants. Participants with normal grief had slightly higher MMSE scores (score 28.2 v. 28.0, 95% CI 0.04–0.33, p = 0.01) than no-grief participants. No other differences were found. When participants with complicated grief were compared with those with normal grief, we found that they had significantly lower MMSE scores (score 27.7 v. 28.2, 95% CI −0.7 to −0.13, p = 0.004).

Also, participants with complicated grief had lower scores in the LDST (Z-score −0.16 v. 0.03, 95% CI −0.36 to −0.02, p = 0.02) and the Word Fluency Task (Z-score −0.15 v. 0.03, 95% CI −0.36 to −0.003, p = 0.04) than participants with normal grief. No differences in the Stroop Test or word learning test, immediate and delayed recall, were observed between these two groups.

Next we compared the brain tissue volumes and white matter lesions across the three groups (Table 3). Of the 3607 study participants, 3148 were classified as experiencing 'no grief', 373 as 'normal grief' and 86 as 'complicated grief'. When compared with no-grief participants, complicated-grief participants were more likely to have smaller total brain volume (volume 933.5 ml v. 952.4 ml, 95% CI −37.67 to −0.10, p = 0.04). Indeed, all the brain volume comparisons between non-grieving persons and those with complicated grief were consistently negative, indicating a smaller volume associated with complicated grief. No differences in brain tissue volumes between participants with normal grief

and those without grief were observed. When we entered both cognitive and brain tissue volume parameters in a single model, the association between complicated grief and smaller total brain volume disappeared; however, the association between lower cognitive functioning and complicated grief remained (data not shown). When we performed analyses using a cut-off of 30 to define complicated grief, we found that participants with complicated grief had lower MMSE scores than non-grievers (score 27.4 v. 28, 95% CI −1.00 to −0.16, p = 0.007) and normal grievers (score 27.4 v. 28.2, 95% CI −1.18 to −0.31, p = 0.001). As in the analyses using a less stringent cut-off, consistent differences in cognitive para-meters (LDST, Word Fluency Task) were found, suggesting that persons with complicated grief perform worse in these tasks independent of the case definition.

Using the cut-off of 30 on the ICG to define complicated grief, we found that participants with complicated grief had less gray matter (volume 512.78 ml v. 533.60 ml, 95% CI −37.90 to −3.72, p = 0.017) and less white matter volume (volume 387.14 ml v. 414.74 ml, 95% CI −46.92 to −8.28, p = 0.005) than participants with no grief. The total brain volume of participants with complicated grief was smaller (volume 903.31 ml v. 952.42 ml, 95% CI −80.50 to −17.71, p = 0.002) than that of participants with no grief. The continuous analyses in persons with grief showed that higher scores on the ICG were associated with lower MMSE score (B=−0.02, 95% CI −0.03 to −0.01, p = 0.002) and smaller total brain volumes (B = −1.28, 95% CI −2.16 to −0.40, p = 0.004).

Table 1. Baseline characteristics of the study population (n = 5501)

Characteristics	No grief (n = 4731)	Grief (n = 615)	Complicated grief (n = 155)
Mean age, years (S.D.)	60.7 (8.6)	62.4 (9.3)[a]	61.9 (8.1)
Women, n (%)	2529 (53)	433 (70)[a]	121 (78)[b]
Education, n (%)			
Primary	446 (9)	74 (12)	16 (10)
Intermediate	3147 (66)	405 (66)	103 (66)
High	1062 (22)	123 (20)	36 (23)
Alcohol consumption, n (%)			
Low (0 to 2 glasses per day)	3585 (76)	497 (81)	134 (86)
Moderate (3 to 4 glasses per day)	919 (19)	95 (15)	16 (10)
High (5 or more glasses per day)	227 (4.8)	23 (3.7)	5 (3.2)
Lives alone, n (%)	472 (10)	70 (11)	17 (11)
Who died?, n (%)			
Partner	–	127 (20)	40 (26)
Child	–	35 (5.7)	25 (16)
Parent	–	177 (29)	36 (23)
Brother/sister	–	90 (14)	22 (14)
Others	–	186 (30)	32 (21)
Mean MMSE scorec (S.D.)	28 (1.6)	28.1 (1.6)	27.7 (1.8)[a,d]
Depressive symptoms, CES-D >16, n (%)	285 (6)	64 (10)[a,d]	41 (26)[b,d]
History of depression, n (%)	341 (7.2)	74 (12)[a,d]	29 (19)[b,d]
History of anxiety, n (%)	309 (6.6)	69 (11)[a,d]	24 (16)[b,d]
History of cerebrovascular accident, n (%)	85 (2.0)	9 (1.5)	4 (3.0)
Diabetes, n (%)	258 (5)	40 (6.5)[a]	14 (9)[b]
Mean systolic blood pressure, mmHg (S.D.)	139 (20)	142 (20)	137 (19.7)
Mean diastolic blood pressure, mmHg (S.D.)	81.7 (10.9)	81.5 (11.0)	80.7 (11.7)

S.D., Standard deviation; N.A., not applicable; MMSE, Mini-Mental State Examination; CES-D, Center of Epidemiological Studies Depression scale.

Group comparisons were performed with $\chi 2$ or analysis of variance.

[a]Comparison of no-grief participants with grief participants ($p < 0.05$). [b]Comparison of no-grief participants with complicated grief participants ($p < 0.05$). [c]Participants with MMSE <23 were excluded. [d]Comparison of grief participants with complicated grief participants ($p < 0.05$).

Table 2. Cognition in participants with no grief, normal grief and complicated grief (n = 5501)

Cognitive tests[a]	No grief (n = 4731)	Grief (n = 615)	Comparison with no grief		Complicated grief (n = 155)	Comparison with no grief	
	Estimated mean	Estimated mean	Difference (95% CI)	p	Estimated mean	Difference (95% CI)	p
Global measures							
Global cognition compound score[b]	0.01	−0.00	−0.01 (−0.07 to 0.04)	0.58	−0.07	−0.08 (−0.19 to 0.01)	0.08
MMSE	28.0	28.2	0.2 (0.04 to 0.33)	0.01	27.7	−0.2 (−0.51 to 0.03)	0.08
Individual test scores							
Letter–Digit Substitution Test	0.04	0.03	−0.01 (−0.09 to 0.07)	0.79	−0.16	0.20 (−0.36 to −0.04)	0.01
Stroop Test, reading	0.02	0.003	−0.01 (−0.11 to 0.06)	0.60	−0.07	−0.10 (−0.27 to 0.06)	0.23
Stroop Test, color naming	0.03	−0.01	−0.04 (−0.13 to 0.04)	0.33	−0.11	−0.14 (−0.31 to 0.02)	0.09
Stroop Test, interference	0.03	−0.002	−0.03 (−0.12 to 0.05)	0.46	−0.07	−0.10 (−0.27 to 0.05)	0.19
Word Fluency Task	0.03	0.03	−0.0 (−0.09 to 0.09)	0.99	−0.15	−0.18 (−0.35 to −0.02)	0.02
Word learning test, immediate recall	−0.03	0.01	0.04 (−0.05 to 0.13)	0.43	−0.12	−0.09 (−0.27 to 0.07)	0.27
Word learning test, delayed recall	0.02	0.01	−0.01 (−0.10 to 0.08)	0.80	−0.01	−0.03 (−0.20 to 0.12)	0.65

CI, Confidence interval; MMSE, Mini-Mental State Examination

[a]All individual cognitive tests scores have been standardized. Those for the Stroop Test have additionally been inverted to indicate poorer performance with lower scores. All analyses are adjusted for age, sex, level of education, blood pressure, history of depression, history of anxiety, history of stroke, diabetes, alcohol consumption and Center for Epidemiologic Studies Depression scale score >16. [b]Global cognitive function (average of three trials of the Stroop Test; Letter–Digit Substitution Test, Word Fluency Task, 15-word learning test – immediate and delayed recall).

Table 3. Brain volume differences in participants with no grief, normal grief and complicated grief (n = 3607)[a]

Brain parameters	No grief (n = 3148)	Grief (n = 373)	Comparison with no grief		Complicated grief (n = 86)	Comparison with no grief	
	Estimated mean	Estimated mean	Difference (95% CI)	p	Estimated mean	Difference (95% CI)	p
Global							
Total brain volume[b], ml	952.4	949.1	−3.3 (−13.06 to 6.49)	0.51	933.5	−18 (−37.67 to −0.10)	0.04
Separate brain tissue classes							
White matter volume, ml	414.7	411	−3.73 (−9.74 to 2.28)	0.22	403.6	−11.1 (−22.66 to 0.44)	0.05
Gray matter volume, ml	533.6	534.0	0.45 (−4.87 to 5.77)	0.87	526.5	−7.06 (−17.28 to 3.15)	0.17
Brain lesions							
White matter lesions, ml	4.11	4.07	−0.04 (−0.72 to 0.65)	0.92	3.38	−0.73 (−2.05 to 0.59)	0.28

CI, Confidence interval.

[a]All analyses are adjusted for age, sex, level of education, blood pressure, history of depression, history of anxiety, history of stroke, diabetes, alcohol consumption and Center for Epidemiologic Studies Depression scale score >16. [b]Total brain volume = gray matter + normal white matter + white matter lesions.

Discussion

In this population-based study we investigated whether persons with complicated grief differ in cognitive function and structural brain changes from participants with normal grief and a control group without grief. Compared with either normal-grief or no-grief groups, participants with complicated grief performed worse in domains of executive function, and information processing speed, and had a lower total brain volume as measured by structural brain imaging.

The few previous studies of bereavement and cognition demonstrated poorer memory performance and attention in persons with normal grief, but did not specifically examine complicated grief (Xavier et al. 2002; Ward et al. 2007; Rosnick et al. 2010; Corruble et al. 2011). Two other studies examined the emotional Stroop Test in persons with complicated grief, demonstrating that participants have more cognitive interference compared with no-complicated grief participants (Maccallum & Bryant, 2010; O'Connor & Arizmendi, 2014). However, the mechanisms supposedly underlying the association between normal grief and poor cognition may also explain our observations in persons with complicated grief. First, individuals with grief or complicated grief may perform worse in the cognitive tests because they find it more difficult to direct their attention (Maccallum & Bryant, 2010; Rosnick et al. 2010; O'Connor & Arizmendi, 2014). Interestingly, no differences in Stroop Tests 1 and 2 were observed between complicated grief participants and non-grievers in the present study, suggesting that attention problems cannot easily explain our findings. However, we did not perform the emotional Stroop Test, which uses death-related and neutral cue words. Second, depressed mood, which is common in persons with normal grief, may interfere with cognitive performance in the bereaved (Boelen & Prigerson, 2007). However, we excluded participants with major depression and adjusted our analyses for depressive symptoms.

Participants with complicated grief were characterized by more brain atrophy, whereas white matter lesion volumes, which reflect vascular brain damage, did not differ between those with complicated grief, normal grief or no grief. We argue that the absence of specific compartmental differences between the groups is most likely due to a non-specific process in participants with complicated grief. Our study cannot establish the temporal sequence, but if this is not a

chance finding, our results suggest that differences in structural brain volumes are linked to complicated grief.

The observed brain volume loss could be a consequence or a precipitating factor of complicated grief. If poorer cognitive performance in persons with complicated grief is a consequence of the brain loss, the observed atrophy may reflect a vulnerability to developing complicated grief. This interpretation is in accordance with the results of the cognitive testing. The Word Fluency Task is considered to be related to the intact function of the frontal cortices and the medial temporal areas (Pihlajamäki et al. 2000; Funahashi, 2001), and the LDST is sensitive to brain dysfunction (Lezak et al. 2004).

The Word Fluency Task and LDST were affected most in persons with complicated grief. In our study, participants with complicated grief had lower brain volumes. It is recognized that as people get older, their brain volume decreases and that different brain regions decrease in volume at different rates (Romanowski & Wilkinson, 2011). The brain volume decrease implies neuronal loss that may disrupt the microstructural integrity of the fascicles connecting the prefrontal cortex with the cortical (frontal, temporal and occipital lobes) and the subcortical areas (amygdala and hippocampus), and even in the functioning of corticostriatal circuitry (Elliot, 2003; Shimada et al. 2012).

A lack of cerebral connectivity could explain a more prolonged resolution of grief in older adults as well as the poor performance in cognitive tests.

Alternatively, cognitive impairment may be a consequence of complicated grief. Recently a study showed that the regional brain activation to grief cues frequently includes the dorsal anterior cingulate cortex and the insula, as well as the posterior cingulate cortex (O'Connor, 2012). Some researchers have proposed that the anterior cingulate cortex, specifically the anterior cingulate gyrus, is part of an executive attention network, and its main role is to regulate the processing of information from other networks, both sensory modalities, and emotional (Ochsner & Gross, 2005; Posner & Rothbart, 2007; Posner et al. 2007; Nelson et al. 2010; Pearson et al. 2011). The regional neural activation of the dorsal anterior cingulate gyrus, insula, and posterior cingulate cortex in persons with complicated grief could cause a deregulation in the network processing the information.

Complicated grief is also perceived as a continuous and chronic stress. Chronic

stress has long-lasting negative effects on cognitive performance (Rosnick et al. 2010). This stress can act in two forms: first, precipitating the neuronal loss and resulting in atrophy of the brain and cognitive decline. Second, glucocorticoids can cause a pronounced loss of synapses which are independent of volume brain loss, producing a disconnection among the brain areas (Tata et al. 2006). Importantly, these explanations for the possible causal process need not be exclusive.

In our study, we found no differences in cognition and structural brain changes between persons with normal grief and the controls. These findings were not unexpected if one views grief as a normal life event, unrelated to pre-existing vulnerabilities such as structural brain volume loss. The previous studies finding poorer cognition in participants with normal grief could be explained by the lack of distinction between normal grief and complicated grief; i.e. previous studies combined persons with normal grief and complicated grief.

Strengths of our study include the very large sample size, the population-based setting and the volumetric quantification of brain tissue volumes. A control group of non-grieving persons was used to provide a contrast to participants with normal grief and those with complicated grief. Also, we controlled for clinically relevant depressive symptoms and excluded persons with major depression.

Some limitations of the current study should also be mentioned. First, it is not possible to evaluate if these associations were causal due to the cross-sectional design of the study. Second, we used a slightly modified version of the original ICG. Third, we focused on selected cognitive domains (e.g. global, memory, information processing speed, and executive function), and could not examine cognitive domains such as visuospatial processing, visuoperceptual tasks, or naming. Fourth, we studied global tissue volumes, but not subcortical or lobar tissue volumes. Fifth, we could not examine if complicated grief was associated with poorer self-care or if there were nutritional deficiencies.

Conclusion

In conclusion, we found that participants with complicated grief had poorer cognitive performance than non-grievers and normal grievers, and lower total brain volume than non-grievers. Our study underscores the importance of assessing social, neuropsychological and biological factors that may underlie the occurrence of complicated grief or may result from prolonged exposure to a normal grief reaction. The neuropsychological differences between persons with and without complicated grief were more modest than in clinical studies (Xavier et al. 2002; Ward et al. 2007; Maccallum & Bryant, 2010; Rosnick et al. 2010; Corruble et al. 2011; O'Connor & Arizmendi, 2014).

Thus, any clinical implications must be inferred cautiously. We suggest that physicians should monitor patients who are in a prolonged grieving process closely and test these persons for possible cognitive deficits. Our analyses with a cut-off of 30 showed a more marked difference between participants with complicated grief and persons without grief, suggesting that clinical definitions of complicated grief describe a cognitively more compromised group. Support techniques for complicated grievers could include cognitive support and treatment or prevention of vascular risk factors, as these can slow the process of brain atrophy. Similarly, patients with known cognitive deficits should be offered support for cognitive problems in addition to psychological and social support if confronted with the loss of a loved one to prevent complicated grief.

We carefully controlled for cognitive decline by adjusting for the MMSE score. However, our results suggest that complicated grief, like severe depressive symptoms, may in some persons be prodromal of dementia. To address the temporality of the associations observed, we are planning to follow the participants and conduct a longitudinal study of the cognitive changes in participants with complicated grief.

Acknowledgements

The Rotterdam Study is supported by: the Erasmus Medical Center and the Erasmus University Rotterdam; The Netherlands Organization for Scientific Research (NWO); The Netherlands Organization for Health Research and Development (ZonMw); the Netherlands Genomics Initiative; the Ministry of Education, Culture and Science; the Ministry of Health, Welfare and Sports; and the European Commission (DGXII). H.C.S.P was supported by an Erasmus Columbus (ERACOL) grant. R.F.P. is supported by a National Health and Medical Research Council (NHMRC) Early Career (ECR) Fellowship (1053666). H.T. was supported by the Vidi grant of ZonMw (2009-017.106.370). The funders had no role in the study design or data collection and analysis. The authors report no competing interests. We want to thank all the persons who contributed to this paper.

H.C.S.P. performed the statistical analyses and wrote the manuscript. M.A.I. supervised the collection of cognition and imaging data. N.D. helped with depression data and the writing of the article. R.F.P. assisted with the writing of the article. H.G.P. reviewed the manuscript and provided expertise on complicated grief. B.F.J.V. supported the analyses of cognition. A.H. is the guarantor of the Rotterdam Study. M.V. supervised the imaging data analyses. H.T. formulated the hypothesis, designed the method, reviewed the manuscript and provided overall supervision. H.C.S.P. and H.T. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Declaration of Interest
None.

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CHAPTER 3

Prolonged Grief and Cognitive Decline: A Prospective Population-Based Study in Middle-Aged and Older Persons

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Am J Geriatr Psychiatry. 2018 Apr;26(4):451-460. doi: 10.1016/j.jagp.2017.12.003. Epub 2017 Dec 12.

Abstract

Objective: *Bereavement can result in unresolved and prolonged grief, often termed prolonged grief disorder (PGD). The impact of PGD on cognitive functioning is poorly understood. The aim of the study was to compare the cognitive decline, assessed by repeated measures of different cognition domains, between persons with normal and PGD and a non-grieving reference population in a 7-year follow-up study.*

Methods: *The study sample comprised 3126 non-demented persons, mean age: 64 years, of the Rotterdam Study. Participants were classified into three groups: no grief (reference group, N = 2,582), normal grief (N = 418), and prolonged grief disorder (N = 126). Partici-pants were assessed with the Complicated Grief Inventory and underwent cognitive testing (Mini-Mental State Examination [MMSE], Letter-Digit Substitution test, Stroop test, Word fluency task, Word learning test). Analyses were adjusted for baseline cognition and depressive symptoms; persons with major depressive disorders were excluded.*

Results: *Compared with the reference group, participants with PGD showed a decrease in global cognitive function, MMSE scores, and World learning test (immediate and delayed) over time. Participants with normal grief did not show a stronger cognitive decline in any of cognitive tests than the reference group.*

Conclusions: *Participants with PGD showed a stronger cognitive decline than the reference group during 7 years of follow-up. This suggests that PGD is a risk factor for cognitive decline, but this study cannot detect the psychobiological mechanism underlying this longitudinal association. (Am J Geriatr Psychiatry 2018; 26:451–460).*

Key Words: Grief, prolonged grief disorder, cognition, cognitive decline, elderly persons, population-based

Highlights

- Bereavement can result in unresolved and prolonged grief.
- Prolonged grief is defined as present when mourners have symptoms of separation distress (e.g., yearning, searching) and traumatic distress (e.g., disbelief, troubling accepting the death, bitterness) for at least 6 months, to the point of functional impairment.

- Prolonged grief is a condition that has a great impact on the quality of life, but the impact of prolonged grief on cognitive functioning is poorly understood.

- The aim of the current study was to compare the cognitive decline, assessed by repeated measures of different cognition domains, between persons with normal and prolonged grief, and a non-grieving reference population in a 7-year follow-up study.

- Our results demonstrate that prolonged grief is a risk factor for cognitive decline.

INTRODUCTION

The human reaction to bereavement is characterized by a variety of feelings, thoughts, and behaviors, of which grief is regarded as the most common.[1] Grief may have negative consequences that typically resolve within months, including exacerbation of preexisting levels of depression,[2] increased risk of suicide,[3] physical health problems, higher rates of disability, medication use, hospitalization,[4] and possibly a short-term decline in cognitive functioning.[5–8] Unresolved and prolonged grief is also observed in some individuals.

Prolonged grief disorder (PGD) is defined as a clinical condition present when mourners have symptoms of separation distress (e.g., yearning, searching) and traumatic distress (e.g., disbelief, troubling accepting the death, bitterness) for at least 6 months, to the point of functional impairment.[9] The disorder occurs in about 5% to 25% of the population experiencing bereavement, but the prevalence varies with age and social, cultural, and clinical background.[10,11] The symptoms of PGD are distinct from those of depression and anxiety,[12–14] despite high levels of comorbidity. PGD is a condition that has a great impact on quality of life,[15,16] and has been related cross-sectionally to sleep disturbances[17] and a higher risk of suicidal ideation.[18] Prospectively, it has been related only to depression, anxiety,[15] and deteriorating health.[16]

The impact of the PGD on cognitive functioning, in particular, is not well known. Several studies investigated the relation of (acute) grief with cognitive impairment,[6–8] but few researchers have examined the impact of PGD on cognitive decline. Recently, a longitudinal study of 1,138 participants with 6 weeks of follow-up compared cognitive impairment between bereaved subjects and major depressive disorder (MDD) subjects. The authors observed that in both groups memory performance declined (immediate and delayed recall) during 6 weeks of follow-up.[6]

Two cross-sectional studies also showed that bereaved persons performed worse in cognitive tests than non-bereaved persons. One of these, a study of 211 older adults, found that bereavement was associated with poorer memory performance in men[7]; the other study showed that bereaved persons performed worse in tests of attention, information processing speed, and verbal fluency.[8]

Recently, in a cross-sectional study of 5,530 participants, we showed that persons with PGD had lower scores

for executive function and information processing speed function when compared with those with normal grief. Participants with PGD also had smaller total brain volumes, suggesting that pre-existing brain changes may contribute to the occurrence of PGD.[19]

The temporal relation between grief and cognition could not be determined, however. Whether cognitive function in persons with PGD declines over the time remains to be studied. The aim of the current study was to compare the cognitive decline, as assessed by repeated measure of different cognition domains, between persons with normal grief and PGD and a non-grieving reference population in a 7-year follow-up study. We hypothesized that participants with PGD will experience a cognitive decline during follow-up, in contrast to participants with normal grief, who will not differ from the non-grieving reference group.

METHODS

Study Participants

The current study is based on the Rotterdam Study, a large prospective population-based cohort designed to examine the occurrence and risk factors of chronic diseases. The study has been described in detail elsewhere.[20] The medical ethics committee of the Erasmus University of Rotterdam approved the study, and informed consent was obtained from all participants. The current study combines two cohorts from the Rotterdam Study. The first was the original cohort, which commenced in 1990–1993. At this time all in-habitants aged over 55 years living in the Ommoord district of Rotterdam were invited to participate; of these, 7,983 (78%) participated. In 2000, people who had become 55 years of age or moved into the study district were added as a second cohort of 3,011 (67% response rate) participants.

The measurement of PGD through the Inventory of Complicated Grief (ICG) was introduced in the fourth follow-up examination (2002–2004) of the original cohort and in the second follow-up examination of the additional cohort (2004–2005). These two examination rounds were identical across the cohorts and constitute the baseline. All data for this study were collected during an interview at the participants home. The baseline interview comprised 5,939 participants. For the present study we selected participants with no grief, participants with grief (ICG <22), and with PGD (ICG ≥22). Participants with Mini-Mental State Examination (MMSE) scores less than 23 (N = 689), with MDD (N = 91), or with incomplete cognitive tests (N = 211) were excluded. Of the remaining 4,948 participants, 4,750 completed the

ICG. These 4,750 non-demented elderly persons aged over 55 years (mean age: 65 years, 57% women) were included in the cross-sectional analyses at baseline.

From March 2009 to July 2012 we reinvited 4,580 of the participants for a second examination (170 died). From these 4,580 participants, 66 persons did not participate because of complaints of physical symptoms, 123 became demented, 56 could not be contacted, 94 had moved outside Rotterdam, 350 refused to participate in the interview without reasons, 230 did not undergo cognitive testing during follow-up examination, and 535 had incomplete cognitive test. This resulted in 3,126 non-demented elderly people for follow-up analysis. Participants who were not assessed at follow-up were older, less educated, had more depressive symptoms, higher blood pressure, and a lower MMSE score than those included.

Assessment of Prolonged Grief

All participants were asked if in the past someone had died who they still mourn. If the answer was positive, we asked formal follow-up questions: "When did this person die?" and "Who was this person?" The last question had several answer options (spouse, partner, parent, child, brother, sister, another family, good friend, other, or several persons).

The participants who answered the first question affirmatively were assessed for PGD with the Dutch version of the ICG.

At least six months of duration of symptoms was required in accordance with recommendations for diagnostic criteria and to exclude an acute stress reaction.[21] Questions represent symptoms of PGD on the basis of the most recently proposed criteria. The ICG is considered the criterion standard for measurement of PGD in older adults because it has high internal consistency, good convergence, and criterion validity.[22] The inventory represents a single underlying construct of PGD. As described before,[10] 17 questions were asked and responses were provided on a 5-point scale to reflect an increase in severity (0-never, 1-seldom, 2-sometimes, 3-often, and 4-always).

In the current setting, one item, "I feel bitter over this person's death", was removed from the original inventory as a pilot study revealed that this sentiment had a very similar meaning within the Dutch language as the included item: "I feel anger over this person's death". Two further items (relating to seeing and hearing the deceased) were combined into one because of their similarity and a pilot study indicating these symptoms were low in frequency and too often

overlapped ("I hear the voice of or see the person who died").

A summary score for the ICG was calculated by totaling each individual item score (responses from 0-never to 4-always) across the 17-items providing a potential score range of 0 to 68. We defined PGD based on the severity of symptoms. Participants with an ICG score of less than 22 were classified as "normal grievers", in line with previous studies.[10]

Participants with a score of 22 or greater due to bereavement experienced at least 6 months before were considered to have PGD. Participants with an ICG score of 22 or greater who had been grieving for less than 6 months were classified as participants with normal grief. This cut-off was based on the cut-off in the original version of the ICG (original cut-off of 25 from 19 items). Thus we classified participants into three groups: no grief (reference group), persons with normal grief (experiencing no prolonged grief as shown by an ICG score <22) and those with PGD (ICG score ≥22).

The non-grieving reference group included persons who had experienced bereavement in the past but were not grieving at the time of interview. Likewise, persons mourning a pet or someone with severe disease who was still alive were included in the reference group.

Assessment of Cognitive Decline

Participants underwent the same neuropsychological tests at the baseline and at the follow-up examinations: the MMSE, the Stroop test, the Letter-Digit Substitution Task (LDST), the Word Fluency Task (WFT), and a 15-word verbal learning test (WLT). For each participant, we calculated z-scores for the tests at baseline and follow-up, except for the MMSE. We constructed a compound score for global cognitive function as the average of all individual tests except the MMSE.[23]

The MMSE is a widely used test for screening for dementia and it provides a reliable measure of global cognitive functions. Scores on this test range from 0 to 30, with higher scores indicating a better cognitive performance.[23]

The LDST was used to measure processing speed. Participants make as many letter digit combinations as possible within 60 seconds, following an example that shows the correct combinations.[23]

The Stroop Test measures attention and concentration and consisted of three trials. In trial 1, the card contains color names printed in black and participants

are asked to name the printed word. In trial 2, the card contains colored blocks and participants are asked to name the printed color. In trial 3, the card contains color names printed in a different color than the color name and participants are asked to name the color of the ink. The outcome variable is the time needed to finish the trial.[23] The WFT was used to test verbal fluency. Participants are asked to name as many animals as possible within 60 seconds.[23]

The WLT tests memory functions with immediate recall and delayed recall components. Participants are given a list of 15 unrelated words repeated over five different trials and are asked to repeat them. Another list of 15 unrelated words is given and the participant is asked again to repeat the original list of 15 words and again after 30 minutes.[24]

Assessment of Other Variables

Potential confounders were selected on the basis of previous publications, and included important determinants of grief or cognitive decline.[6–8] Information was collected in home interviews and during physical examination at baseline.[20] The following variables were tested as possible confounders: age (continuously per year), sex, level of education, blood pressure, diabetes mellitus, cerebrovascular accident history, and depressive symptoms.

Except age and sex, all variables were imputed. The missing covariate values, on average less than 2%, were imputed using median imputation.

Level of education was assessed during the home interview and was classified into primary education only, intermediate education, and high education (university studies). Blood pressure was measured twice at the right arm in a sitting position using a random-zero sphygmomanometer. The average of the two values measured at one occasion was used. Diabetes mellitus was defined as a fasting serum glucose level of 7.0 mmol/L or greater and/or the use of blood glucose-lowering drugs. A cerebrovascular accident was defined according to World Health Organization criteria as a syndrome of rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than of vascular origin. Depressive symptoms were assessed with a validated Dutch version of the Centre for Epidemiologic Studies Depression (CES-D) scale (range: 0–60).[25] Scores of 16 or greater are interpreted as suggestive of clinically significant depressive symptoms. In addition to adjustment for depressive symptoms, participants with MDD at baseline were excluded based on previous

studies showing that symptoms of PGD are distinct from those of depression but that there is very substantial comorbidity between PGD and MDD.[12–14] To this aim, all participants with clinically relevant depressive symptoms (CES-D score ≥16) were interviewed using the Present State Examination, a semistructured psychiatric interview included in the Schedules for Clinical Assessment in Neuropsychiatry. All interviews were conducted by one of two experienced clinicians. Major depression was classified according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition.[26]

Statistical Analysis

We investigated the association of PGD with measures of cognitive performance. We evaluated the cognitive performance on different levels of aggregate: global cognitive measures (global cognitive function and MMSE), and we also analyzed the associations with individual cognitive tests (LDST, WFT, Stroop test, and WLT immediate and recall). Information on demographic characteristics was compared among the groups using a $\chi 2$ test for categorical data and an analysis of variance for continuous variables.

First, we investigated the cross-sectional baseline association of PGD with the measures of cognitive performance. To this aim, we compared the scores of the reference group to those participants with normal grief and to those of participants with PGD using analysis of covariance (ANCOVA).

Second, we investigated the longitudinal association of PGD with the cognitive performance change with linear regression. We examined the association of grief and PGD with cognitive change defined by the global cognitive measures and the individual tests. We tested the difference in cognitive decline over the follow-up period between persons in the reference group and those with normal grief as well as the difference between persons in the reference group and those with PGD.

The analyses were adjusted for age and sex, level of education, blood pressure, diabetes, history of cere-brovascular accident, depressive symptoms (CES-D), and the score on the respective baseline cognitive measure. The adjustment for baseline cognition maximizes the statistical power in the analyses of cognition at follow-up and is considered a more reliable method than difference scores and percentage change.[27] We also calculated the effect size of the associations using Cohen's d. We performed a non-response analysis to evaluate if participants

not assessed at follow-up significantly differed from those included at baseline.
Several sensitivity analyses were conducted. First, we additionally adjusted for antidepressants, psychostimulants, and antidementia drugs, as these medications may be prescribed subsequent to the occurrence of grief but can also confound results. Second, we additionally adjusted for anxiety disorders. Anxiety disorders were diagnosed with an adapted version of the Munich version of the Composite International Diagnostic Interview.[28] Third, we performed analyses including participants with MDD. Fourth, we tested a time frame of 12 months instead of 6 months since loss as criterion to diagnosis PGD.

We used the SPSS statistical package (IBM, Armonk, NY) to perform our analyses. Two general hypotheses were tested, and p values were Bonferroni-corrected accounting for the three groups. were tested, and p values were Bonferroni-corrected accounting for the three groups.

RESULTS

Table 1 presents the characteristics of the study sample for longitudinal analysis. The longitudinal study sample comprised 2,582 people with no grief (reference group), 418 with normal grief, and 126 with PGD. The mean age of the participants at baseline was 64 years (SD: 5.3) and 58% were women. Supplemental Table S1 provides the characteristics of the sample in the cross-sectional analyses.

Table 1 show that the main cause for bereavement in those with prolonged grief was death of a partner (49%), followed by the death of a child (19%). In participants with normal grief this was death of a partner (27%) and brothers/sisters (20%). Participants with prolonged grief more often had diabetes, history of a cardiovascular event, and clinically relevant depressive symptoms than persons with normal grief. No differences were found in any of the other characteristics.

Table 2 shows the cross-sectional association of cognitive test scores across the three groups at baseline, which we tested with ANCOVA. Participants with PGD had lower scores in the LDST compared with the reference group without grief. Participants with normal grief had lower scores on the Stroop test than the reference group. No other differences were found at baseline.

Next, we examined the cognitive decline in these participants over on average 7.3 years with ANCOVA. Table 3 shows the decline in the different cognitive test

scores across the three groups at follow-up. Participants with PGD showed a stronger decrease in global cognitive function and MMSE scores than the reference group. Participants with PGD had a particularly strong decline in the WLT (immediate and delayed). Participants with normal grief did not show a stronger decline in any of the cognitive tests if compared with the reference group. We also compared participants with PGD to those with normal grief. Participants with PGD showed a greater decline in MMSE ($F(1,531) = 3.869$, $p < 0.05$) and in the WLT (delayed recall) ($F(1,413) = 4.108$, $p < 0.04$) across follow-up than persons with normal grief. We did not observe any difference over time between persons with normal grief and persons with PGD in any of the other cognitive tests.

Table 1. Baseline Characteristics of the Study Population for Longitudinal Analysis (N = 3126)

	No Grief N = 2,582		**Grief N = 418**		**Prolonged Grief Disorder N = 126**	
Characteristic	**N**	**(%)**	**N**	**(%)**	**N**	**(%)**
Sex, F	1,405	(54)	313	(75)	95	(75)
CES-D positive	138	(5)	4v0	(9)	40	(31)
History of cerebrovascular accident	246	(9)	35	(8)	13	(10)
Diabetes	59	(2.2)	17	(4)	7	(5.5)
Education						
Primary	455	(18)	79	(18)	27	(21)
Intermediate	1,884	(72)	304	(73)	89	(70)
High	243	(9)	35	(8)	10	(7.9)
Who died?	n.a	n.a				
Partner	–	–	115	(27.5)	62	(49)
Child	–	–	27	(6)	24	(19)
Parent	–	–	63	(15)	12	(9.5)
Brother/sister	–	–	82	(19.6)	8	(6)
Other	–	–	129	(30)	20	(15.8)
Characteristics (continuous)	**Mean**	**(SD)**	**Mean**	**(SD)**	**Mean**	**(SD)**
Age, years	64	(5.5)	64.3	(5.2)	64.8	(5.2)
MMSE	28	(1.6)	28	(1.6)	28	(1.5)
Systolic blood pressure, mm Hg	135	(19)	135	(22)	133	(18)
Diastolic blood pressure, mm Hg	77	(11)	76	(11)	75	(10)

Notes: Participants with grief (ICG <22) and with prolonged grief disorder (ICG ≥22) were included. Participants with MMSE score <23 and with major depressive disorder were excluded.
CES-D: Center for Epidemiologic Studies-Depression; SD: standard deviation; MMSE: Mini-Mental State Examination.

We compared the Cohen's d between participants with PGD and the reference group on global cognition (Cohen's d = 0.21), MMSE (Cohen's d = 0.17), WLT delayed (Cohen's d = 0.20), and WLT immediate (Cohen's d = 0.17). These effect sizes must be considered small. Participants that were not assessed at follow-up were older, less educated, had more depressive symptoms, higher blood pressure, and a lower MMSE score than those included.

We performed several sensitivity analyses. First, we additionally adjusted the analyses for antidepressants, psychostimulants, and anti-dementia drugs. The results remained essentially unchanged, all associations with cognitive tests remained except that between the WLT immediate and prolonged grief ($F(1,2128) = 3.372$, $p < 0.06$). In another sensitivity analysis we adjusted for anxiety disorders. Compared with the reference group, participants with PGD no longer showed a significantly stronger cognitive decline in MMSE score ($F1.26=3.713$, $p<0.05$).

The other associations remained. We performed a third sensitivity analysis in which we included 91 participants with MDD. The results only very marginally changed, although again some associations were no longer significant.

Participants with PGD did not show a significant global cognitive change ($F(1,1976) = 2.746$, $p < 0.09$) or an MMSE score decline ($F(1,2734) = 3.385$, $p < 0.06$).

We also tested a timeframe of 12 months since loss as criterion to diagnose PGD. Compared with the reference group, participants with PGD did not show a cognitive decline in MMSE score in these additional adjusted analyses ($F(1,2682) = 1.61$, $p < 0.20$). This difference partly reflects a reduced effect size and reduced power as fewer people had complicated grief with this more stringent definition. The other results did not change meaningfully.

DISCUSSION

In this population-based study of middle-aged and older persons, we showed that participants with PGD were, on average, more likely to have a decline in global cognitive functioning over 7 years follow-up than participants with no grief.

A few previous cross-sectional studies of bereavement and cognition demonstrated poor memory performance and attention in persons with normal grief.

Table 2. Cross-Sectional Analyses of Cognitive Tests in Participants with No Grief, Grief, and Prolonged Grief disorder at baseline (N = 4,750)

Compound	No grief N = 3,878	Grief N = 659	Comparison Grief—No Grief		Prolonged Grief Disorder N = 213	Comparison Prolonged Grief Disorder—No Grief	
Cognitive Tests	Estimated mean	Estimated mean	Difference with reference (95% CI)	F-statistic/p-value	Estimated mean	Difference with reference (95% CI)	F-statistic/p-value
Global measures							
Global Cognitive Function, z-score	0.08	0.03	−0.05 (−0.11, 0.07)	F(1,3927) = 2.97 0.08	0.008	0.022 (−0.08, 0.13)	F(1,3541) = 2.941 0.68
MMSE, score	27.78 (1.6)	27.81 (1.6)	0.03 (−0.11, 0.65)	F(1,4523) = 0.171 0.69	27.69 (1.5)	0.12 (−0.13, 0.37)	F(1,4080) = 1.048 0.36
Individual cognitive tests							
Letter-Digit test, z-score	0.05	0.07	0.01 (−0.06, 0.08)	F(1,4413) = 0.146 0.69	−0.08	0.15 (0.01, 0.29)	F(1,3972) = 5.111 0.03
Stroop test, z-score	0.07	−0.03	−0.10 (−0.17, −0.02)	F(1,4077) = 7.102 0.008	0.02	−0.05 (−0.19, 0.08)	F(1,3681) = 1.086 0.46
Word Fluency test, z-score	0.06	0.01	−0.05 (−0.12, 0.03)	F(1,4501) = 1.472 0.22	−0.01	0.02 (−0.12, 0.17)	F(1,4060) = 1.648 0.71
Word learning test, immediate recall, z-score	0.05	−0.004	−0.05 (−0.14, 0.02)	F(1,4093) = 1.939 0.16	0.034	−0.03 (−0.18, 0.11)	F(1,3703) = 0.345 0.62
Word learning test, delayed recall, z-score	0.05	−0.003	−0.06 (−0.14, 0.02)	F(1,4093) = 2.134 0.14	−0.035	0.03 (−0.12, 0.18)	F(1,3703) = 2.307 0.67

Notes: The table presents estimated means, which are adjusted values. ANCOVA and F tests were performed. Participants with grief (ICG <22) and with prolonged grief disorder (ICG ≥22) were included. Participants with MMSE <23 and with MDD were excluded. All analyses are adjusted for age, sex, level education, blood pressure, history of stroke, diabetes, and CES-D score ≥16. CES-D: Center for Epidemiological Studies-Depression scale; MDD: major depressive disorder.

Table 3. The Prospective Association Between Grief, Complicated Grief and Cognitive Decline (N = 3,126)

Compound	No grief N = 2,582	Grief N = 418	Comparison Grief—No Grief			Prolonged Grief Disorder N = 126	Comparison Prolonged Grief Disorder—No Grief		
Cognitive Tests	Estimated mean	Estimated mean	Difference with reference (95% CI)	F-statistic/p-value		Estimated mean	Difference with reference (95% CI)	F-statistic/p-value	
Global measures									
Global Cognitive Function, z-score	−0.02	−003	−0.007 (−0.05, −0.04)	F(1,2161) = 0.075	0.78	−0.13	−0.10 (−0.19, −0.01)	F(1,1956) = 4.983	0.02
MMSE, score	27.42	27.42	0.05 (−0.20, 0.31)	F(1,2986) = 0−155	0.69	26.87	−0.48 (−0.95, −0.00)	F(1,2696) = 3.988	0.04
Individual cognitive tests									
Letter-Digit test, z-score	−0.08	−0.12	−0.03 (−0.09, 0.03)	F(1,2716) = 0.998	0.31	−0.11	−0.03 (−0.14, 0.08)	F(1,2443) = 0.205	0.65
Stroop test, z-score	−0.06	−0.03	0.025 (−0.05, 0.10)	F(1,2522) = 0.352	0.55	−0.06	−0.010 (−0.15, 0.13)	F(1,2282) = 0.018	0.89
Word Fluency test, z-score	−0.06	−0.08	−0.019 (−0.10, 0.06)	F(1,2821) = 0.205	0.65	−0.18	−0.12 (−0.27, 0.02)	F(1,2543) = 2.82	0.09
Word learning test, immediate recall, z-score	−0.07	−0.05	0.015 (−0.07, 0.10)	F(1,2355) = 0.109	0.74	−0.23	−0.16 (−0.32, −0.03)	F(1,2133) = 3.978	0.04
Word learning test, delayed recall, z-score	−0.07	−0.014	0.05 (−0.14, 0.02)	F(1,2354) = 1.376	0.24	−0.25	−0.17 (−0.32, −0.01)	F(1,2132) = 4.84	0.02

Notes: The table presents estimated means, which are adjusted values. ANCOVA and F tests were performed. Participants with grief (ICG <22) and with prolonged grief disorder (ICG ≥22) were included. Participants with MMSE < 23 and with MDD were excluded. All analyses are adjusted for age, sex, level education, blood pressure, history of stroke, diabetes, and CES-D score ≥16. CES-D: Center for Epidemiological Studies-Depression scale; MDD: major depressive disorder

However, none of these studies examined cognitive decline or assessed PGD.[5,6]

These results are typically interpreted as cognitive impairment due to grief. Our baseline analyses suggest that such findings may largely be accounted for by persons with severe or PGD. Our study also indicates that in cross-sectional studies reverse causality cannot easily be ruled out that is, those with pre-existing cognitive problems cannot cope with grief. At baseline, the participants with PGD already performed poorly in the Letter-Digit Substitution Task.

The mechanisms underlying the association between PGD and poor cognition are not clear, but several explanations must be discussed. First, bereaved individuals may perform worse in the cognitive tests because they find it more difficult to direct their attention to the tests. There is evidence that persons with PGD find it particularly difficult to concentrate and suffer from intrusive negative thoughts, which interfere with attention and performance during testing.[29]

Also, they may utilize their attention resources during cognitive tests, to try to suppress intrusive and unwanted thoughts.[7] Likewise, depressed mood may interfere with cognitive performance in the bereaved.[15,30] However, we excluded participants with MDD and adjusted our analyses for depressive symptoms.

Second, because of the persistence of the bereavement symptoms and the resistance of the related psychological problems to treatment and to non-medical interventions, PGD subjects the bereaved person to high levels of stress.[31–33] Sustained levels of stress produce activation of the hypothalamic-pituitary-adrenal axis with an increase of glucocorticoids concentrations, which have been linked to a reduction of the hippocampal volume.[34–37] The hippocampus is essential for many types of memory, such as episodic memory and spatial memory.[38,39]

Third, the observed memory decline may be due to a pre-existing cognitive impairment[40] that could make a grieving person more vulnerable to develop PGD. To offset for this, we adjusted for baseline MMSE scores and excluded persons with scores below 23. This means that any pre-existing cognitive impairment at baseline must have been very subtle and, for example, an early stage of any mild cognitive impairment (MCI).

In our study, we showed an overall decline in global cognitive function and MMSE but also observed that the WLT

(both immediate and delayed recall) contributed strongly to the observed decline of the global cognition.

In contrast, participants with PGD experienced no further decline in the performance in the LDST, WFT, or the Stroop test over the 7-year follow-up period. This finding can be carefully interpreted as supporting MCI in those with PGD. Studies have shown that the delayed recall battery has a high sensitivity and specificity to differentiate early stages of cognitive impairment from cognitive performance in normal elderly, and is more accurate than the MMSE.[41–43] In particular, total immediate recall and delayed recall were found to be robust predictors of MCI converting to Alzheimer disease within 3 years of follow-up.[44]

The estimated effect sizes in our study were small, implying that PGD by itself is not a major cause of cognitive decline and should not be a focus of intervention in all grieving persons but probably contributes meaningfully to cognitive impairment only in vulnerable persons.

We did not find any further decline in cognitive functioning in this longitudinal study of participants with only normal grief. These findings were in line with our hypothesis, that grief is a normal life event, unrelated to pre-existing vulnerabilities, from which the majority of the persons recover.

The findings of previous studies showing poor cognition in participants with normal grief might be explained by the lack of distinction between normal grief and PGD in their population.[6–8] These studies jointly analyzed persons with normal grief and PGD, who are characterized by more severe symptoms. Also, these studies mostly did not exclude or adjust for MDD, which is strongly related to poor cognitive performance and grief severity.

A strength of our study is the very large sample size and the population-based setting. A reference group of non-grieving persons was used to provide a contrast to participants with normal grief and those with PGD. We controlled for depressive symptoms and history of cerebrovascular accident. Some limitations of the current study should also be mentioned. First, 34% of the baseline group of participants had no follow-up assessment, but this is not uncommon in prospective studies of elderly people.[45] Second, we used a single question to screen for possible grief. This may introduce some misclassification, although a detailed history of all meaningful persons lost in this elderly population was not feasible and may

also introduce recall bias with regard to mourning. Third, we did not assess grief symptoms at follow-up and thus cannot establish whether PGD, which persisted at follow-up, explains the findings.

Fourth, we focused on selected cognitive tests, and could not examine cognitive domains such as visuospatial processing, visuoperceptual tasks, or naming. Fifth, we used a static imputation as the frequency of missing data was very low, although this can marginally reduce the standard error.

In our study, we found a decline in global cognitive function, MMSE, and the WLT (immediate and delayed recall) in participants with PGD. The measure of the effect size for each cognitive test was small. The decline in the delayed-recall component of the WLT was most marked. Our results suggest an association between PGD and cognitive decline, but we cannot infer causality from this study or detect the psychobiological mechanism underlying this longitudinal association.

Also, we cannot conclude whether PGD persistent across the follow-up period underlies the observed results. PGD has a high incidence in any elderly population, as more than 30% of all women and 10% of men lose a partner.[46] In our study, we found that the prevalence of PGD in the sample was 4%, and the prevalence of PGD in the grieving population was 23%. Our findings suggest that physicians should closely monitor patients who are in a prolonged grieving process, test these persons for possible cognitive deficits, and consider cognitive support techniques for the most vulnerable persons. Similarly, patients with known cognitive deficits should be offered additional psychological and social support if confronted with the loss of a loved one to prevent PGD.

The Rotterdam Study is supported by the Erasmus Medical Centre and the Erasmus University Rotterdam, The Netherlands Organization for Scientific Research (NWO), The Netherlands Organization for Health Research and Development (ZonMw); the Netherlands Genomics Initiative; and the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports, and the European Commission (DGXII). HSP was supported by an ERACOL grant (EACEA/13/09-Lot21b). HT was supported by a VICI grant of ZonMw (2009-017.106.370, 016.VICI.170.200). The funders had no role in the study design or data collection and analysis. Authors report no competing interests.

APPENDIX: SUPPLEMENTARY MATERIAL

Supplementary data to this article can be found online at doi:10.1016/j.jagp.2017.12.003.

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CHAPTER 4

The Longitudinal and Cross-Sectional Associations of Grief and Complicated Grief with Sleep Quality in Older Adults

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Behav Sleep Med. 2019 Jan-Feb;17(1):31-40.
doi: 10.1080/15402002.2016.1276016. Epub 2017 Jan 20.

ABSTRACT

Objective/Background: About 15% of grievers experience complicated grief. We determined cross-sectional and longitudinal relations of grief and complicated grief with sleep duration and quality in the general population of elderly adults.

Participants: We included 5,421 men and women from the prospective population-based Rotterdam Study.

Methods: The Inventory of Complicated Grief was used to define grief and complicated grief. We assessed sleep with the Pittsburgh Sleep Quality Index.

Results: After 6 years, 3,511 (80% of survivors) underwent the follow-up interview. Complicated grief was cross-sectionally associated with shorter sleep duration and lower sleep quality. These associations were explained by the presence of depressive symptoms. The prospective analyses showed that sleep duration and sleep quality did not decline further during follow-up of persons who experienced grief or complicated grief.

Conclusion: In community-dwelling, middle-aged and older adults, persons with normal and complicated grief had both a shorter sleep duration and a lower sleep quality, mainly explained by depressive symptoms. However, prospective analyses showed that sleep quality and sleep duration do not decline further in persons with normal grief and complicated grief.

INTRODUCTION

The death of a loved one is a common life event in older adults (Boelen & Hoijtink, 2009; Boelen& van den Bout, 2008; M. K. Shear, 2015). Very few persons make it through old age without having to cope with this kind of loss, once or several different times.

The loss of a partner, child, parent, or close family member can be very distressing (Monk, Germain, & Reynolds, 2008). However, even if it is experienced as a traumatic event, after a delimited period of grief, the majority of people recover. An estimated 15% of bereaved people continue to grieve for an extended period; they experience disbelief and are preoccupied by the deceased (Prigerson et al., 1995). This state is known as complicated grief (Prigerson et al., 2009). Complicated grief is an important mental health issue for the aging population, affecting social functioning and well-being (Newson, Boelen, Hek, Hofman, & Tiemeier, 2011). However, our knowledge about complicated grief is limited.

Previous studies suggest that symptoms of complicated grief are distinct from those of depression and anxiety and have incremental validity predicting impairments in social and interpersonal daily functioning (Boelen, van de Schoot, van den Hout, de Keijser, & van den Bout, 2010; Newson et al., 2011; Prigerson & Jacobs, 2001). In addition, the severe emotional strain of the loss of a loved one can trigger profound changes in lifestyle. These changes often induce reductions in financial security, perceived personal safety, and freedom of action. All of these facets of grief could lead to changes in sleep patterns. Several studies (Hall et al., 1997; Kowalski & Bondmass, 2008; Monk, Begley, et al., 2008) suggest that grief is associated with significant sleep impairment. However, our knowledge regarding the associations of complicated grief with sleep is limited, as only a few studies with small sample size and a cross-sectional design have been conducted (Boelen & Lancee, 2013; Germain, Caroff, Buysse, & Shear, 2005; Maytal et al., 2007; Monk, Begley, et al., 2008; Purebl, Pilling, Konkoly, Bodizs, & Kopp, 2012; Spira, Stone, Beaudreau, Ancoli-Israel, & Yaffe, 2009). An exploratory study of the effects of complicated grief on sleep by McDermott et al. (1997) conducted analyses on 65 bereaved persons.

The results showed mild subjective sleep impairment is associated with complicated grief, but no effect was detected using

the electroencephalographic sleep measures. Germain et al. (2005) evaluated the severity of sleep disturbances in a group of 105 adults meeting criteria for complicated grief. They showed an association of complicated grief with an overall poor sleep quality. Comorbid depression (Adrien, 2002; Germain et al., 2005; Hall et al., 1997; Maytal et al., 2007; Monk, Begley, et al., 2008; Nutt, Wilson, & Paterson, 2008; Purebl et al., 2012; Spira et al., 2009), but not posttraumatic stress disorder, further worsened sleep quality.

Taking into account the lack of high-powered longitudinal studies in normal populations of elderly adults, we aim to determine whether in adults aged 55 years and above, grief or complicated grief was related to sleep duration and sleep quality, cross-sectionally and longitudinally.

We hypothesized that if studied cross-sectionally, persons with grief and complicated grief have shorter sleep duration and a lower sleep quality than persons who did not experience grief due to the stress that death of the loved one brings to person's life. Second, we hypothesized that complicated grief remains a risk factor for further decline of sleep duration and poor sleep quality over time due to coping mechanisms that may not always be successful.

METHODS

Settings and Study Population

This study was embedded in The Rotterdam Study, an ongoing prospective cohort of older adults designed to examine the occurrence and risk factors of chronic diseases. The study design and objectives are described in Hofman et al. (2013).

The Rotterdam Study comprises two cohorts, which were combined in the current analysis. Between 2002 and 2005, complicated grief and sleep quality were assessed during a home interview, referred to as baseline. The baseline interview was conducted in 5,481 participants. Of these participants, 60 persons did not complete the grief or sleep questionnaire.

This left 5,421 participants with assessment of grief and sleep characteristics for cross-sectional analysis. In part of the follow-up examination (2009– 2011), both components of sleep (duration and quality) were assessed at the research center. After an average of 6.33 years (SD = 0.42), 3,511 (80%) of the 4,601 surviving participants underwent the follow-up interview for sleep duration,

and 3,003 (71%) for sleep quality. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus Medical Center (Erasmus MC) and by the Ministry of Health of the Netherlands, implementing the Wet Bevolkingsonderzoek: ERGO (Erasmus Rotterdam Gezondheid Onderzoek; Population Studies Act: Rotterdam Study). All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

Assessment of Complicated Grief

Complicated grief was diagnosed at the baseline examination (2002 and 2005), with a Dutch version of the 17-item Inventory of Complicated Grief (ICG) originally constructed by Prigerson et al. (which contains 19 items). First, participants were asked if they were currently grieving. If a positive answer was received, the ICG was administered, but if not, they were categorized as nongrievers (the reference group). The ICG is the most widely used instrument to measure complicated grief.

Questions represent symptoms of complicated grief such as those in the most recent proposed criteria for the condition suggested by (Prigerson et al., 2009). Some of the symptoms include intense yearning for the lost person, anger over the death, distrust and detachment from others as a consequence of the death, survivor guilt, and loneliness. The measure has high internal consistency and convergent and criterion validity and it is considered the gold standard for measurement of complicated grief in older adults.

The inventory is shown to represent a single underlying construct of complicated grief (Boelen & Hoijtink, 2009). The Dutch version of the Inventory of Complicated Grief contains 17 items and has been previously validated (Boelen et al., 2003). These 17 questions were asked and responses were provided on a 5-point scale to reflect an increase in severity (0-never, 1-seldom, 2-sometimes, 3-often, 4- always). In the current study one item from the original inventory, "I feel bitter over this person's death," was removed from the original ICG because a pilot study revealed that this sentiment had the same meaning within the Dutch language as the included item, "I feel anger over this person's death." Two further items (relating to seeing and hearing the deceased) were collapsed into one due to their similarity and to a pilot study indicating these symptoms were low in frequency and often overlapped ("I hear the voice of, or see, the person who died"). Several studies give further details on the interpretation of ICG (Boelen et

al., 2010; Newson et al., 2011; Prigerson & Jacobs, 2001).

We divided all interviewed participants into nongrievers (reference group), normal grievers, and complicated grievers. Complicated grief symptoms were assessed as present among participants who scored equal or greater than 22 on the ICG score and grieved longer than 6 months (Newson et al., 2011; Saavedra Perez et al., 2015).

Assessment of Sleep

Sleep duration and sleep quality were measured with the Pittsburgh Sleep Quality Index (PSQI), a self-reported questionnaire (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989). The PSQI consists of 19 self-rated questions. Questions are grouped into seven component scores, each weighted equally on a 0–3 scale. The seven component scores are then summed to yield a global PSQI score, which is used in all further analyses. This score has a range of 0–21; higher scores indicate worse sleep quality. The seven components are subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction. In the current study, we used total sleep time in hours to indicate sleep duration, and a total score of PSQI to indicate sleep quality. Finally, we presented a sample of PSQI (Supplement A).

Assessment of potential confounders

Age, sex, education, cognitive functioning, activities of daily living, body mass index (BMI), and depressive symptoms were considered as potential confounders. Education was assessed routinely in the home interview and subdivided into low, intermediate, and high education. Cognitive functioning was measured using the Mini-Mental State Examination (Folstein, Folstein, & McHugh, 1975) during one of the visits to our center. The ability to perform activities of daily living was measured with the Stanford Health Assessment Questionnaire (Bruce & Fries, 2003; Fries, Spitz, & Young, 1982). Height and weight were measured without shoes and heavy clothing to calculate the BMI (kg/m2). Depressive symptoms were measured with the Center for Epidemiological Studies Depression scale (CES-D). In our baseline table, we also showed the presence of depressive symptoms among participants who scored 16 or above, suggesting clinically relevant depressive symptoms on the Center for Epidemiological Studies Depression scale.

Statistical Analyses

To explore the association between grief and sleep parameters we used linear

regression. Model 1 was adjusted for age and sex. Model 2 was additionally adjusted for education, cognitive functioning, activities of daily living, and BMI. Model 3 was further adjusted for depressive symptoms. In the longitudinal analyses, to examine whether grief status was prospectively associated with sleep duration and sleep quality, we used sleep duration and sleep quality assessed during the follow-up as outcomes. We selected the same covariates as in the cross-sectional analyses and adjusted for the respective baseline values of sleep duration or sleep quality.

We conducted a series of sensitivity analyses. First, we reran the analysis, not only for depressive symptoms, but to exclude all patients with major depression disorder at the baseline. We evaluated the presence of Major Depressive Disorder in those with a CES-D score, or above the established screening cutoff of 16, using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) interview (Wing et al., 1990). This semistructured clinical assessment was performed by trained clinicians to determine which participants fulfilled the DSM-IV major depressive disorder. We performed this sensitivity analysis in order to minimize the depressive disorders on sleep quality. Also, in our study, Major Depressive Disorder (MDD) was assessed at baseline only (prevalence of 2%). Second, to test the effect of the more recent sleep, we performed longitudinal analysis restricted to those who experienced the bereavement in the years prior to baseline assessment. Third, we repeated the cross-sectional linear regression only in those participants who had attended the follow-up assessment, to test whether any between cross-sectional and longitudinal analysis reflected a selection effect. Adjustments for these analyses were conducted as in the main linear regressions. Fourth, since sleep duration component of PSQI, we performed a sensitivity analyses calculating the PSQI total without the component of sleep duration. Fifth, in order to explore the possibility of reverse causality, in the longitudinal analysis, we excluded people who had poor sleep at the baseline (defined as the total score of all components of PSQI greater than 5 points). Sixth, we explore whether, cross- sectionally and longitudinally, sleep duration and sleep quality differ between grievers (reference) and complicated grievers. We also explored the association between grief status and depressive symptoms (CES-D). In multivariable linear regression models, we examined whether baseline grief status was prospectively associated with CES-D at follow-up, further adjusted for the baseline value of depressive symptoms.

With respect to the remaining data, missing values were imputed using multiple imputations (Rubin, 2004). In the present study, for each missing value five draws were performed providing five substituted items of data, which in turn created five completed data sets. Analyses were performed separately on each completed data set and there after combined into one pooled estimate. The percentage of missing values within the population for the analyses was lower than 20% (ranging from 0 to 18%). Age and sex had no missing values, education had 18%, cognitive score had 8%, activities of daily living score had 1%, BMI had 14%, and CES-D had 0.2%. Analyses were performed using SPSS Statistics (version 20; SPSS, Chicago, IL, USA).

RESULTS

Of the 5,421 eligible participants, 4,378 (80%) were classified as experiencing "no grief," 795 (15%) as experiencing "normal grief," and 248 (5%) as experiencing "complicated grief" at baseline. Table 1 presents the characteristics of the study population. Participants classified as experiencing complicated grief were older, were more likely to be female, had a lower level of education, and were more likely to have clinically relevant depressive symptoms. The main cause for grief was death of a partner (36% of those with normal grief and 56% of those with complicated grief), or a child (10% of those with normal grief and 22% of those with complicated grief).

Table 1. Baseline Characteristics of Study Participants 2002–2005 (N = 5,421)

Characteristics	Nongrievers N = 4,378 (80%)		Normal grievers N = 795 (15%)		Complicated grievers N = 248 (5%)	
Age, years (SD)	72.4	(7.7)	73.4	(8.1)[a]	74.7	(7.5)b
Women (%)	55		73[a]		72[b]	
Education[a,b]						
Primary (%)	19		22		30	
Intermediate (%)	67		66		63	
High (%)	14		12		7	
Cognitive functioning, score (SD)	27.41	(2.58)	27.39	(2.52)[a]	26.99	(3.04)[b,c]
Depressive symptoms, score (SD)	5.42	(6.67)	8.62	(8.49)[a]	14.12	(11.35)[b,c]
Clinically relevant depressive symptoms (%)	4		8[a]		25[b,c]	
Who died?						
Partner (%)	–		36		56	
Child (%)	–		10		22	
Parent (%)	–		12		6	
Brother/sister (%)	–		17		7	
Others (%)	–		25		9	
Activities of daily living, score (SD)	0.51	(0.57)	0.59	(0.57)[a]	0.66	(0.60)[b]
Body mass index (kg/m2), (SD)	27.56	(4.03)	27.67	(4.34)	27.75	(4.38)
Sleep duration (hours), (SD)	6.91	(1.30)	6.69	(1.36)[a]	6.44	(1.50)[b,c]
Sleep quality, score (SD)	3.37	(2.91)	4.28	(3.20)[a]	5.08	(3.67)[b,c]

Group comparisons were performed with χ^2 (categorical variables) or t-test (continuous variables) for independent samples. [a]Comparison of nongrieving participants with grieving participants ($p < 0.05$). [b]Comparison of nongrieving participants with complicated-grief participants ($p < 0.05$). [c]Comparison of grief participants with complicated-grief participants ($p < 0.05$).

Table 2 shows the cross-sectional associations of grief with sleep duration and sleep quality. In the age-and-gender adjusted analysis, we found a consistent association pattern of grief and complicated grief with sleep duration as well as with sleep quality. Further, adjustment for education level, activities of daily living, cognitive functioning, and body mass index did not change these associations. However, the association between grief and sleep indicators was explained by depressive symptoms (model 3).

In Table 3 we present the prospective association of grief with sleep duration and sleep quality (both assessed at follow up exam after 6.33 years on average (SD = 0.42)). We did not find an association of grief or complicated grief with changes in sleep duration or sleep quality, either in the age-and-gender adjusted or in the fully adjusted analyses. Next, we performed a series of sensitivity analyses. First, we excluded persons with major depression from our study population and reran the analysis; our result remained essentially unchanged. Then we limited the cases to those who experienced the bereavement leading to complicated grief in the last 2 years prior to baseline assessment (Supplement B). Our result showed no association between more recent lost event and sleep parameters in the longitudinal analysis. Also, to test whether the differences between cross-sectional and longitudinal analysis reflect a selection effect, we reran the cross-sectional analysis in participants who attended the follow-up assessment. Results remained essentially unchanged; the cross-sectional associations of complicated grief with sleep duration and sleep quality were similar to our original cross-sectional findings (data shown in Supplement C). Also, the results did not change when we reran the analysis calculating the PSQI score without including the sleep duration component (Supplement D). Next, in the longitudinal analysis, exclusion of subjects who had poor sleep quality at baseline did not change the results (Supplement E). Furthermore, we did not find any difference in sleep duration or sleep quality between grievers and complicated grievers in both cross-sectional and longitudinal analysis (data not shown). Last, we did not find an association between baseline grief status and depressive symptoms at follow-up (grievers: $\beta = -0.22$, 95% CI: −1.51—1.07, $p = 0.74$; complicated grievers: $\beta = 1.00$, 95% CI: −1.30—3.31, $p = 0.39$).

Table 2. Cross-Sectional Associations of Grief and Complicated Grief With Sleep

	Model I					*Model II*				*Model III*		
	N	B	95%	CI	P	B	95%	CI	P	B	95%	CI
Sleep duration (PSQI score) Grieving status												
Nongrievers (ref.)	4378	–	–	–	–	–	–	–	–	–	–	–
Grievers	795	−0.15	−0.25	0.05	0.003	−0.15	−0.25	−0.05	0.004	−0.05	−0.15	0.05
Complicated Grievers	248	−0.41	−0.57	−0.24	≤ 0.0001	−0.40	−0.57	−0.24	≤ 0.0001	−0.12	−0.30	0.05
Sleep quality (PSQI score) Grieving status												
Nongrievers (ref.)	4378	–	–	–	–	–	–	–	–	–	–	–
Grievers	795	0.64	0.39	0.90	≤ 0.0001	0.63	0.38	0.88	≤ 0.0001	0.15	−0.08	0.39
Complicated Grievers	248	1.52	1.09	1.95	≤ 0.0001	1.48	1.06	1.90	≤ 0.0001	0.08	−0.33	0.48

Abbreviations: PSQI (Pittsburgh Sleep Quality Index) Model I was adjusted for age and sex; Model II was adjusted for age, sex, education, activities of daily living, cognitive functioning, BMI, and the respective baseline values of sleep duration and Pittsburgh Sleep Quality Index to model change; Model III included factors in Model II and further adjustment for depressive symptoms.

Table 3. The Longitudinal Associations of Grief and Complicated Grief With Sleep

	Model I					*Model II*				*Model III*			
	N	B	95%	CI	P	B	95%	CI	P	B	95%	CI	P
Sleep duration (hours) Grieving status													
Nongrievers (ref.)	2876	–	–	–	–	–	–	–	–	–	–	–	–
Grievers	495	0.02	−0.10	0.14	P	0.02	−0.10	0.14	0.72	0.01	−0.11	0.13	0.91
Complicated Grievers	140	0.06	−0.15	−0.27	0.57	0.06	−0.15	0.27	0.57	0.01	−0.20	0.23	0.91
Sleep quality (PSQI score) Grieving status													
Nongrievers (ref.)	2482	–	–	–	–	–	–	–	–	–	–	–	–
Grievers	413	−0.09	−0.39	0.21	0.55	−0.09	−0.39	0.20	0.53	−0.07	−0.37	0.23	0.66
Complicated Grievers	108	0.06	−0.49	0.61	0.83	0.07	−0.49	0.62	0.82	0.15	−0.42	0.71	0.61

Abbreviations: PSQI (Pittsburgh Sleep Quality Index). Model I was adjusted for age and sex; Model II was adjusted for age, sex, education, activities of daily living, cognitive functioning, BMI, and the respective baseline values of sleep duration and Pittsburgh Sleep Quality Index to model change; Model III included factors in Model II and further adjustment for depressive symptoms.

DISCUSSION

In this large population-based study of middle-aged and elderly persons, we investigated whether persons with grief or complicated grief had a different sleep duration and sleep quality than participants without grief. Our cross-sectional findings showed that normal and complicated grief were associated with shorter sleep and lower sleep quality.

These associations were mainly explained by the presence of depressive symptoms. No further changes in sleep duration and sleep quality between the groups were observed after an average follow-up of more than 6 years. Complicated grief can be regarded as a bereavement situation for which sleep duration is likely to be affected (Monk, Germain, et al., 2008). A cross-sectional study of duration of sleep among unselected grievers, that is, most probably including persons with complicated grief, has been reported previously (Monk, Germain, et al., 2008). The authors conducted a laboratory study of sleep and circadian rhythm in 38 spousal bereaved seniors (≥ 60 years) observed 4 or more months after their loss event. On average, the bereaved seniors achieved only about 6 hr of sleep. In a large Japanese population-based prevalence study of 1,871 participants conducted by Doi, Minowa, Okawa, and Uchiyama (2000), the authors showed that being widowed or without a partner was associated with lower sleep quality.

There is evidence suggesting that behavioral changes associated with grief such as decreased activity levels or overall changes in social rhythm stability could lead bereavement to sleep disturbances. After the loss of a loved one, there are profound changes in lifestyle, often accompanied by reductions in financial security, perceived personal safety, and freedom of action (Monk, Germain, et al., 2008), all of which are likely to lead to sleep disruption. Also, the loss of a loved one is associated with psychological problems such as rumination or anxiety, which are shown to impair sleep (Carney, Edinger, Meyer, Lindman, & Istre, 2006; K. Shear et al., 2007). Sleep disturbances are particularly prevalent in depressed bereaved persons; even bereaved persons who fail to meet a formal diagnosis of depression have measurable sleep impairment (Reynolds et al., 1992). Indeed, our cross- sectional analysis showed that the association between grief and complicated grief with sleep indicators was largely explained by depressive symptoms.

However, we ran multivariable linear regression models to see if baseline grief status was prospectively associated with CES-D at follow-up and found no association, providing support that depressive symptoms are not a mediator in the association between grief and sleep parameters. Further, reversed causality should be taken into account. Since relatively few studies have yet examined sleep difficulties as a risk factor for post-loss psychopathology, we cannot rule out that existing sleep problems make individuals vulnerable to more severe or prolonged grief or complicated grief. Indeed, as Boelen and Lancee (2013) pointed out, poor sleep quality is a known risk factor for many different forms of a psychopathology, including depression and PTSD.

We did not find a prospective association between grief and sleep parameters. Different explanations for these null findings are possible: First, the lack of findings can reflect the insufficient power to detect an association. Although fewer participants could be included in the longitudinal analyses, sufficient power to detect any effect similar to that observed in the cross-sectional analyses remained. Thus, these findings suggest that there was no further change in sleep duration and sleep quality once a person had reported bereavement at our baseline assessment. We carefully infer that the results could be explained with mechanisms of adaptive coping (S. S. Rubin, 1999; Stroebe & Schut, 1999) developed by the grieving participants during prolonged exposure to grief.

Possibly, persons grieving reached a "stable state," that is, with no further change of sleep quality, when participating in the follow-up assessment on average 6 years after the event. Sleep quality might have been affected before the occurrence of complicated grief.

Due to the lack of prebereavement sleep assessment, it is not possible to evaluate the directional effects in the cross-sectional analysis, that is, whether bereavement triggered the decline of sleep duration and quality or whether sleep impairment preceded the grief reaction. However, our sensitivity analysis in which we excluded participants with poor sleep quality provides no evidence for reverse causality. Also, grief was assessed only at baseline. Consequently, we cannot account for the change in grief status, whether the feelings of grief remitted, persisted, or worsened.

However, the majority of clinical diseases and conditions are characterized by a progression of symptoms and their consequences; against this background,

we had hypothesized a continuous decline of sleep problems, having in mind that sleep duration and quality among persons with complicated grief is of potential value for prognosis of grieving persons and potentially even of relevance for therapeutic interventions that rely on the cognitive behavioral interventions focused on sleep difficulties as discussed by Boelen and Lancee (2013). It suggests that the impact of grief on this important aspect of well-being is not accumulating over time and can potentially be overcome.

To the best of our knowledge, other longitudinal studies have not been performed previously in the general population. Also, our study is characterized by a long follow-up period and a large sample size. Furthermore, a middle-aged and elderly sample was used, which is the main vulnerable population for complicated grievers, as late-life loss of a loved one is among the most common life events. However, some limitations of the current study should be mentioned. First, in a population-based study, it is not feasible to ascertain grief and sleep directly after a loss event. Most important, complicated grief cannot be diagnosed if the event occurred less than 6 months before. Therefore, we performed sensitivity analyses restricting the study population to those who experienced the bereavement more recently (in the last 2 years before the baseline assessment). Second, we miss information on whether these persons are still suffering from complicated grief or MDD at follow-up. Further studies should be performed including this kind of prospective reassessment.

CONCLUSION

In community-dwelling middle-aged and older adults, persons with normal and complicated grief had both a shorter sleep duration and a lower sleep quality, mainly explained by depressive symptoms. However, prospective analyses showed that sleep quality and sleep duration do not decline further in persons with normal grief and complicated grief.

ACKNOWLEDGMENTS

We want to thank all the persons who contributed to this article.

FUNDING

The Rotterdam Study is supported by the Erasmus Medical Center and the Erasmus University Rotterdam, The Nederland's Organization for Scientific Research (NWO), The Nederland's Organization for Health Research and Development, the Ministry of Health, Welfare, and Sports, and the European Commission (DGXII). Jelena Milic was supported by an Erasmus Mundus (ERAWEB) grant. Henning Tiemeier was supported by the Vidi grant of the Netherlands Organization for Health Research and Development (ZonMw 2009-017.106.370). The funders had no role in the study design or data collection and analyses.

SUPPLEMENTAL MATERIAL

Supplemental data for this article can be accessed on the publisher's website.

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CHAPTER 5

The Impact of complicated grief on diurnal cortisol levels two years after loss: A population-based study

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Psychosom Med. 2017 May;79(4):426-433.
doi: 10.1097/PSY.0000000000000422.

ABSTRACT

Objective: Few studies have focused on the effect of complicated grief—unresolved and prolonged grief—on the neuroendocrine systems. The present study examined the association of complicated grief and normal grief with the diurnal cortisol patterns in a large population-based study.

Methods: This study was set in the Rotterdam Study and comprised 2084 persons aged older than 55 years (mean [SD] age, 64.9 [5.5] years). Participants were assessed with the Complicated Grief Inventory and classified into no grief (n = 1922), normal grief (n = 131), or complicated grief (n = 31) if they experienced the loss in the past 2 years. Saliva samples were collected to measure cortisol levels. Morning cortisol and summary measures (area under the curve and the slope) were studied to account for the diurnal pattern of cortisol. Persons with depressive disorders were excluded, and analyses were additionally adjusted for depressive symptoms.

Results: Compared to normal grievers, participants with complicated grief showed lower levels of morning cortisol (11.26 vs 15.51 nmol/L; difference, −4.24; 95% confidence interval [CI] = −7.87 to −0.62; p = .022), and lower levels of overall diurnal cortisol (6.89 vs 8.98 nmol/L; difference, −2.09; 95% CI = −3.81 to −0.37; p = .017). No difference was observed in slope between both groups. Participants with complicated grief also showed lower levels of morning cortisol than the nongrievers (11.26 vs 14.71; difference, −3.46; 95% CI = −6.78 to −0.13; p = .042). In contrast, cortisol secretion patterns did not differ between persons with normal grief and nongrieving controls.

Conclusions: Participants with complicated grief showed low levels of morning cortisol and low overall diurnal cortisol levels characteristic for a chronic stress reaction.

Key words: grief, complicated grief, complicated grief, cortisol, hypothalamic-pituitary-adrenocortical axis, population based, coping.

INTRODUCTION

Bereavement is defined as having experienced a significant loss (1). Its most common reaction is grief (2).

Grief knows many different manifestations and courses, but grief itself is not a mental disorder. However, approximately 9% to 20% of the population experiencing bereavement show symptoms of an unresolved and prolonged grief termed "complicated grief" (3). Complicated grief has been referred to as "traumatic grief," "complicated grief disorder," and "prolonged grief " and is distinctly different from "depression accounted for by bereavement," as mentioned in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (4). Recently, complicated grief has been included within the section "conditions for further study" in DSM-V section as persistent complex bereavement disorder (5).

Complicated grief includes a set of symptoms such as persistent intense yearning for the deceased, intense feelings of loneliness, feelings of disbelief or that life is empty, being emotionally numb or troubling accepting the death, bitterness, intrusion and rumination of thoughts or images of the dead person, and hearing or seeing the deceased person, lasting for at least 6 months (4,6,7).

The etiology of complicated grief is not well established, although psychosocial risk factors have been described, such as an unexpected death or suicide of the deceased (8,9), lack of social support, or fewer preloss coping resources (lower selfperceived coping efficacy; lower religiosity) (10,11), excessive dependence on the deceased (12), and pessimistic temperament (13).

The death of a loved one can be one of the most stressful events a person must endure (14). Under conditions of stress, the hypothalamic-pituitary-adrenocortical (HPA) axis is stimulated, and it activates the secretion of cortisol into the bloodstream. Acute psychosocial

ACTH = adrenocorticotropic hormone, AUC = area under the curve, BMI = body mass index, CES-D = Centre for Epidemiologic Studies of Depression, CG = complicated grief, HPA = hypothalamic-pituitary-adrenocortical axis, ICG = inventory of complicated grief, MMSE = mini mental score, RS = Rotterdam Study, SD = standard deviation.

stress is typically accompanied by increased secretion of cortisol, as an adaptation to the stressor, and then decreases to normal levels; but it is the chronic dysregulation of cortisol that is implicated in a host of psychological and physical health conditions (15). A recent meta-analysis found that compared to nonstressed controls, chronically stressed persons more often had a dysregulated pattern of cortisol secretion. This pattern was characterized by lower morning secretion and higher secretion across the rest of the day, yielding a flattened diurnal pattern (16). In depressed persons, higher levels of cortisol secretion have been reported widely, although in chronic depression and in community-dwelling depressed persons, low levels of morning cortisol have also been observed (17). Likewise, low cortisol levels are reported in studies of chronic fatigue syndrome (18–23) and in studies of post-traumatic stress disorder and after traumatic events (24–26).

Previous studies of grief and cortisol showed that the loss of a loved one is associated with more dysregulated cortisol patterns and an increased mortality risk of the bereaved person (14,16). Richardson et al. studied bereaved spouses and the effect of a prolonged forewarning of the death (i.e., knowing at least 1 month before the death that the person is going to die in the coming months). The group of bereaved persons who reported a forewarning of death showed higher cortisol levels at 6 month than those bereaved who did not experience prolonged forewarning (27).

Few studies have investigated the relationship between cortisol and complicated grief. One study compared 12 women with complicated grief to 12 women with normal grief, showing a flatter slope across the day in those with complicated grief (28). Another study compared 56 depressed adults, divided into three groups of nonbereaved, bereaved without signs of complicated grief, and bereaved with complicated grief symptoms. Interestingly, the depressed bereaved persons had lower levels of log-cortisol at wake and flatter diurnal slopes compared with the depressed nonbereaved independent of complicated grief symptoms (29). Against this background and given the phenotypical correlation of complicated grief and chronic depression and post-traumatic stress disorder (PTSD), both of which have been related to lower cortisol values, we examined the association between complicated grief and cortisol measures.

We focused on persons with duration of complicated grief up to 2 and 5 years after the loss (30). The aim of our study was to examine the association of the morning

cortisol and summary cortisol measures with grief and complicated grief. We tested two hypotheses. First, persons with complicated grief symptoms have a lower diurnal cortisol secretion than those without grief or with normal grief. Second, persons with normal grief have a similar cortisol secretion pattern as those without grief.

METHODS

Study Participants

This study was set in the Rotterdam Study, a large prospective population-based cohort designed to examine the occurrence and risk factors of chronic diseases. The study has been described in detail elsewhere (31). In 1990, all residents in a district of Rotterdam who were aged 55 years and older were invited to participate. Every 4 years, participants undergo an extensive home interview and physical examination at a research center. The Medical Ethics Committee of the Erasmus University of Rotterdam approved the study, and informed consent was obtained from all participants. The current study is based on the fourth examination of the original cohort members (n = 7983). The examination was performed in 2002–2004 with 3550 participants (74% response rate), and it assessed complicated grief and salivary cortisol levels.

For the present study, we excluded participants with incomplete complicated grief inventory (n = 187), those using corticosteroids (n = 78), without salivary cortisol measures (n = 840), and persons with complicated grief less than 6 months after loss (n = 18) or grieving more than 2 years (n = 313). This resulted in 2084 elderly persons aged older than 55 years (M [SD] age, 64.9 [5.5] years; 55% women) for the analyses of 2 years after loss. Another 130 grieving persons were additionally included in the analyses of all persons that experienced grief in the past 5 years.

Assessment of Complicated Grief

All participants were asked if they were currently grieving (3).If the answer was positive, we asked whom they were grieving over (spouse, partner, child, parent, sibling, other family member, close friend, other, several people, including or excluding spouse) and the time elapsed since the death. The participants, who answered the first question affirmatively, were assessed for complicated grief with the Dutch version of the Inventory of Complicated Grief (ICG).

In the present study, we used two different cut-offs to limit the time since loss. The primary analyses used a 2-year cut-off since bereavement and included all participants with grief (≤2 years) and

complicated grief (≥6 months to 2 years). The second and contrasting analyses used a 5-year cut-off since bereavement, and included all participants with grief and complicated grief up to 5 years after bereavement. However, at least 6-month duration of symptoms was required in accordance with recommendations for diagnostic criteria and to exclude acute stress reaction (32). The choice of the 2-year postloss cut-off was made to study the association with more recent complicated grief in line with a previous study (3) and the cut-off has also been used in previous analyses of the present cohort (33).

However, we also present analyses including all persons (N = 292) with grief or complicated grief up to 5 years after loss. Complicated grief was diagnosed with the Dutch version of the ICG. Questions represent symptoms of complicated grief based on the most recent proposed criteria. The ICG is considered the criterion standard for measurement of complicated grief in older adults because it has high internal consistency, good convergent and criterion validity (34).

The inventory represents a single underlying construct of complicated grief. As described before (3), 17 questions were asked, and responses were provided on a five-point scale to reflect an increase in severity (0, never; 1, seldom; 2, sometimes; 3, often; and 4, always). In the current setting, one item, "I feel bitter over this person's death," was removed from the original inventory, as a pilot study revealed that this sentiment had a very similar meaning within the Dutch language as the included item: "I feel anger over this person's death." Two further items (relating to seeing and hearing the deceased) were combined into one due to their similarity, and a pilot study indicating these symptoms were low in frequency and too often overlapped ("I hear the voice of, or see, the person who died").

A summary score for the ICG was calculated by totaling each individual item score (responses from 0, never, to 4, always) across the 17 items providing a potential score range of 0 to 68. Participants with a score of less than 22 were considered as participants with grief symptoms in line with previous studies (3).

We defined complicated grief based on the severity of symptoms and did not define severity by duration of symptoms. Participants with a score of less than 22 were considered participants with grief symptoms in line with previous studies (3). Participants with a score of 22 or greater and with symptoms lasting after

for at least 6 months were considered to have complicated grief. This cut-off was based on the cut-off in the original version of the ICG (original cut-off of 25 from 19 items). We classified participants into three groups; no grief (control group), persons with normal grief (experiencing non-complicated grief as shown by an ICG score <22) and those with complicated grief (ICG score ≥22). The non-grieving control group included persons who had experienced bereavement in the past but were not grieving at the time of interview. Likewise, persons mourning over someone with severe disease, but who were still alive or a pet, were included in the control group.

Salivary Cortisol Protocol

Saliva samples were collected on awakening (T1), 30 minutes after awakening (T2), at 5:00 PM (T3), and at bedtime (T4). Salivary cortisol concentrations were measured using a commercial immunoassay with chemiluminescence detection (IBL Hamburg, Hamburg, Germany). Intra-assay and inter-assay coefficients of variation were less than 6% and 9%, respectively. The lower limit of detection was 0.4 nmol/L. Data were screened for quality of cortisol measurements. For each time point, cortisol values that were above the 98th percentile in the original cortisol data set were excluded from the final data set to normalize the distribution of cortisol measurements and to exclude misclassification due to possible measurement errors. After this exclusion, cortisol levels followed a normal distribution (35). The individual measures of cortisol were combined in summary measures to provide valid information about the diurnal pattern of cortisol. We calculated the area under the curve with respect to the ground (AUCg) and the slope. The AUCg summarizes overall diurnal cortisol exposure, and was calculated as the local AUC from the individual cortisol measures on the Y-axis and the time between cortisol measures on the X-axis. It takes into account both sensitivity (the difference between the single measurements from each other) and intensity (the distance of these measures from ground) (36,37). In order not to include the effect of the morning rise as part of the AUC, we did not include T2 in the AUC calculation. Diurnal decline was assessed by a slope, which was calculated by fitting a linear regression line for each participant, which predicted the cortisol values from time since awakening. A greater decline in the daytime cortisol means that the slope of the regression line is steeper, whereas a lesser decline means that the slope is flatter.

Assessment of Other Variables

Age, sex, education level, smoking status, body mass index, cognitive status, major

depression, depressive symptoms, and anxiety symptoms were evaluated as potential confounders based on previous publication (38–41). The time between subsequent cortisol measurements within the same day was used as a covariate in the analyses of the AUC and the slope. Information was collected in home interviews and physical examination at baseline (42).

Level of education was assessed during home interview and was classified into those with primary education only, intermediate education, and high education (university studies). Participants were asked about their smoking status (never, current, and former). Current smokers were asked how many cigarettes they smoked daily and how long they had been smoking. Former smokers were asked about their smoking history (39). Height and weight were measured in participants without shoes and heavy clothes, and body mass index was calculated as kilogram per square meter (kg/m2) and used as a continuous variable (43). Cognitive status was evaluated with the Mini Mental Status Examination (44) and used as a continuous variable. Depressive symptoms were assessed with a validated Dutch version of the Centre for Epidemiologic Studies Depression (CES-D) scale (range, 0–60) to obtain a continuous measure of depressive symptoms (45,46). All participants with clinically relevant depressive symptoms (i.e., those with a scores of 16 or greater on the Centre for Epidemiologic Studies Depression), were interviewed using the Present State Examination, a semistructured psychiatric interview included in the Schedules for Clinical Assessment in Neuropsychiatry. All interviews were conducted by two experienced clinicians. Major depression was classified according to the DSM-IV (47).

Anxiety disorders were diagnosed with an adapted version of the Munich version of the Composite International Diagnostic Interview to assess the following anxiety disorders: generalized anxiety disorder, panic disorder with or without history of agoraphobia, agoraphobia, social phobia, and specific phobia. Obsessive-compulsive disorder and PTSD, which are part of the anxiety disorders in the DSM-IV, were not assessed. The Composite International Diagnostic Interview was specifically designed to obtain DSM-IV diagnoses of mental disorders (48).

Assessment of Exclusion Criteria

Participants using corticosteroid were excluded. Pharmacy records were used to collect data on systemic corticosteroid use (39).

Statistical Analysis

Information on demographic characteristics was compared among the groups using a $\chi 2$ test for categorical data and an analysis of variance for continuous variables.

We investigated the association of complicated grief with cortisol, the single (T1 morning cortisol) and the summary measures (AUC and slope). The primary analyses included as cases only persons who grieved after a loss up to 2 years ago, as this cut-off was used previously (30).

The second analysis included persons who were grieving after bereavement 5 or less years ago. We compared cortisol measures of the reference population to participants with normal grief and to those with complicated grief using analysis of covariance.

The analyses were adjusted for age and sex, level of education, smoking status, body mass index, Mini Mental State Examination score, current depressive symptoms, and time between cortisol measures (for TI, T2, T3, and T4). Covariates were imputed using the expectation-maximization algorithm. All covariates had less than 3% missing values. Missing values were imputed based on the entire baseline population (n = 3550).

Although the ICG is designed as a screening measure and not to assess severity of grief, we performed continuous analyses in persons grieving using the scores on the ICG to test whether there is a dose-response relationship between the grief symptoms score and cortisol levels independently of the predefined cut-off.

RESULTS

The study population comprised 1922 persons with no grief, 131 with normal grief, and 31 with complicated grief of maximal 2 years duration. In the sensitivity of persons with up to 5 years after loss, 210 persons with normal grief, and 82 persons with complicated grief were included.

Table 1 presents the characteristics of the study population. The sample was composed of 55% women, and the M (SD) age of the participants was 64.9 (5.5) years. Participants with complicated grief were more often women, and they were older than persons without grief.

The main cause of grieving in those with complicated grief was death of the partner (61%), and in those with normal grief, it was death of a partner (29%) or a sibling (22%). Participants with complicated grief had more clinical relevant depressive symptoms (18.4%), major depression

(13.2%), and anxiety symptoms (18.4%) than persons without grief or those with normal grief.

Table 2 shows the cortisol saliva measures (morning cortisol, AUC, and slope) of the participants with no grief, normal grievers, and complicated grievers who experienced the loss in the past 2 years.

Participants with complicated grief had lower levels of morning cortisol than normal grievers (11.26 vs 15.51 nmol/L; difference, −4.24; 95% CI = −7.87 to −0.62; p = .02). Persons with complicated grief also had lower overall diurnal cortisol levels (AUCg) (6.89 vs 8.98 nmol/L; difference, −2.09; 95% CI = −3.81 to −0.37; p = .017). The difference in the slope of cortisol observed between both groups did not reach significance. Consistently, participants with complicated grief were found to have lower levels of morning cortisol than nongrievers (11.26 vs 14.71; difference, −3.46; 95% CI = −6.78 to −0.13; p = .042). Neither the difference in overall cortisol levels nor the difference in cortisol slope reached statistical significance, although the effect estimates suggest a meaningful difference.

The table does not show the formal contrast between those with grief and without grief in the table; none of the group means between those with normal grief and those without grief reached significance (data not shown). However, the table clearly shows that the means of all cortisol measures are very similar in these two groups.

Table 1. Baseline Characteristics of the Study Sample, N = 2084

Characteristic	No Grief n = 1922	Grief n = 131	Complicated Grief n = 31
Sex, women	1056 (55)	89 (68)	21 (68)
Continuous variables, M (SD)			
Age, M (SD), years,	63.5 (5.6)	65.3 (6.3)	65.9 (4.7)
CES-D score, M (SD)	5.1 (6.1)	7.6 (6.6)	13.8 (9.8)
BMI, M (SD), kg/m2	27.4 (3.9)	27.2 (4.2)	26.1 (2.4)
MMSE score, M (SD)	27.5 (2.2)	27.4 (2.5)	27.6 (1.9)
Education			
Primary	515 (27)	37 (28)	10 (32)
Intermediate	1380 (72)	90 (69)	21 (68)
High school	27 (1.4)	4 (3.1)	0 (0)
Smoking status			
Never smoked	603 (31)	49 (37)	14 (45)
Current smoker	234 (12)	12 (9)	3 (10)
Former smoker	1085 (57)	70 (53)	14 (45)
Anxiety symptoms	144 (7.5)	12 (9)	6 (19)
Who died?			
Partner	—	38 (29)	19 (61)
Child	—	7 (5)	1 (3)
Parent	—	4 (3)	0 (0)
Brother/sister	—	29 (22)	4 (13)
Others (another family member, good friend, several)	—	52 (40)	7 (23)

SD,standard deviation; CES-D, Center of Epidemiological Studies-Depression scale; BMI, body mass index; MMSE,Mini Mental State Examination.

Data are presented as n (%) unless otherwise indicated.

Participants with grief (ICG < 22) from 0 month to 2 years after loss and participants with complicated grief (ICG ≥ 22) from 6 months to 2 years after loss were included. Participants using corticoids were excluded. Chi-square test and analysis of variance were performed.

Table 2. Cortisol Saliva Summary Measures in Persons Up to 2 years After Loss

Outcome Salivary Cortisol Measure 2 years After Loss	No Grief Reference Category N = 1922 Estimated mean	Grief N = 131 Estimated mean	Complicated Grief N=31 Estimated mean	Comparison Complicated Grief - No Grief Difference With Reference (95% CI)	P	Comparison Complicated Grief - Grief Difference With Reference (95% CI)	P
Morning cortisol, nmol/L	14.72	15.51	11.26	−3.46 (−6.78, −0.13)	.042	−4.25 (−7.87, −0.62)	.22
Area under the curve, nmol/L	8.31	8.98	6.89	−1.42 (−2.99, 0.16)	.078	−2.01 (−3.81, −0.37)	.17
Slope, nmol/L per hour	−0.83	−0.83	−0.64	0.19 (−0.04, 0.06)	.113	0.19 (−0.06, 0.44)	.134

The table presents estimated means, which are adjusted values. The analyses included persons who grieved after a loss up to 2 years ago. Analysis of covariance was performed, and adjusted values are presented. Participants using corticosteroid were excluded. All analyses were adjusted for age, sex, level of education, smoking, body mass index, CES-D score, MDD, anxiety symptoms, and Mini Mental State Examination score. P values are Bonferroni corrected accounting for the three groups.

CES-D, Center of Epidemiological Studies—Depression scale; MDD, major depressive disorder.

Table 3 presents the results of our sensitivity analyses. The groups now include all persons that experienced grief or complicated grief up to 5 years ago (those experiencing grief 2 to 5 years were added in these analyses). No differences between the participants with grief, complicated grief, and those without any grief in the cortisol morning levels or the two sum measures were observed anymore, whatever the reference and comparison group. Consequently, we tested if the cortisol measures of persons with complicated grief differed depending on time since bereavement. To this aim, we defined exclusive groups of complicated grievers less than 2 years and between 2 and 5 years since bereavement. Both the AUCg (CG 2 years, 6.84 nmol/L; CG >2–5 years, 9.23 nmol/L; difference, −2.393; CI = −4.21 to −0.57; F-statistic, 3.33; p = .01) and the morning cortisol levels (CG 2 years, 11.85 nmol/L; CG >2–5 years, 16.44 nmol/L; difference, −4.58; CI = −8.46 to −0.70; F-statistic, 2.69; p = .021) differed in persons with complicated grief depending on the time since bereavement.

The continuous analyses in persons with grief experienced in the past 5 years showed that higher scores on the ICG were associated with lower morning cortisol (B = −0.270; 95% CI = 11.94 to −0.72; p = .027).

DISCUSSION

In this population-based study, we studied whether diurnal cortisol secretion of persons with complicated grief or persons with normal grief differed from those not experiencing grief in the past 2 years. As hypothesized, the present study demonstrated that participants with complicated grief 2 years after loss showed lower level of morning cortisol and lower overall diurnal cortisol exposure than the participants with grief or without grief. In contrast, persons with grief showed similar cortisol secretion patterns as those without grief.

Cortisol is frequently referred to as the "stress hormone." Stressful life circumstances stimulate hypothalamic-pituitary-adrenocortical (HPA) axis, activating a hormonal response system that results in increased blood levels of cortisol (45). However, hypocortisolism has been observed in patients, who developed PTSD (21,46,47) and has also been reported in patients with bodily disorders, such as burnout with physical complaints, chronic fatigue syndrome, fibromyalgia, and chronic pelvic pain (16,17,20,22,48,49). Similar findings have been reported for healthy individuals living under conditions of chronic stress, chronic depression, as well as for patients with rheumatoid arthritis and asthma

(13,14,49). Our observations in persons with complicated grief extend these latter findings. Whereas a previous study of complicated grief and diurnal cortisol showed that those with complicated grief have a flatter slope across the day (25), morning cortisol or AUC has not been examined in persons with complicated grief.

Several mechanisms may be involved in the development of hypocortisolism. First, reduced biosynthesis of cortisol as part of an adaptive down-regulation has been postulated in persons experiencing severe trauma (48,50). Second, increased sensitivity of the HPA axis for negative feedback (51,52) and corticotropin-releasing factor hyper secretion from the hypothalamus are discussed, as these can result in reduced adrenocorticotropic hormone and lower cortisol levels (49).

Third, a very different but not necessarily exclusive mechanism is suggested by prior work from our group. We have previously shown that persons with complicated grief are characterized by more brain atrophy than persons with grief. Neuronal loss may disrupt the microstructural integrity of the fascicles connecting pre-frontal cortex with the subcortical areas (amygdala and hip-pocampus) (30,53), which are involved in the inhibitory regulation of the HPA axis (52,54,55). Morphological brain changes have also been described in patients with PTSD, such as a reduced volume of the hippocampus, which is predominantly involved in the inhibitory regulation of the HPA axis (52,54,55).

Our data, however, suggest also that any such mechanisms behind hypocortisolism may sometimes be reversible. In the present study, no effect of grief or complicated grief on cortisol secretion patterns was observed, if persons with longer periods since the loss were included. This suggests that with longer follow-up time, the association between grief and cortisol may attenuate. We can only carefully speculate about coping in this interval. Participants with more than 2 years after loss may have adapted to the loss and the resulting changes, instead of avoiding the loss. Several studies proposed that complicated grief reactions only persist when people engage in avoidance behaviors trying to impede habituation to painful memories and interfering with the integration of the loss (56–58).

In our study, we found no differences in diurnal secretion cortisol and persons with normal grief and the controls. These findings were not unexpected if one views grief as a normal life event, unrelated to preexisting vulnerabilities. Effects on the HPA axis may be visible after several months or even years only if a person develops complicated grief

Table 3. Cortisol Saliva Summary Measures in Persons Up to 5 years After Loss

Outcome Salivary Cortisol Measure 2 years After Loss	No Grief Reference Category N = 1922 Estimated mean	Grief N = 210 Estimated mean	Complicated Grief N=82 Estimated mean	Comparison Complicated Grief - No Grief Difference With Reference (95% CI)	P	Comparison Complicated Grief - Grief Difference With Reference (95% CI)	P
Morning cortisol, nmol/L	14.72	15.18	14.44	−0.26 (−2.34, 1.81)	.802	−0.74 (−3.09, 1.60)	.535
Area under the curve, nmol/L	8.31	8.57	8.39	0.07 (−0.91, 1.05)	.884	−0.18 (−1.29, 0.95)	.743
Slope, nmol/L per hour	−0.83	−0.83	0.04	0.04 (−0.10, 0.18)	.596	0.19 (−0.12, 0.20)	.636

The table presents estimated means, which are adjusted values. The analyses included persons who grieved after a loss up to 2 years ago. Analysis of covariance was performed and adjusted values are presented. Participants using corticosteroid were excluded. All analyses were adjusted for age, sex, level of education, smoking, body mass index, CES-D score, MDD, anxiety symptoms and Mini Mental State Examination score. P-values are Bonferroni corrected accounting for the three groups.

CES-D, Center of Epidemiological Studies-Depression scale; MDD, major depressive disorder.

or has risk factors for complicated grief such as a dependent relationship with the dead person, unexpected dead, lack of social support, and the loss of someone who was ambivalently loved (30). Our results suggest that persons with normal grief may have a healthier coping style. Alternatively, they experience less of those grief-related feelings and behaviors, which tend to dominate severe bereavement and may affect the HPA axis (59).

Strengths of our study include the sample size, the population-based setting, and the multiple daily cortisol measurements. A large control group of nongrieving persons was provided the reference for participants with normal grief or complicated grief. In addition, we controlled for clinically relevant depressive symptoms and major depression.

Some limitations of the current study should also be mentioned. First, it is not possible to evaluate if these associations were causal due to the cross-sectional design of the study. In particular, we do not have cortisol assessments before grief, nor in the next examination round. Thus, we cannot rule out that existing alterations in HPA axis activity made some persons more susceptible to develop complicated grief. Second, not all the participants with ICG participated in the saliva sampling. Third, selection effects may have led to the inclusion of more grievers with healthy coping style in those persons whose loss events occurred long ago. This would have diluted the effects. Fourth, it is not possible to account for complicated grief with PTSD because in the Rotterdam Study, this was not assessed. Fifth, we do not have longitudinal data to study the factors that might be involved on the reversibility of the cortisol secretion. In addition, we cannot rule out that existing alterations in HPA axis activity made some persons more susceptible to develop complicated grief.

The importance of our study is that cortisol, which is involved in cognitive functions such as memory performance and executive function and regulates the inflammatory responses, is altered in persons experiencing complicated grief (12,48,49,60). This implies that persons with complicated grief may be more vulnerable to develop cognitive problems (30), depression, and medical conditions than persons with normal grief. Follow-up studies are needed, however, to demonstrate the clinical consequences of our observations.

Source of Funding and Conflicts of Interest: The Rotterdam Study is supported by the Erasmus Medical Centre and the Erasmus University Rotterdam, The Netherlands Organization for Scientific

Research (NWO), The Netherlands Organization for Health Research and Development (ZonMw); the Netherlands Genomics Initiative; and the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports, and the European Commission (DGXII). HSP was supported by an ERACOL grant. HT was supported by the VIDI grant of ZonMw (2009–017.106.370). The funders had no role in the study design or data collection and analysis. The remaining authors report no conflicts of interest.

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CHAPTER 6

Silent brain infarcts: A cause of depression in the elderly?

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Psychiatry Res. 2013 Feb 28;211(2):180-2. doi: 10.1016/j.pscychresns.2012.06.005. Epub 2012 Nov 13.

Abstract

The present study included 1047 elderly participants. At baseline, brain magnetic resonance imaging (MRI) was performed to detect infarcts and white matter lesions; further, depressive disorders were assessed. Participants were followed up during 3.6 years to determine incident and recurrent depression. We found an increased risk of recurrent depression associated with silent brain infarcts.

1. Introduction

A relationship between cerebrovascular disease and depression in the elderly has been established (Baldwin and O'Brien, 2002). However, it remains unclear to what extent depression is a direct consequence of vascular damage or a psychological reaction to physical disability caused by stroke.

Studies investigating the relation between cerebral white matter lesions (WMLs) and depression provided evidence that subclinical cerebrovascular disease is associated with depression (Grool et al., 2011), although not consistently (Ikram, 2010). Also, silent brain infarcts (SBIs) detected by magnetic resonance imaging (MRI) in the absence of clinical stroke are frequent in depressed patients (Vermeer, 2007).

The association between SBIs and depression in the elderly has not been studied longitudinally; only the prognosis of prevalent depression in patients with silent brain infarct has been examined (Yamashita et al., 2010). Furthermore, previous studies were based on small, clinical samples. Previous research on the relationship between SBI and the poor prognosis of patients with depression or lack of response to antidepressant treatment prompted us to investigate the possible relationship between SBI and recurrent depression (Yamashita et al., 2010, 2001).

Therefore, we aimed to assess whether SBIs and WMLs in community-dwelling elderly increase the risk of incident and recurrent depression.

2. Methods

2.1. Setting

The current study is embedded in the Rotterdam Scan Study in the elderly, a large population-based neuroimaging study. We randomly selected participants from two large ongoing population-based studies, the Zoetermeer Study and the Rotterdam Study (Vermeer et al., 2003). After 2000, only the Rotterdam Study subcohort was followed. Together with non-participation and death of respondents, this substantial reduction in participants, precluded longer follow-up analysis. The study was approved by the medical ethics committee of Erasmus Medical Center and participants gave written informed consent.

2.2. Participants

The study sample consisted of 1077 non-demented elderly persons aged over 60 years (mean age 70 years, 52% women), who were screened for depressive symptoms

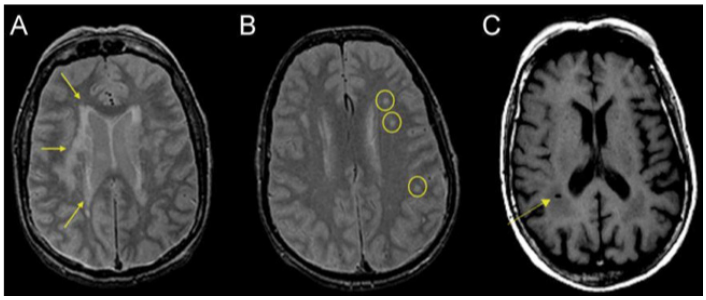

Fig. 1. Silent brain infarct and white matter lesions in participants of the Rotterdam Study at baseline. Vascular brain changes on 1.5 T MRI are shown. Arrow indicates the abnormality in the image. (A) Periventricular white matter lesions on T2 weighted sequence. (B) Subcortical white matter lesions on T2 weighted sequence. (C) Lacunar infarct on T1 weighted sequence.

and underwent structural MRI to assess cerebral changes at baseline in 1995–1996. Participants were continuously monitored by reviewing medical records. Follow-up was complete until March, 2000.

Participants with no information on depression (n =16), those who screened positive but without psychiatric diagnostic interview (n =6), and those diagnosed as having other psychiatric disorders during follow-up (n =8) were excluded.

2.3. Brain infarcts and white matter lesions

Axial T1-weighted, T2-weighted, and proton density scans were performed on 1.5 T MRI scanners. Infarcts were defined as focal hyperintensities on T2-weighted images, Z3 mm (Fig. 1). A physician scored infarcts, their location and size at baseline. We obtained a history of stroke and transient ischemic attack (TIA) by self-report and medical records. Subsequently, an experienced neurologist categorized the MRI-defined infarcts as silent or symptomatic (Vermeer et al., 2007).

We defined SBIs as evidence of one or more infarcts on MRI, without a history of a corresponding stroke or TIA.

WMLs were considered if visible as hyperintense on proton-density and T2-weighted images, without prominent hypointensity on T1-weighted scans. Two raters scored periventricular and subcortical WMLs independently. Severity of periventricular WMLs was rated semi-quantitatively in three regions (grade range 0–9) (de Leeuw et al., 2001).

2.4. Assessment of depressive disorders

At baseline, we assessed depressive

symptoms with a validated Dutch version of the Centre for Epidemiologic Studies Depression (CES-D) scale and by checking indications of prescribed drugs in all the participants. Prevalent depressive disorders were defined as depressive symptoms (CES-D scores >=16) or the use of antidepressant medication at baseline.

Information on recurrent and incident depressive disorders during follow-up was established by re-examining 787 participants with the CES-D scale between 1999 and 2000. Screen positives were assessed by a psychiatrist with a Dutch version of the Schedules for Clinical Assessment in Neuropsychiatry (SCAN), to ascertain a Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (DSM-IV) depression diagnosis. In addition, we continuously monitored the general practitioner's medical records and the Regional Institute for Ambulatory Mental Health records for depressive episodes. Medicals records allow the identification of depressed participants during the interval between assessments. Incident depressive disorders were defined as depressive disorders during follow-up without depression at baseline. Recurrent depressive disorders were defined as a depressive disorder during follow-up in persons with prevalent depression at baseline. A history of depression was defined as a depressive episode before the baseline examination lasting more than 2 weeks.

2.5. Covariates

Age, sex, education and cognitive function assessed by Mini-Mental State Examination (MMSE) score were used as possible confounders.

2.6. Data analysis

We tested whether the presence of any brain infarcts and the severity of WMLs were associated with incident or recurrent depression with logistic regression. Analyses were adjusted for age, sex, education, and baseline MMSE score.

Separate analyses were run to test the association of symptomatic brain infarcts, SBIs, periventricular and subcortical WMLs and depression. Severe periventricular and subcortical WMLs were defined as >= 5 points for periven-tricular WMLs and >= 2 ml for subcortical WMLs.

Table 1. Brain infarcts and white matter lesions and the risk of incident depression or recurrent depression.[a]

Brain lesions	Incident depressive disorders (60/961)		Recurrent depressive disorders (32/86)	
	OR*	95% CI**	OR	95% CI
All infarcts	1.1	(0.6–2.9)	2.1	(0.8–5.2)
Asymptomatic infarcts	1.0	(0.5–1.8)	2.9	(1.0–8.2)
Symptomatic infarcts	1.1	(0.7–1.7)	0.8	(0.1–5.9)
White matter lesions	1.1	(1.0–1.2)	1.1	(0.8–1.4)
Severe periventricular	1.3	(0.6–2.6)	1.0	(0.8–1.2)
Severe subcortical	2.1	(1.1–3.9)	0.9	(0.3–2.7)

34. All analyses were adjusted for age, sex, education and baseline Mini-Mental State Examination (MMSE) score.
[n] OR=odds ratio.
[nn] CI=confidence interval.
Additionally, in sub-analyses participants with any history of depression were excluded (n=249). We also repeated the analyses after exclusion of participants who developed dementia during follow-up (n=34).

3. Results

At baseline, of the 961 participants without depressive symptoms, 36 persons had symptomatic brain infarcts and 182 had SBIs. Of the 86 participants with depressive symptoms, seven had symptomatic brain infarcts and 25 had SBIs.

During a mean follow-up of 3.6 years, 60 (6.2%) participants without depressive symptoms and 32 (37%) participants with depressive symptoms at baseline were diagnosed with depressive disorders.

Symptomatic brain infarcts and SBIs were not associated with incident depressive disorders in longitudinal analyses. However the presence of SBIs at baseline almost tripled the risk of recurrent depressive disorders (Table 1).

Severe subcortical WMLs doubled the risk of incident depressive disorders whereas severe periventricular WMLs were not related to incident depression. WMLs were not associated with recurrent depression (Table 1).

Exclusion of participants with history of depression and exclusion of participants who developed dementia during follow-up did not change the results (data not shown).

4. Discussion

We found that older adults with SBIs had an almost three-fold risk of recurrent depression. In contrast SBIs were not associated with incident depression.

So far only an association between SBIs and the prognosis of clinical depression has been studied (Vermeer, 2007; Yamashita et al., 2001), showing a poor long-term prognosis and a decreased remission rate in hospitalized patients with SBIs. Our findings are in line with these studies and suggest that SBIs cause depression immediately and lastingly but are not associated with a higher risk of incident depressive episodes during follow-up.

There are several possible explanations for the association between SBIs and recurrent depression in older adults. First, depressive disorders may be a direct consequence of ischemic brain damage. It is known that damage to small vessels supplying subcortical pathways disrupts the neurotransmitter circuitry that is involved in mood regulation (Zgaljardic et al., 2003). Also, the location and nature of these lesions may affect individuals differently. When the accumulation of the infarcts exceeds a certain threshold, people could become more vulnerable to depression. Second, it has been suggested that depression may contribute to the evolution of vascular risk factors, which in turn increase the risk of cardiovascular disease. Following this reasoning, the underlying vulnerability to depression precipitates the future episode independent of the vascular burden.

Our findings showing the association between severity of subcortical WMLs, which are generally seen as indicating ischemia damage, and incident depression are in accordance with most other studies.

The observed findings remained after exclusion of participants, who developed dementia during follow-up (n=34), suggesting that associations of SBI and WML with depression are not due to dementia. Moreover, these associations were independent of a history of depression, suggesting that vascular brain damage precedes the depressive disorder, rather than being a consequence of depression.

A potential methodological limitation of our study is the possibility of misclassification. Participants tend to

underreport depressive symptoms, and physicians probably underdiagnose depressive disorders, which may have resulted in an under-estimation of the true numbers of events. However, in addition to information from medical records, we actively screened participants for the presence of depressive symptoms.

Strengths of this study are its large number of elderly people and its prospective population-based design.

In summary, our data show that among elderly persons the presence of SBIs is associated with recurrent depressive disorders.

Acknowledgments

Dr. Saavedra was supported by the ERACOL Grant. Dr. Tiemeier was supported by a VIDI grant of the National Medical Research Council of The Netherlands (NWO/ZonMW 2009-017.106.370).

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CHAPTER 7

Markers of cerebral small vessel disease and severity of depression in the general population

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Psychiatry Res Neuroimaging. 2016 Jul 30;253:1-6.
doi: 10.1016/j.pscychresns.2016.05.002. Epub 2016 May 7.

Abstract

The vascular depression hypothesis postulates that cerebral small vessel disease can cause or exacerbate depression in elderly persons. Numerous studies explored the association of imaging markers of cerebral small vessel disease including white matter lesions (WMLs) and lacunar infarcts with depressive symptoms or disorders. However, cerebral microbleeds have not been tested in depression. In the current study, we aimed to explore the association of WMLs, lacunar infarcts and cerebral microbleeds with depression continuum in a large population-based sample, the Rotterdam Study. Study population consisted of 3799 participants (aged 45 or over) free of dementia. WML volumes, lacunar infarcts and cerebral microbleeds were measured with brain magnetic resonance imaging. Depressive symptoms, depressive disorders and co-morbid anxiety disorders were assessed with validated questionnaires and clinical interview. WML volumes and lacunar infarcts were associated with depressive symptoms and disorders. Cerebral microbleeds, especially in deep or infratentorial brain regions, were related to depressive disorders only. Our results indicate that WMLs and lacunar infarcts might be non-specific vascular lesions seen in depressive symptoms and disorders. Association of cerebral microbleeds with more severe forms of depression may indicate impaired brain iron homeostasis or minor episodes of cerebrovascular extraversion, which may play a role in depression etiology.

1. Introduction

The co-occurrence of cerebrovascular diseases and depression led researchers to propose the vascular depression hypothesis in late-onset depression. Since the hypothesis was first described by Alexopoulos et al. (1997) and Krishnan et al. (1997), research on the etiology of vascular depression has been advanced along two conceptually different lines. Some researchers have focused on the localization of the vascular lesions to explain the etiology of the vascular depression (Sneed and Culang-Reinlieb, 2011). Others have explored the cognitive deficits due to the vascular cerebral lesions predisposing to depression. In the last decade, this approach has led researchers to define "the depression-executive dysfunction syndrome" in which symptoms of executive dysfunctions such as difficulty with planning, organizing, abstracting are seen as part of clinical depression (Sneed and Culang-Reinlieb, 2011; Taylor et al., 2013b). Despite the supporting evidence, it is still debatable whether the vascular depression exists as a clinical entity. Extracerebral vascular diseases seem to be less consistently associated with depression in elderly than to cerebral vascular diseases (Almeida, 2008; Almeida et al., 2007). While vascular risk factors, lesions and diseases are very common in elderly, the prevalence of depression does not increase in parallel. If the vascular depression hypothesis was of public health importance (i. e. vascular factors strongly predispose, precipitate, and perpetuate depression), the prevalence of depression in elderly would be expected to rise more strongly with age (Almeida, 2008).

Early studies have tested the link between depression and clinically overt vascular events. In these patients, the effects of the functional deficits on depression as a result of the vascular event are difficult to control for.

In a recent report of the Rotterdam Study, myocardial infarction was related to depression in men, only when recognized that supporting the importance of psychosocial effects of an overt disease (Jovanova et al., 2016).

Nowadays, a narrower definition of vascular depression hypothesis is used in which the vascular component is considered as clinical or non-clinical cerebrovascular events. Non-clinical vascular events are consisted of imaging findings of cerebral small vessel disease. Such findings occur as a result of hypertension, arteriolosclerosis, inflammation or amyloid deposition in small arteries, arterioles or venules of the brain (Pantoni, 2010). These imaging

findings are common in the elderly people. The most commonly explored imaging findings of cerebral small vessel disease are white matter lesions (WMLs) and lacunar infarcts. For decades, WMLs have been explored in different severity degrees of depression. Cross-sectional and longitudinal association of WMLs with depressive symptoms, major depressive disorder (MDD), poor treatment response, and occurrence and recurrence of MDD (Arnone et al., 2012; Baldwin, 2005; de Groot et al., 2000; Godin et al., 2008; O'Brien et al., 1998; Olesen et al., 2010; Saavedra Perez et al., 2013; Taylor et al., 2013a) and comorbid anxiety disorders have been demonstrated (Fiedorowicz et al., 2011). Similarly, lacunar infarcts has been related to depressive symptoms (Grool et al., 2013; Wu et al., 2014), MDD, and recurrence of MDD (Saa-vedra Perez et al., 2013).

Cerebral microbleeds are now recognized as an imaging phenotype of the cerebral small vessel disease. (de Jong et al., 2002; Pantoni, 2010; Poels et al., 2012; Vernooij et al., 2008c). Cerebral microbleeds are perivascular collections of hemosiderin induced by prior tiny hemorrhage. Similar to the WMLs and lacunar infarcts, cerebral microbleeds are detected commonly in elderly (Poels et al., 2010). In general, there are two types of cerebral microbleeds on the basis of location: deep or infratentorial microbleeds and lobar microbleeds. Deep or infratentorial microbleeds were generally related to vascular risk factors whereas lobar microbleeds were associated with cerebral amyloid angiopathy (Vernooij et al., 2008b). Cerebral microbleeds and their locations have not yet been extensively tested in depressive disorders. Studies assessing the relation between cerebral microbleeds and depressive disorders are mostly limited to the stroke cases in clinical settings (Tang et al., 2014a, 2011a, 2011b, 2014b). In a recent longitudinal study, it was shown that cerebral microbleeds were not related to incident depressive symptoms (van Sloten et al., 2015). However, the relation of cerebral microbleeds with different severity degrees of depression including depressive symptoms, depressive disorders and comorbid conditions of depressive disorders have not been tested in general population.

The link between cerebral small vessel disease and depressive symptoms has been tested in large population-based studies. However, the association between cerebral small vessel disease and depressive disorders was assessed mostly in small clinical studies.

In the current study, we aimed to test the associations of several imaging

phenotypes of cerebral small vessel disease with different severity degrees of depression including depressive symptoms, depressive disorders and depressive disorders with comorbid anxiety disorders in general population. We hypothesized that WMLs, lacunar infarcts, and cerebral microbleeds are all related to the depression continuum.

2. Methods

2.1. Study sample

This study was embedded in the Rotterdam Study, a prospective population-based cohort of middle-aged and elderly persons (Hofman et al., 2015). From 2005 to 2008, a random sample within the Rotterdam Study was formed for the regular research center visits. They were invited for brain magnetic resonance imaging (MRI).

In total 3855 participants were involved. Of these, 44 (1.1%) had no depression assessment and 10 (0.3%) persons with dementia were excluded. This left 3799 people in the study sample.

Of the 3799 persons in one or more analysis, 3741 had a valid WML measurement, 3742 had data on microbleeds and 3701 participants had data on lacunar infarcts.

The Rotterdam Study has been approved by the institutional review board of the Erasmus University Medical Center and by the review board of The Netherlands Ministry of Health, Welfare and Sports. All participants provided written informed consent after complete description of the Rotterdam Study.

2.2. Assessment of depressive symptoms and anxiety disorders

We diagnosed depressive disorders with a two-step procedure. First, we tested all participants for depressive symptoms using the Center for Epidemiological Studies-Depression (CES-D) scale during the home interview at study entry. A cut-off of 16 was used to define "clinically significant depressive symptoms". This cut-off score has a very high sensitivity for major depression in older adults in the Netherlands (Beekman et al., 1997; Radloff, 1977).

In the second step, we invited the participants with a CES-D score of 16 or greater to a semistructured interview, the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) (Wing et al., 1990). Clinicians were conducted the interviews in close proximity in time to screening. Clinical depressive disorders included DSM-IV-TR–defined major depressive disorder and dysthymia. Thus,

both clinically significant depressive symptoms and DSM-IV depressive disorders were assessed in this study.

To determine participants with depressive disorders and comorbid anxiety disorders, we used a slightly adapted version of the Munich version of the Composite International Diagnostic Interview (M-CIDI). The interview was performed by trained interviewers (Wittchen et al., 1998). All DSM-IV anxiety disorders including generalized anxiety disorders, panic disorder with or without agoraphobia, social phobia and specific phobia were assessed except obsessive-compulsive disorder and posttraumatic stress disorder (rare and difficult to diagnose reliably in the general population). We grouped anxiety disorders into a category of "any anxiety disorder".

2.3. Brain MRI

Brain MRI was performed on a 1.5-T scanner (GE Healthcare, Milwaukee, WI) with an 8-channel head coil including T1-weighted, proton-density weighted, fluid-attenuated inversion recovery, and T2*-weighted gradient echo sequences (Ikram et al., 2011).

Post-processing steps have been described elsewhere and include a conventional k-nearest-neighbor brain tissue classifier extended with WML segmentation (Ikram et al., 2011). Using this classifier, we obtained quantitative measures of WML volume and intracranial volume (in mL). (Vernooij et al., 2007).

We defined lacunar infarcts as focal hyperintensities that are >=3 mm on FLAIR and T2-weighted images. An experienced physician scored all infarcts. All scans were reviewed by 1 of 5 well-trained raters who were blinded to the clinical data. Intra-observer (n=500, 1 rater) and inter-observer (n=300) reliabilities were κ=0.87 and κ=0.85, which indicates very good agreement. In line with previous studies, we defined categories of microbleeds restricted to a lobar location (strictly lobar microbleeds) and microbleeds in a deep or infratentorial location (Poels et al., 2011; Vernooij et al., 2008a).

2.4. Covariate assessment

Age, sex, education, smoking status, hypertension, diabetes mellitus, body mass index, total and HDL cholesterol concentrations and cognitive function were used as potential covariates on the basis of prior literature (Luijendijk et al., 2008). Education was grouped into eight categories in an ordinary scale from primary education (1) to

university level (8) on the basis of the Standard Classification of Education. For the analyses, we further categorized it into three categories; low, intermediate, and high. Smoking status was coded in categories as never, former and current smokers. Systolic and diastolic blood pressures were calculated as the average of two consecutive measurements.

Hypertension was defined as a systolic blood pressure >=140 mm Hg or a diastolic blood pressure >= 90 mm Hg or the use of antihypertensive medication according to pharmacy records. Data on diabetes mellitus was collected on the basis of the general practitioners' reports and the assessment at the research center. Participants were considered as having prevalent diabetes when they had at least one of the following four criteria: plasma glucose concentration >= 7.0 mmol/L, random plasma glucose concentration >= 11.1 mmol/L, antidiabetic medication, and/or diabetes treatment by diet. Height and body weight were measured. Body mass index was calculated as weight in kilograms divided by height in meters squared. Cholesterol levels were evaluated with an automated enzymatic procedure.

Cognitive function was assessed with the Mini-Mental State Examination (MMSE) (Folstein et al., 1975).

2.5. Statistical analyses

We log-transformed the WML volumes to achieve a normal distribution. WML volumes were used as continuous measures and lacunar infarcts and cerebral microbleeds were used as binary variables.

To evaluate the cross-sectional associations of the imaging phenotypes of cerebral small vessel disease with depression continuum, we performed a series of analyses. First, we modeled depressive symptoms score continuously (the CES-D score) and tested the associations between the cerebral small vessel disease and depressive symptoms score with linear regression analyses. Second, we used a predefined cut-off of 16 for the CES-D to detect participants with clinically significant depressive symptoms and tested the associations of cerebral small vessel disease with clinically significant depressive symptoms using logistic regression analyses.

Third, we examined the associations of the cerebral small vessel disease with the DSM-IV depressive disorders (major depressive disorder and dysthymia) with multinomial logistic regression. In these latter analyses, participants below the CES-D score of 16 formed the reference category. All analyses were adjusted for

age and gender. Analyses were further adjusted for education, smoking status, hypertension, diabetes mellitus, total and HDL cholesterol concentrations and MMSE score except for lacunar infarcts because of small sample size. Analyses of cerebral microbleeds were additionally adjusted for WML volume and intracranial volume. Analyses of WML volume were further adjusted for intracranial volume.

Next, we tested whether any associations of cerebral microbleeds with depression depended on the localization of microbleeds using strictly lobar and infratentorial microbleeds categories.

In the sensitivity analyses, we explored if the associations of cerebral small vessel disease with MDD differed between persons with a comorbid anxiety disorder and those with MDD only; we used the group of persons without depressive symptoms as the reference group for the multinomial logistic regression analyses testing this difference. These sensitivity analyses were adjusted for age and gender.

Analysis of WML volume was adjusted for intracranial volume and analysis of cerebral microbleeds was adjusted for intracranial volume and WML volume.

We imputed the missing values on covariates using expectation maximization algorithm. Missing values on covariates were minimal (maximum, 3.1%). The SPSS software (version 20; SPSS, Chicago, Illinois) was used for statistical analyses.

3. Results

Characteristics of the study population (n=3799) are presented in Table 1. Mean age was 58.7 years (standard deviation [SD] =07.8) and 2070 (54.5%) participants (n=2070) were women. In total 322 (8.5%) participants had clinically significant depressive symptoms, 60 (1.6%) participants had prevalent depressive disorders according to the DSM-IV. Of the 60 participants with major depressive disorder, 31 (51.7%) had a comorbid anxiety disorder.

Table 1. Characteristics of the study population.

Characteristics	Study sample N=3799
Age, years, mean (SD)	58.7 (7.8)
Women, n (%)	2070 (54.5)
Education, n (%)	
Low	438 (11.5)
Intermediate	2411 (63.5)
High	950 (25)
Smoking status, n (%)	
Current smoker	1429 (37.6)
Former smoker	1152 (30.3)
Never smoked	1218 (32.1)
Diabetes mellitus (yes), n (%)	304 (8)
Hypertension, n (%)	1834 (48.3)
Lipid lowering medication, n (%)	831 (21.9)
Total serum cholesterol, mmol/l, mean (SD)	5.6 (1)
Serum HDL cholesterol, mmol/l, mean (SD)	1.4 (0.4)
Body mass index (kg/m2), mean (SD)	27.5 (4.2)
Mini mental state examination score, mean (SD)	28.1 (1.7)
Depressive symptom score, mean (SD)	5.3 (7)
Clinically significant depressive symptoms, n (%)	322 (8.5)
Depressive disordersa, n (%)	60 (1.6)
Cerebral microbleeds (yes), n (%)	538 (14.4)
Strictly lobar microbleeds, n (%)	371 (9.9)
Deep or infratentorial microbleeds, n (%)	167 (4.5)
White matter lesion volume, ml, mean (SD)	4.3 (7)
Intracranial volume, ml, mean (SD)	1125.7 (123.4)
Lacunar infarcts (yes), n (%)	184 (4.8)
Cortical infarct (yes), n (%)	98 (2.6)

[a] Depressive disorders category includes persons with major depressive disorder or dysthymia.

A larger WML volume was positively related with the severity of depressive symptoms as indicated by the higher CES-D scores in the fully adjusted model (regression coefficient per ml increase in WML volume=0.44; 95% Confidence Interval [CI] =00.14; 0.75; P =0.005). Presence of lacunar infarcts was related with high CES-D scores (regression coefficient for yes versus no=1.07; 95% CI=0.04–2.11, P =0.04). There was no association of the presence of cerebral microbleed (regression coefficient for yes versus No=0.17; 95% CI= 0.48 0.81; P =0.61) with the severity of depressive symptoms.

Second, we assessed the associations of the cerebrovascular determinants with clinically significant depressive symptoms (CES-D Z16). Table 2 shows that, consistent with the continuous analyses, WML volume was positively associated with clinically significant depressive symptoms. Neither lacunar infarcts nor cerebral microbleeds were related with clinically significant depressive symptoms.

Next, we evaluated the association of the imaging phenotypes of cerebral small vessel disease with DSM-IV depressive disorders.

An increase in WML volume was associated with an increased likelihood of DSM-IV depressive disorders (Odds Ratio [OR] per ml=1.40; 95% CI=1.20–1.62; P<0.001). Presence of microbleeds increased the likelihood of having DSM-IV depressive disorders 40% in the fully adjusted model (OR=1.40; 95% CI=1.01–1.94; P=0.04). Presence of lacunar infarcts was related to depressive disorders (OR=1.78; 95% CI=1.07–2.95, P =0.03). In the follow-up analyses, we categorized cerebral microbleeds as deep/infratentorial cerebral microbleeds (n=167, 4.5%) or strictly lobar cerebral microbleeds (n=371, 9.9%). We found a borderline association of deep/infratentorial cerebral microbleeds with depressive disorders (OR=1.41; 95% CI=1.0–1.99, P =0.05). There was no association between strictly lobar cerebral microbleeds and depressive disorders even though the effect size was similar with the results of the deep/infratentorial cerebral microbleeds and depressive disorders (OR=1.47; P=0.72).

3.1. Sensitivity analyses

We examined whether markers of cerebral small vessel disease were related to depressive disorders with comorbid anxiety disorders. Presence of cerebral microbleeds was related to increased likelihood of depressive disorders with (OR=2.15; 95% CI=1.65–2.81, P<0.001). WML volume and presence of lacunar

infarcts were not related to depressive disorders with comorbid anxiety.

Discussion

In this population-based study, the imaging markers of cerebral small vessel disease were differently related to depressive symptoms and disorders. WML volumes were consistently associated to depressive symptoms and MDD. Cerebral microbleeds were related to depressive disorders only, in particular to depressive disorders with comorbid anxiety. In the last two decades, WMLs have been studied in persons with depression. Investigators repeatedly demonstrated the association of WMLs with clinical and non-clinical presentations of depression. This suggests that WMLs are relatively non-specific vascular pathologies seen in people with depressive symptoms or clinical depression and even in other common psychiatric disorders including anxiety and dementia (Arnone et al., 2012; Costanza et al., 2012; de Groot et al., 2000; Liao et al., 2014; Menzies et al., 2008; Saavedra Perez et al., 2013; Taylor et al., 2013a; Teodorczuk et al., 2007, 2010). Also, lacunar infarcts were related to depressive symptoms and depressive disorders in the current study. Previous studies mostly explored the association of lacunar infarcts with depressive symptoms. In contrast, studies of DSM-IV depressive disorders are limited. Silent lacunar infarcts, especially when located in the basal ganglia, were related to depressive symptoms in a small clinical study (Wu et al., 2014). A recent population-based study showed that subcortical infarcts predict incident depressive symptoms (van Sloten et al., 2015). Another study of patients with atherosclerosis demonstrated that lacunar infarcts in deep white matter were related with an increase of depressive symptom severity and a more fluctuating course of depressive symptoms during follow-up (Grool et al., 2013). In the Rotterdam Study, we previously reported that silent brain infarcts predict the recurrence of clinical depression (Saavedra Perez et al., 2013).

The underlying mechanism for the relation of WMLs and lacunar infarcts with depression might be the detrimental local effects of cerebral small vessel disease in the frontostriatal and limbic regions. These local damages disrupt the neurotransmitter circuitry involving in mood regulation (Alexopoulos, 2002; Baldwin and O'Brien, 2002). This is supported by observation in the current and previous studies that the subcortical WMLs and lacunar infarcts are consistently related to depression (de Groot et al., 2000; Herrmann et al., 2008; Saavedra Perez et al., 2013).

Cerebral microbleeds have not been tested in relation to different severity degrees of depression in a non-clinical sample. Previously, Tang and colleagues (Tang et al., 2011a, 2011b) explored cerebral microbleeds in patients with stroke and found that the presence of cerebral microbleeds was related to post-stroke depression. In a longitudinal study of elderly people in general population; van Sloten et al. detected no association between cerebral microbleeds and incident depressive symptoms (vanSloten et al., 2015). In our population-based study, we observed an association of cerebral microbleeds with depressive disorders in participants free of stroke. Also, there was a relation between cerebral microbleeds and depressive disorder with comorbid anxiety, which indicates a more severe form of depression.

This effect was not found when we test the association of cerebral micro bleeds with depressive symptoms although continuous analyses generally have more power to detect the associations. This suggests that cerebral microbleeds may be a specific vascular pathology only seen in the most severe form of depression.

Localization of cerebral microbleeds is of importance because they do not only signal an impact on different anatomical regions but this may also reflect different underlying pathological processes of the vascular pathology. Lobar microbleeds are generally related to cerebral amyloid angiopathy whereas deep infratentorial cerebral microbleeds are associated with hypertensive microangiopathy (Vernooij et al., 2008b). We found that deep/infratentorial microbleeds, which are related to cardiovascular risk factors, were associated to depressive disorders. In contrast, lobar microbleeds indicating an amyloid angiopathy were not associated with depressive disorders even though the effect size was similar with the analyses of deep/infratentorial microbleeds. Overall, our findings support a vascular etiology of depression rather than an involvement of the amyloid pathway in the pathology of depression.

These explanations discussed above imply that the direction of mechanisms is from cerebral small vessel disease to depression (Direk et al., 2012; Herrmann et al., 2008; Saavedra Perez et al., 2013; Teodorczuk et al., 2010). However, it has also been suggested that there is a bidirectional association between small cerebral vascular disease and depression. The mechanisms by which depression may contribute developing cerebral small vessel disease increase are not completely understood, but biological (atrophy in different brain regions, the hypothalamic-pituitary-adrenal axis

dysfunction, inflammation) and lifestyle changes (poor health, smoking, less exercise) during depressive episodes are thought to be risk factors to cerebral small vessel disease (Gothe et al., 2012; Poels et al., 2010; Schmidt et al., 2013; Wardlaw et al., 2013).

The current study has several strengths. First, the study was based on general population, enhancing the external validity of the findings. Second, we evaluated both depressive symptoms and depressive disorders. Depressive disorders were diagnosed with a clinical psychiatric interview in this population-based study. Additionally, depressive disorder with comorbid anxiety disorder that is considered a more severe form of depression was tested.

Evaluating anxiety disorders is not common in population-based studies. Third, large study sample in the analyses of depressive symptoms allowed us to test several covariates. Finally, we were able to evaluate different imaging markers of cerebral small vessel disease, which allowed us to explore different aspects and mechanisms of cerebral small vessel disease such as ischemia and hemorrhage in the same sample.

There are also some limitations that should be considered when interpreting the results. Firstly, this study was not longitudinal. We discussed above that the direction of the temporality is unclear. In the further studies, cerebral microbleeds needs to be tested longitudinally to interpret if these associations are causal. Secondly, number of participants with depressive and anxiety disorders was low, yet we had enough power to detect the association of cerebral small vessel disease and depressive disorders.

In conclusion, our study supports vascular depression hypothesis. Three imaging markers of cerebral small vessel disease in later life were consistently associated to depressive symptoms and depressive disorders. Our study suggests that deep infratentorial microbleeds can index impaired brain iron homeostasis or minor episodes of cerebrovascular extraversion in persons with depressive disorders but more studies are needed to provide evidence on the exact mechanisms and the nature of cerebral small vessel disease in depression.

Table 2. Associations between the indicators of cerebral small vessel disease and depressive symptoms and clinical depression.

	Clinically significant depressive symptoms[a] N¼322				DSM-IV depressive disorders[b] N¼60		
	N	OR	95% CI	P	OR	95% CI	P
White matter lesion volume, ml	3741						
Age and gender adjusted		1.28	1.10–1.48	0.001	1.37	1.18–1.59	<0.001
Fully adjusted[c]		1.25	1.08–1.46	0.004	1.40	1.20–1.62	<0.001
Cerebral microbleeds	3742						
Age and gender adjusted		1.14	0.82–1.58	0.44	1.45	1.02–2.06	0.04
Fully adjusted[c]		1.09	0.78–1.53	0.61	1.40	1.01–1.94	0.04
Lacunar infarcts[d]	3701						
Age and gender adjusted		1.22	0.71 2.08	0.47	1.78	1.07–2.95	0.03

- Clinically significant depressive symptoms category consisted of participants with a CES-D score Z16. Participants without depressive symptoms were used as a reference group.
- Depressive disorders category consisted of participants with a DSM-IV major depressive disorder or dysthymia. Participants without depressive symptoms were used as a reference group (n=3477).
- Analyses of WML volume were adjusted for age, sex, education, smoking status, hypertension, diabetes mellitus, BMI, MMSE score and ICV. Analyses of cerebral microbleeds were adjusted for age, sex, education, smoking status, hypertension, diabetes mellitus, BMI, MMSE score and ICV and WML volume.
- Because of the small sample size, age and gender-adjusted model was performed only.

Authorship contribution

ND analyzed the data. ND and HT drafted the manuscript. HT, SA, BFJV, HSP, AH, WJGH, MWV and MAI provided critical revisions of the manuscript. All authors approved the final version for publication. ND had full access to all of the data and takes the responsibility for the integrity of the data and the accuracy of the data analysis.

The funding support

The Rotterdam Study is supported by Erasmus Medical Center and Erasmus University Rotterdam, the Netherlands Organization for Scientific Research (NWO), the Netherlands Organization for Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Netherlands Genomics Initiative, the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam.

Dr. Direk was supported by the Netherlands Consortium for Healthy Ageing. Dr. Tiemeier was supported by the VIDI grant of ZonMw (2009–017.106.370). The funders had no role in the study design or data collection and analysis.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Acknowledgements

We would like to thank everyone involved in the data collection process of the Rotterdam Study and the study participants for their time and effort.

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CHAPTER 8

Discussion

SUMMARY

In this thesis, I investigated the associations between prolonged grief disorder (PGD) and different domains of cognition and with brain volumes assessed by MRI in a middle-aged and elderly population. I also compared cognitive decline, morning cortisol and summary cortisol measures, and sleep quality as well as sleep duration between persons with normal grief, persons with PGD, and persons from a non-grieving reference group. Finally, I aimed to test the association of non-clinical cerebral small vessel disease with depression longitudinally in general population.

In this chapter, I will review the main findings of the studies. Next, I will address some methodological considerations, clinical implications, and make suggestions for future research.

MAIN FINDINGS

Prolonged grief and cognition

Worldwide there are more than 60 million deaths each year (1), and an average of four survivors per death (2). Bereavement and the experience of grief are considered one of the most stressful life's events (3). Despite this, most bereaved adults will cope with the loss after the initial weeks and months following the loss (4); however, about 9-20% of the persons cannot cope with the death of a loved one and will present a chronical condition with suffering pain and disruption for years after the loss (5,6).

This condition were first called complicated grief (CG) by experts, and later prolonged grief disorder (PGD). The association of grief with cognitive deficits has been studied for many years (7-9). Only a few years ago, the relation of PGD with cognitive functioning attracted the interest of scientists, but up to this day the cognitive impact of PGD on a person's health is not well-known.

In the Rotterdam Study, the most common cause of grief and PGD was the death of a partner. Grieving participants were more likely to be female, were older, had more depressive symptoms, poorer cognitive functioning, and were more

likely to have a history of depression, stroke and diabetes.

In chapter 2, I explored the cross-sectional association of PGD with cognitive performance, and tested whether there are differences in brain volumetric measures between persons with and without symptoms of prolonged grief. In a population of 5501 non-demented persons, I found an association between PGD and poor cognitive performance. If compared to non-grievers and normal grievers, persons with PGD had lower scores on Letter-Digit Substitution test and Word Fluency Task.

In addition, I found that persons with PGD were more likely to have a smaller total brain volume. It is important to mention that in this study I excluded participants with major depression and controlled the analyses for depressive symptoms, thus ensuring that depression, which is related both to cognitive performance and brain characteristics did not confound the results.

These results, for the first time, provide evidence for an underlying neurobiological mechanism for the relation between PGD and cognitive functioning. The Word Fluency Task is considered to be related to the intact function of the frontal cortices and the medial temporal areas (13,14), and the LDST is sensitive to more global changes in brain function (15); performances in both tests were affected in persistently grieving persons in our study. Participants with PGD were also characterized by more brain atrophy, whereas the white matter lesion volumes, which reflect vascular brain damage, did not differ between the groups. These results could suggest that differences in structural brain volumes could be a consequence or a precipitating factor of PGD. The observed atrophy may reflect a vulnerability to developing PGD, as previous studies showed atrophy is related to cognitive functioning and coping (13,14). The smaller total brain volume and the cognitive differences imply that neuronal loss may have disrupted the microstructural integrity of the fascicles connecting the prefrontal cortex with the cortical (frontal, temporal and occipital lobes) and the subcortical areas (amygdala and hippocampus), and even in the functioning of corticostriatal circuitry (16). Reduced cerebral connectivity could explain the prolonged resolution of grief in older adults as well as the poor performance in cognitive tests.

However, equally, the cognitive impairment may be a consequence of PGD. In 2012, O' Connor showed that the regional brain activation to grief cues frequently includes the dorsal anterior cingulate cortex and

the insula, as well as the posterior cingulate cortex (17). The anterior cingulate cortex, specifically the anterior cingulate gyrus, is part of an executive attention network, its main role is to regulate the processing of information from other networks, both sensory and emotional modalities (17-22). The regional neural activation of the dorsal anterior cingulate gyrus, insula, and posterior cingulate cortex in persons with PGD could be the cause of a persistent deregulation in the network processing grief.

In chapter 3, I compared cognitive decline between persons with normal grief or PGD, and a non-grieving reference population in a 7-year follow-up study. As in my first study, the main cause for grieving in participants with PGD was the death of a partner. Although previous cross-sectional studies of bereavement and cognition demonstrated poor cognitive performance in persons with grief and PGD (7-9,12, 23); none of these studies examined cognitive decline on PGD over time. The participants with PGD showed a stronger cognitive decline (decrease in global cognitive function, MMSE scores, and World learning test (immediate and delayed)) over time than the reference group. The delayed recall test has a high sensitivity and specificity to differentiate early stages of cognitive impairment from cognitive performance in normal elderly, and is more accurate than the MMSE (24-25). In particular, total immediate recall and delayed recall were found to be robust predictors of mild cognitive impairment before converting to Alzheimer's disease within 3 years of follow-up (26). However, I found no evidence that persons with normal grief, thus after the exclusion of persons with PGD from this group, show evidence of faster cognitive decline. These findings were in line with our hypothesis, that grief, if unrelated to pre-existing vulnerabilities, is a normal life event from the persons typically recover.

In chapter 4, I determined the association of PGD with sleep duration and sleep quality. Studies have shown that bereavement was associated with lower sleep quality (27-30). In this study, PGD was associated cross-sectionally with shorter sleep duration and lower sleep quality, in accordance with previous studies (31,32), but no association was found longitudinally. The sleep disturbances were mainly explained by depressive symptoms. The measure of depressive symptoms is not specific, thus the sleep disturbances might also be explained by the difficulty imagining and planning the future, a subjective sense of having a hopeless or foreshortened future (33-35), the intrusive memories about the deceased (36,37) or rumination (i.e., a style of perseverative thought on negative

emotions and the meaning of those emotions) (38-40).

In chapter 5, I examined the association of morning cortisol and summary cortisol measures with prevalent grief and prolonged grief hat occurred in a period of two years. I found that participants with PGD had lower levels of morning cortisol and lower overall diurnal cortisol levels. In contrast, persons with normal grief showed similar cortisol secretion patterns as those without grief. Cortisol is frequently referred to as the "stress hormone", and is secreted when the hypothalamic–pituitary–adrenocortical axis HPA is stimulated (41). However, hypocortisolism has also been observed in patients, who developed post-traumatic stress disorder (PTSD) (42,43), or chronic diseases (44-46). Persons with PGD experience severe stress and perceive their loss like a major trauma. The results confirm my hypothesis based on prior studies that persons undergoing prolonged grief have an adaptive down-regulation of cortisol. I postulate that this is due to reduced biosynthesis leading to hypocortisolism (42,44). Another explanation would be the effects of chronic stress on neuronal loss. In chapter 2, I demonstrated that persons with PGD had lower brain volumes, and that chronic stress could reflect neurodegeneration. Neuronal loss may disrupt the microstructural integrity of the fascicles connecting prefrontal cortex with the subcortical areas (amygdala and hippocampus) (45,46), which are involved in the inhibitory regulation of the HPA axis (44,47,48). In addition, the smaller brain volumescould also affect struictures like the hippocampus or hypothalamus, which are involved in the inhibitory regulation of the HPA axis (49,59).

No association of grief or PGD with cortisol secretion patterns was observed, if persons with longer periods since the loss were included. This suggests that participants with more than two years post loss may have adapted to the loss and the resulting changes.

Several studies proposed that CG reactions only persist when people engage in avoidance behaviors trying to impede habituation to painful memories and interfering with the integration of the loss (51). It is important to highlight that cortisol is involved in cognitive functions such as memory performance and executive function (52,53). My results thus might imply that persons with PGD may be more vulnerable to develop cognitive impairment. In summary, the results in this chapter offer an intriguing explanations for earlier findings.

In chapter 6, I tested the association

of non-clinical cerebral small vessel disease with depression longitudinally in the general population. A relationship between cerebrovascular disease and depression in the elderly has been established two decades ago (54). Studies provided evidence that subclinical cerebrovascular disease is associated with depression (55-57). In accordance to these results, I found that older adults with silent brain infarcts had an almost three-fold risk of recurrent depression episode during a mean follow-up of 3.6 years. The study suggests that the associations of silent brain infarcts and white matter lesions with depression is not due to dementia; and is independent of a history of depression, suggesting that vascular brain damage precedes the depressive disorder, rather than being a consequence of depression. The damage to small vessels supplying subcortical pathways disrupts the neurotransmitter circuitry that is involved in mood regulation (58). Also, when the accumulation of the infarcts exceeds a certain threshold, people could become more vulnerable to depression.

In chapter 7, I explored crossectionally the association of WMLs, lacunar infarcts and cerebral microbleeds with different severity degrees of depression. White matter lesions volumes and lacunar infarcts were associated with depressive symptoms and major depressive disorders. Cerebral microbleeds, especially in deep or infratentorial brain regions, were related to depressive disorders in stroke-free participants. Deep/infratentorial cerebral microbleeds have been related to cerebrovascular risk factors and hypertension, whereas strictly lobar microbleeds are related to cerebral amyloid angiopathy. Alterations or defects in the autoregulation of the cerebral blood flow can cause vascular lesions many years before the clinical onset of a cerebrovascular disease. Deficits in cerebral perfusion may affect protein synthesis important for cognitive networks and synaptic plasticity (59,60). The results indicate that WMLs and lacunar infarcts might be non-specific vascular lesions seen in depressive symptoms and disorders; and are consistent with previous studies that showed an association between WML and depression (61-63).

Similarly, silent brain infarcts related with depression in late life has been demonstrated in previous studies (64-66).

Taken together, these results provide little evidence for specific effects of grief, prolonged grief disorder or depression on brain morphology. Rather, persons with signs of neurodegeneration are likely to be more susceptible for grief and depression. Furthermore, many of our

findings could be explained by external factors, in particular pre-existing disease. This is encouraging news for many elderly persons experiencing episodes of poor mental health, as this suggests that typically, these will not have lasting neurodegenerative effects as can be determined with current neuroimaging techniques.

Methodological considerations

Normal grief is experienced as a distressing event, usually with an initial state of shock followed by acute emotional or somatic discomfort and social withdrawal. But eventually, most mourning persons learn to accept the loss and reassume their previous levels of functioning, find a sense of meaning and purpose, enjoy social activities, feel hopeful about the future, and in general, function without significant impairment or acute distress. In contrast, persons with prolonged grief disorder remain severely distress for several months or years following the death (67).

At the beginnings of the 90s, the absence of a scale to assess symptoms of complicated grief (CG) made it difficult to investigate its' incidence, prevalence, risk factors and consequences. The most commonly used grief scales were too broad or over-inclusive with respect to CG. The assessment of CG was confounded by the inclusion of measures of general grief, depression, and anxiety. Scales such as the Texas Revised Inventory of Grief (TRIG) (68) included only benign symptoms of grief, leaving out symptoms of CG. The Grief Measurement Scale (GMS) (69) included symptoms associated with anxiety disorders and contained depressive items. At the same time, grief scales were underinclusive with respect to symptoms of CG. Scales such as the TRIG and the GMS omitted most of the more potentially threatening symptoms of prolonged grief disorder. Symptoms such as survivor guilt, bitterness over the death, jealousy of others who have not experienced a similar loss, distraction to the point of disruption in the performance of one's normal activities, and lack of trust in others because of the loss were not assessed in existing scales of grief (70).

All this led to the need to develop a tool that could accurately identify grief-related symptoms that could help to discriminate between uncomplicated and prolonged grievers.

In 1995, Prigerson et al (70) developed the Inventory of Complicated Grief (ICG). The instrument consists of 19 first-person statements concerning the immediate bereavement-related thoughts and behaviors of the client. There are five response options (0 = never; 1 = rarely;

2 = sometimes; 3 = often; 4 = always) with which they describe currently experienced each of the emotional, cognitive, and behavioral states described in the PGD. Asking respondents to report the frequency of an emotional or cognitive state is an effective means by which to assess the impact of events (71).

The evaluated symptoms on the ICG include symptoms of separation distress (intrusive thoughts or preoccupation for the deceased, yearning for the deceased, searching for the deceased or loneliness), and symptoms of traumatic distress (loss of identity, emptiness, avoidance of memories, anger, sensation that life has no meaning, numbness or detachment, feeling shocked or dazed, bitterness, disbelief about the death). The constellation of symptoms needed to persist a minimum of 6 months, since longitudinal studies indicate that symptoms have generally stabilized by this point (67).

The ICG is considered the 'gold standard' for measurement of CG in older adults because it has high internal consistency, with an alpha coefficient of .94, and good convergent and criterion validity. In addition, this scale has a well-validated clinical cut point. Persons who score over 25 are significantly more impaired in social, general, mental and physical health functioning and in bodily pain than those with ICG scores less than or equal to 25.
We asked 17 questions. One item from the original English inventory, 'I feel bitter over this person's death', was removed as a pilot study revealed that this sentiment had a very similar meaning within the Dutch language as the included item: 'I feel anger over this person's death'. Two further items (relating to seeing and hearing the deceased) were combined into one due to their low frequency of endorsement by largely the same persons.

A summary score for the ICG was calculated by totaling each individual item score across the 17 items providing a potential score range of 0 to 68. Participants with a score of less than 22 were consider as participants with grief symptoms. Participants with a score of 22 or greater and with symptoms reported for at least 6 months were considered to have CG, The non-grieving control group included persons who had experienced bereavement in the past but were not grieving at the time of interview. Likewise, persons grieving for a pet or a loved one with a severe disease were included in the control group. This cut-off was based on the cut-off in the original version of the ICG. The original cut-off was 25 from 19 items) (72).

Subsequently the term prolonged grief

disorder (PGD) is introduced. An additional short assessment instrument with 13 items (including two severity questions) has been introduced to establish PGD . Seven of the eleven items correspond to items in the ICG.
The terms CG and PGD were proposed to be included in the 5th edition of the DSM and 11th edition of the ICD. Based on proposals for PGD and CG, DSM-5 introduced criteria for persistent complex bereavement disorder (PCBD) representing a mixture of PGD and CG (73).

Prolonged grief disorder and depression
In our studies PGD often did not have a predictive value independent of depression. Several studies have been conducted to examine whether PGD symptoms are distinct from depression. The etiology, symptomatology, course, response to treatment, and adverse outcomes of PGD have repeatedly been shown to be different from major depressive disorder.

First, studies have shown that yearning loads highly on the grief factor, but not on depression or anxiety factors, whereas sadness loads highly only on a depression factor, and feeling nervous and worried loads highly only on an anxiety factor (70,74). One study with functional magnetic resonance imaging, demonstrated that both, normal grievers and persons with PGD, showed pain related neural activity in response to reminders of the deceased, but only persons with PGD showed reward-related neural activity in the nucleus accumbens, which was positively associated with self report yearning (75).

Second, studies of negative cognitions among bereaved persons found that being overwhelmed by the loss and an intense preoccupation related to the loss of the loved one - are a cognition specific to prolonged grief disorder, but not specific to depression. In contrast, persons with depression exhibit a generalized misery and a pessimistic rumination. Likewise, global guilt or a sense of personal worthlessness, common in depression, is not part of PGD, were guilt is specific only to the circumstances of the death event (76,77).

Third, attachment issues are associated with vulnerability to develop PGD. Studies show that feelings of emotional dependency on the dying patient are associated with symptoms of grief, but not with symptoms of depression in recently bereaved persons. Moreover, at least one risk factor, separation anxiety in childhood, uniquely predicts PGD but not major depressive disorder, generalized anxiety disorder, or posttraumatic stress

disorder, following bereavement later in life (77,78).

Fourth, three studies investigated the facets of negative self-processing in PGD, and revealed that PGD is characterized by self-devaluation and negative self-related cognitions about the future; showing a cognitive-affective processing profile that is distinct from major depression disorder (79).

Fifth, the course and response to treatment of persons with PGD differ from those with normal grief and depression. Tricyclic antidepressants alone and interpersonal psychotherapy have proven ineffective for the reduction of PGD symptoms, although it produces a therapeutic effect on major depressive symptoms in the bereaved. On the other hand, randomized, controlled trials of specific therapy designed for PGD have demonstrated a favorably response in patients if compared with interpersonal therapy or supportive counseling, and symptoms of prolonged grief showed faster reduction in the groupw receiving grief therapy (77,78).

Prolonged grief disorder and cognition

Although there has been substantial research on the effects of normal grief on cognitive functioning, only a few investigated the relation of prolonged grief disorder with cognitive deficits (9-12). Two of them studied the performance of persons with PGD on an emotional Stroop test, in which persons were presented with death-related and neutral cue words. Both studies showed a slower reaction time to grief related words in the PGD group (10,11). This pattern of findings suggests an attentional bias towards loss-related events.

Another study (12), examined global and domain-specific cognitive functioning in persons with PGD, assessed using the Montreal Cognitive Assessment (MoCA). They show that PGD had lower total MoCA, visuospatial and attention scores relative to control participants.

In our research, we performed several cognitive test (Mini Mental State Examination (MMSE), Letter-Digit Substitution test (LDST), Stroop test, Word fluency task, Word learning test), to evaluate cognitive performance and cognitive decline in our population data. In cross-sectional analyses we found that persons with PGD had lower scores on Letter-Digit Substitution test and Word Fluency Task. Given the cross-sectional nature of this study, we are unable to determine the causal direction between PGD and cognitive functioning. Having prolonged grief disorder may increase cognitive impairment in older adults

or having cognitive impairment may increase the likelihood of developing prolonged grief disorder after the death of the loved one.

Next, we evaluated the cognitive decline longitudinally, showing that participants with PGD had a stronger cognitive decline (decrease in global cognitive function, MMSE scores, and World learning test (immediate and delayed)) over time, compared with the reference group.

At the first glance, we - like others - assumed that a correlation between PGD and poor cognitive functioning implies that PGD is a causal factor for cognitive decline. Not only did we find evidence for an association between PGD and poor cognitive functioning; second, PGD apparently preceded the onset of cognitive decline, and third, we found it unlikely that this was spurious relationship. However, it is not that simple to be certain about the temporality of this relation, as is the case for the relation with many chronic diseases with a not clearly distinguishable clinical prodomic phase, like dementia.

We also found that participants with PGD have smaller total brain volumes, produced mostly by a reduction in white matter. Could our findings thus indicate the presence of a mild cognitive impairment (MCI) before the development of the prolonged grief disorder? Mild cognitive impairment (MCI) is regarded as a precursor to dementia, but not all patients with MCI will develop dementia. Could it be that MCI is a vulnerability factor to develop PGD in older adults? Is important to emphasize that not all individuals with MCI will convert to dementia: up to 40–70% of the patients with a MCI improve, stay stable at follow-up (80) or develop other age-related neuropsychiatric conditions.

In our cross-sectional study, the performance in the Mini Mental State examination (MMSE) was not affected. Some studies revealed that the MMSE is not sensitive to subtle neuropsychological impairment (81). Thus it is noteworthy that in our longitudinal study, we found a cognitive decline and a poor performance in MMSE, indicating that the progression of the cognitive disease is less subtle or this measure is sensitive to change.

I postulate that MCI, with its characteristically poor cognitive function could interfere with the coping flexibility abilities of the persons, leads to vulnerability to develop PGD. Studies suggest that deficits in coping flexibility are indicative of PGD (82).The ability to process a death, and the ability to remain optimistic and look beyond the loss,

are both thought to be effective means of coping with loss and other aversive events. Recently, these seemingly contrary dimensions have been integrated into a model of coping flexibility (82).

RECOMMENDATIONS

Clinical implications

In this thesis, I explored cognitive impairment, cognitive decline, cortisol levels and sleep quality of persons with prolonged grief disorder in a middle aged and older population. The findings invite some clinical reflections.

The death of a loved one is one of the most stressful events in a person's life, but most people learn to cope with the loss and reassume their lives. However, 9-20% of the persons will develop prolonged grief, and will remain severely distressed for years following the death (5,6).

I have found that prolonged grief disorder is cross-sectionally related to poor cognitive impairment and lower total brain volumes, and longitudinally to cognitive decline after 7 years of follow-up. The results suggest that prolonged grief disorder may, in some persons, be part of a mild cognitive impairment. These findings could provide further evidence to promote the close monitoring of patients who are in a prolonged grieving process and test these persons for possible cognitive deficits. Support techniques for prolonged grievers could include cognitive support and treatment or prevention of vascular risk factors, as these can slow the process of brain atrophy. They also must be monitored closely for development of Alzheimer's Disease. Similarly, patients with known cognitive deficits should be offered support for cognitive problems if confronted with the loss of a loved one to prevent prolonged grief disorder.

I also explored the association of prolonged grief disorder with the diurnal cortisol patterns in a large population-based study. I found that they showed low levels of morning cortisol and low overall diurnal cortisol levels. The results suggest that prolonged grief disorder has classic characteristics of a chronic stress reaction.

This finding suggests psychological and social support to persons who are in a prolonged grieving process may be helpful. It also suggests that group therapies focuses on relaxing therapies like mindfulness should be tested to diminish the stress associated with the

loss, and reduce the likelihood of develop cognitive problems.

The thesis also explored the vascular depression hypothesis. I found that early vascular changes such as non-clinical white matter lesions, silent brain infarcts and cerebral microbleeds increase the risk of incident depression and the recurrence of depression. These findings indicate that is very important to strengthen education about vascular health and the prevention programs of vascular diseases in the programs combatting depressive disorders in later-life.

Future directions for research

Future studies should include younger people who lose a loved person. I think that it is important to evaluate cognitive impairment, general health, cortisol levels, and perform brain imaging in early ages. Elsewise reverse causality cannot be ruled out, only this design gives us the possibility to follow them for 10 or 20 years to address the following issues: the extent to which prolonged grief disorder is predictive of cognitive problems, the extent to which prolonged grief disorder is predictive of long- term morbidity; and ultimately the development and study of therapies for its treatment. It would also be interesting to evaluate if prolonged grief disorder could be considered a marker of dementia.

Concluding remarks

Prolonged grief disorder is a pathology not well-known, with an enormous impact on the social, psychological and biological area of a person. Having prolonged grief disorder changes the life of a person enormously, leaving him or her stuck in time, without the desire to continue living and without goals or project for the future. Working on this thesis and having the opportunity to treat patients with prolonged grief disorder, has allowed me to have a broad vision on this pathological condition. I see the need for more studies to be carried out in order to understand its characteristics and its evolution. More epidemiological studies are needed to pave the way for strategies for early detection and novel interventions to help these people have "full" lives.

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CHAPTER 9

Summary

SUMMARY

Experiencing the death of a significant person is an event that most people will encounter multiple times in their lives, and is often perceived as a severely stressing experience. Grief is the most usual reaction to such a loss and thus considered a normal and natural transition. About 9-20% of the persons cannot deal with the loss however, and will show symptoms of complicated and unresolved grief, termed prolonged grief disorder (PGD) (***chapter 1***).

In ***chapter 2***, I explored the cross-sectional association of PGD with cognitive performance, and tested whether there are differences in brain volumetric measures between persons with and without symptoms of complicated grief. I found an association between PGD and poor cognitive performance. Persons with PGD had lower scores on a Letter-Digit Substitution test and a Word Fluency Task. In addition, I found that persons with PGD were more likely to have a smaller total brain volume.

In ***chapter 3***, I aimed to compare the cognitive decline between persons with normal grief or PGD, and a non-grieving reference population in a 7-year follow-up study. Persons with PGD showed a stronger cognitive decline (decrease in global cognitive function, performance on a dementia screener, and learning and recalling words) over time than the non-grieving persons.

Chapter 4 shows the association of PGD with sleep duration and sleep quality. In this study, PGD was associated cross-sectionally with shorter sleep duration and lower sleep quality, but no association was found longitudinally. The high level of prevalent sleep disturbances was mainly explained by depressive symptoms.

In ***chapter 5***, I examined the association of morning cortisol and summary cortisol measures with prevalent grief and recently occurring PGD (e.g., loss occurred in last two years). I found that persons with PGD had lower levels of morning cortisol and lower overall diurnal cortisol levels than those without grief. In contrast, persons with normal grief showed similar cortisol secretion patterns as those without grief. Also, no association of grief or PGD with

cortisol secretion patterns was observed, if persons with longer periods since the loss were included. This suggests that even persons with severe grief may adapt to the loss and the resulting physiological changes normalize after some years.

In ***chapter 6***, I tested the longitudinal association of non-clinical cerebral small vessel disease with depression in the general population. I found that older adults with so-called silent brain infarcts, infarcts that have not been clinically diagnosed, had an almost three-fold risk of recurrent depression episode during a mean follow-up of 3.6 years. This study suggests that the associations of silent brain infarcts and white matter lesions with depression is not due to dementia; and is independent of a history of depression, suggesting that vascular brain damage precedes the depressive disorder, rather than being a consequence of depression.

In ***chapter 7***, I explored cross-sectional the association of white matter lesions, lacunar infarcts and cerebral microbleeds with different severity degrees of depression. White matter lesions volumes and lacunar infarcts were associated with more depressive symptoms and major depressive disorder. Cerebral microbleeds, especially in deep or infratentorial brain regions, were related to depressive disorders in stroke-free participants.

In ***chapter 8***, I discuss the most important findings of the studies and summarize methodological issues and clinical implications.

SAMENVATTING

Bijna iedereen krijgt te maken met het overlijden van een dierbare, waarschijnlijk zelfs meer dan eens. Voor de meesten van ons is dit een emotionele ervaring die gepaard gaat met rouw, verdriet en stress. Rouw is een gebruikelijke reactie op het verlies van een dierbare en een natuurlijk transitie proces. Ongeveer 9 tot 20% ervaart echter problemen met de rouwverwerking, waarbij het verdriet complex en onverwerkt is. Dit wordt ook wel gecompliceerde rouw genoemd (***hoofdstuk 1***).

In ***hoofdstuk 2*** onderzoek ik de cross-sectionele associatie van gecompliceerde rouw met cognitief functioneren en ook of er verschillen in brein volume bestaan tussen mensen met en zonder symptomen van gecompliceerde rouw. Uit mijn onderzoek kwam een verband tussen gecompliceerde rouw en cognitief functioneren naar voren; mensen met gecompliceerde rouw behaalden lagere scores op een substitutie en woord vloeiendheid taak. Daarnaast vond ik dat in mensen met gecompliceerde rouw een kleiner brein volume hadden.

In ***hoofdstuk 3*** vergelijk ik de cognitieve achteruitgang tussen mensen met normale rouw, gecompliceerde rouw en zonder rouw. We volgden deze groep mensen voor 7 jaar en lieten zien dat mensen met gecompliceerde rouw een sterkere cognitieve achteruitgang dan mensen zonder rouw ervoeren over deze 7 jaar, dat wil zeggen een verminderd algeheel cognitief functioneren, verlaagde scores op een dementie screeningsinstrument en een verminderde prestatie bij het leren en ophalen van verbale informatie.

Hoofdstuk 4 demonstreert het verband tussen gecompliceerde rouw en slaap. In onze studie liet gecompliceerde rouw een cross-sectioneel verband met een kortere slaapduur en lagere slaap kwaliteit zien, maar dit verband werd niet longitudinaal gevonden. De veel gerapporteerde slaap problemen werden in plaats daarvan voornamelijk door symptomen van depressie veroorzaakt.

In ***hoofdstuk 5*** heb ik het verband tussen cortisol, gemeten in de ochtend en gemiddeld over de dag, en rouw

onderzocht. Mensen met recente gecompliceerder rouw, dat wil zeggen met een verlies gedurende de voorgaande twee jaar, hadden een lager cortisol niveau in de ochtend en ook een lager gemiddeld cortisol niveau over de gehele dag in vergelijking met mensen die niet in rouw waren. Daarentegen waren de cortisol niveaus van mensen met 'normale' rouw en mensen met gecompliceerde rouw waarbij het verlies meer dan twee jaar geleden was vergelijkbaar met de cortisol niveaus van mensen die niet in rouw waren. Dit suggereert dat ook mensen met langdurige gecompliceerde rouw uiteindelijk adapteren aan hun verlies en dat de bijbehorende fysiologische veranderingen na meerdere jaren normaliseren.

In ***hoofdstuk 6*** test ik de longitudinale associatie van aandoeningen in de kleine bloedvaten met depressieve episoden in de algemene bevolking. Ik vond dat ouderen met zogenaamde 'stille' herseninfarcten, dat wil zeggen herseninfarcten die niet klinisch gediagnosticeerd zijn, een bijna drie keer zo hoog risico hadden op een recidive depressieve episode over een gemiddelde periode van 3.6 jaar. Dit verhoogde risico in mensen met een 'stil' herseninfarct en witte stof laesies kon niet verklaard worden door dementie, en was onafhankelijk van de medische voorgeschiedenis met betrekking tot depressie. Dit lijkt er op te duiden dat vaatschade in de hersenen voorafgaat aan depressie, en niet een gevolg is van de depressie.

In ***hoofdstuk 7*** onderzoek ik het cross-sectionele verband van witte stof laesies, lacunaire infarcten en cerebrale microbloedingen met depressie. Een hoger volume witte stof laesies en meer lacunaire infarcten waren geassocieerd met meer depressieve symptomen en meer depressieve stoornis diagnoses. Het hebben van cerebrale microbloedingen, in het bijzonder diep in het brein en in de infratentoriale regio's van het brein, was eveneens gerelateerd aan meer depressieve stoornissen in deelnemers zonder een voorgeschiedenis van beroerte.

In ***hoofdstuk 8*** bespreek ik als laatste de meest belangrijke bevindingen van mijn werk en vat ik methodologische kwesties en klinische implicaties samen.

CHAPTER 10

PhD portfolio
List of publications
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Acknowledgements

PhD portfolio

Erasmus Summer Programme	**Grade**	**ECTS**
Principles of Research in Medicine (ESP01)	a/p	0.7
Clinical Decision Analysis (ESP04)	a/p	0.7
Methods of Public Health Research (ESP11)	a/p	0.7
Clinical Trials (ESP14)	a/p	0.7
Topics in Meta-analysis (ESP15)	a/p	0.7
Pharmaco- epidemiology (ESP21)	a/p	0.7
Health Economics (ESP25)	a/p	0.7
Cohort Studies (ESP39)	a/p	0.7
Case-control Studies (ESP40)	a/p	0.7
Principles of Genetic Epidemiology (ESP43)	a/p	0.7
Demography of Ageing (ESP59)	a/p	0.7
Markers and Prognostic Research (ESP62)	a/p	0.7
Core Curriculum		
Study Design(CC01)	6.0	4.3
Classical Methods for Data-analysis (CC02)	5.8	5.7
Clinical Epidemiology (CE02)	5.6	5.7
Methodologic Topics in Epidemiologic Research (EP02)	6.0	1.4
Modern Statistical Methods (EP03)	5.6	4.3
Advanced Short Courses		
Psychiatric Epidemiology (EP12)	a/p	1.1
Medical Demography (HS04)	a/p	1.1
Planning and Evaluation of Screening (HS05)	a/p	1.4
Maternal and Child Health (HS09)	a/p	0.9
Skills Courses		
English Lamguaje (SC01)	a/p	1.4
Introduction to medical Writing (SC02)	a/p	1.1
Working with SPSS for Windows (SC04)	exempt	0.15
Research		
Development Research Proposal (DRP)	a/p	2.5
Oral Research Presentation (PRES)	a/p	1.4
Research Period (RP)	GD	29.2

Silent brain infarcts and depressive disorder in elderly people. Décimas Jornadas de Salud Mental. October 24, 2013. Panamá.

Prevention of domestic violence. Seminario de prevención de violencia doméstica. November 25, 2013.

Case of domestic violence with ocular trauma. Docencias médicas de Policlínica Manuel Ferrer Valdes. May 20, 2015. Panamaá.

Mental health disorders in primary care attention. Docencias médicas de Policlínica Manuel Ferrer Valdés. September 22, 2015. Panamá.

Psicosomatic medicine. XII Jornada Nacional de Salud Mental. October 9, 2015. Panamá Complicated grief and cognitive disorders. IV Congreso Científico: Salud Mental en los Profesionales Sanitarios. June 22, 2016. Panamá.

Cognitive disorder in complicated grief. Jornada interior de Atención Primaria en Salud. October 21, 2016. Panamá.

Major problems of mental health in women. XXXIII Congreso de la Alianza Panamericana de Mújeres Médicas. III Congreso de la Asociación de Médicas de Panamá. May 19, 2017. Panamá.

Psychiatric diseases in womens. Instituto de la Mujer. Universidad de Panamá. March 8, 2018. Panamá.

List of publications

1. Cognition, structural brain changes and complicated grief. A population-based study.
2. Prolonged Grief and Cognitive Decline: A Prospective Population-Based Study in Middle-Aged and Older Persons.
3. The Longitudinal and Cross-Sectional Associations of Grief and Complicated Grief with Sleep Quality in Older Adults.
4. The Impact of complicated grief on diurnal cortisol levels two years after loss: A population-based study.
5. Silent brain infarcts: A cause of depression in the elderly?
6. Markers of cerebral small vessel disease and severity of depression in the general population.

About the Author

Heidi Saavedra Pérez was born on November first, 1975, in Panamá City, Panamá. She studied medicine at Panama University, School of Medicine. She obtained her medical degree in 2000 and joined the psychiatry residency program at the Panamá National Mental Health Institute in 2003. After her graduation as a specialist in Psychiatry, she started to work at the Social Security Fund in Panamá City. She pursued studies in psycho-oncology at the Marie Curie Hospital in Argentina, cognitive behavioral therapy at the Albert Ellis Institute, and higher education at the University of Panamá. She has also taken creative writing courses and participated in the publication of her short stories in anthologies. Her last short story was published in Basta, publication aimed at raising awareness about domestic violence.

In 2009, she has started to work at the Department of Epidemiology, Erasmus Medical Center where she has worked on the current thesis. She has worked with the Rotterdam Study under the supervision of Prof. Dr. Henning Tiemeier. During her time at the Erasmus Medical Center, she obtained her degree in Master of Science in Health Sciences (Clinical Epidemiology) in 2010.

Upon her return to Panamá, she has focused her efforts on the prevention of domestic violence by organizing therapy groups for women victims of domestic violence, and promoting education about the topic.

Acknowledgments

The day I made the decision to do a PhD I had no idea what I was getting into. Throughout these years, there were many learning's, not only academic, but also cultural and personal growth. I was happy to live in a country where women are nurtured and men are respectful, to the point of considering them timid in their dealings with women.

From the beginning I was told that the tutor assigned to me was very important, but being in a country where I did not know anyone, I found myself with few possibilities of being able to choose. I still remember the first day I spoke with Henning Tiemeier, after a class, on the ground floor of the Erasmus MC. For some reason I always remember him smiling, disheveled and with many things in his hand (briefcase, papers, folder, coffee). Without knowing, I was talking to the one who would be assigned to me as a tutor. I cannot compare Henning with others, because I have not had more tutors, however I can safely say that it is very unlikely that I could have played a better tutor. I must thank Henning for his joy for life, his enthusiasm, his infinite patience, his tact to tell us when something was wrong, his way of handling crises and his constant desire that we should be integrated into a group. I cannot say that my PhD has been a path of roses, there were very difficult moments, however I always felt in confidence and supported and encouraged by Henning. I can say with certainty that without Henning this PhD would not have been possible.

If we speak of gratitude, there are many people to whom I must thank. First, to Vincent Verlinden. I arrived that first day to that office where mainly Vincent and I were always alone or my sunshine, as I would call him later. Vincent answered all my questions about computers, spss and so on, as well as bringing me hot chocolate every time he went for his coffee. His bright smile cheered my days in that cold and gray room. In addition, as an extra, Vincent learned Spanish and wanted to practice! What more could I ask for?

With Vincent came Ben Verhaaren, serious and very responsible, who helps me with the data. I came, went, and asked many things and Ben was always very kind and patient to answer all my questions. I am pleased to know that Ben married one of my best friends in Holland.

However, there are always changes, and a year later I was moved out of the room and I went from being with men to being with only girls. OMG! There I met the

sweet Karin. How can you be so sweet and beautiful person? I also met AnneMarie, Nese, and Lisette. I will forever remember the athletic and smiling Lizz, with her inexhaustible energy. Love your love story. My deepest affection and thanks to Annemarie and Nese. Without them, I would not have been able to overcome the difficult moments that I had to go through when my father died. Anne Marie you are one of the warmest people I have ever met, and with an enormous capacity to make us feel comfortable and at home. Nese, you have been my first, though not the last, Turkish friend I have ever had. Nese you are fun, irreverent and without judgments, but above all you are empathic and you know how to listen. Infinite thanks for accompanying me on my darkest night and taking charge of details that in my stupor were very difficult to attend. Your silent company was very comforting. In that small space on the 22nd floor, there were laughs, ventures, group therapies, travel plans, internet shopping and dreams. It was very good to have all of you as part of my PhD program.

Arfan Ikram thank you to the kind for reading my articles and helping me in this process.

Rachel Kalf and Mariana, thank for accepting me in your groups and invite me to outside activities, Rachel was always available for a movie outing.

I cannot stop thanking my Latin group with whom I started this trip. To Jorge, Lady Cantor, Dayan and all of us who form this group and in whom I always found support. I leave aside for my dear Mexican friend Ingrid. How many moments lived, how many talks between jobs, shared concerns and walks we enjoyed.

It was wonderful to have had the opportunity to share with so many nationalities.

I also would like to thank all coauthors with whom I have worked together during my PhD. Arfan, Nese, Jelena, Ben, Albert Hoffman, Rachel. Thank you all for your kind co-operation.

Dear Frank, Nano, Jolande and other members of the data management group, you are great on solving problems. Thanks a lot for helping me so generously and always answer my million emails, even from Panama.

Dear Astrid Vraking, Erica, Hetty, Gabriëlle Bakker, Yolanda Bekker-Wilthagen, Solange Degenaar, thanks a lot for your quick help for many administrational issues.

I have to thank to Kaygun, the Dunja coffee owner and his wife for being such wonderful owners and for the delicious dinners I was invited to.

To Joseph, whom I met while working on a tram and turned out to be a generous Dominican whose goal in life was to help Latinos in Rotterdam.

I cannot stop thanking my Panamanian friend Ana Maria, who via telephone heard all my complaints, successes, sadness, despair and so on. I think she knows each of the people that I mentioned

And last, but not least, my parents, who always supported me and agreed with any decision I made. To my father, wherever he is, I know he is happy about this culmination.

This thesis is dedicated to my family, where university studies date from my maternal great-grandfather, one of the first lawyers of the republic, and my maternal grandmother, teacher by profession. This educational lineage has made us see education as a normal step in our lives and not as an obligation. Thanks to my ancestors for this educational heritage.

And finally I thank my cats Ishimei and Roy who patiently prowled around me while I was writing, and in the last year my little dog Pituca Dulcinea, who accompanied me at every moment, every line, every email, every skype, while I typed and thought . At times, I looked at her and her tenderness and unconditional love made me stay a while longer.