THE INTERFACE OF NEUROLOGY AND PSYCHIATRY

Modern Epidemiological approaches

Saira Saeed Mirza

The Interface of Neurology And Psychiatry.

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For My Family

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CHAPTER 1

INTRODUCTION

THE INTERFACE OF NEUROLOGY AND PSYCHAITRY.

MODERN EPIDEMIOLOGIC APPROACHES

Mental disorders comprise a heterogeneous group of disorders, which have in common, their effect on mental well-being. Almost 15% of adults over the age of 60 suffer from a mental disorder.[1] Two of the most common disorders are dementia and affective disorders.[2] Dementia refers to a group of diseases that have effects on memory, thinking, behavior, and daily functioning, in common.[3] The most common causes of dementia are Alzheimer's disease and vascular dementia. Dementia affects 47 million people worldwide, and this burden is expected to double every 20 years because the population is aging rapidly and no treatment is available so far for dementia.[3] Similarly, affective disorders are also a group of disorders that have in common effects on behavior, daily functioning, and thinking. The two most common affective disorders are depression and anxiety. Depression affects approximately 7% of the elderly population and accounts for 1.6% of total disability among over 60 year olds.[1] For both dementia and affective disorders, the frequency increases sharply with age.

Depression, anxiety, and dementia very often co-occur.[4] At the same time, there is an overlap between symptoms of dementia with symptoms of affective disorders, for instance, apathy, loss of interest, social withdrawal, isolation, impaired thinking and concentration, agitation, restlessness, sleep disturbance, hence a demarcation between the two entities is difficult.[5] Comorbid affective symptoms in demented patients lead to poorer prognosis of dementia due to accelerated deterioration,[6] and comorbid affective symptoms in patients with mild cognitive impairment lead to an accelerated progression to dementia.[7] This association between affective disorders and dementia is a complex one, and the boundaries are ill-defined. Many studies suggest that depression and anxiety are risk factors for dementia, but given the long preclinical phase of dementia, it is equally possible that affective symptoms appear in response to the ongoing cognitive impairment, implying reverse causation.[8] Therefore, whether late-onset depression and anxiety are risk factors of dementia, or it's prodrome, remains debated. If depression and anxiety mark the preclinical phase of dementia, meaningful pharmacological or non-pharmacological interventions might be possible. In addition to affective symptoms, the underlying cognitive decline might also reflect in subtle changes in lifestyle or everyday habits of individuals. In view of a rapidly aging population, a better understanding of these disorders is the key to tackle the devastating consequences associated with them. It is also suggested that affective disorders and dementia are consequences of a common pathological process(es).[9] Of the several pathways proposed, vascular impairment is the

most studied, and has been implicated as a risk factor, not only for vascular dementia, but for Alzheimer's disease as well.[10] If dementia and affective disorders have a shared etiology, identification of such common pathways will open avenues for joint etiological research, which may lead to effective prevention or treatment strategies.

The overall aim of this thesis was to study how late-onset affective symptoms relate with dementia. Studies described in this thesis were all embedded within the Rotterdam Study, an established cohort of 14,926 persons aged 45 years and older. In addition to detailed interviews and examinations every 3-4 years, all participants are continuously monitored for all major life events via a computerized linkage of the study database with the files from general practitioners.[11] I used various established and novel methodological techniques to answer my research question, including Latent-Class Analyses and flexible parametric method of survival analysis.

Specifically, in **Chapter 2** of this thesis, I studied the associations of depression and anxiety with dementia. In **Chapter 2.1**, I studied the short- and long-term associations between depression and dementia. However, since depression has a remitting and relapsing nature, risk of dementia might differ with different courses of depression, especially over a long follow-up. Therefore, in **Chapter 2.2** and **2.3**, I studied how risks of dementia and mortality differ across different trajectories of depressive symptoms. In **Chapter 2.4** and **2.5**, I studied the association of anxiety with dementia and mortality. Subsequently, I studied mild cognitive impairment in relation to risk of affective disorders in **Chapter 2.6**.

In **Chapter 3**, I studied the role of lifestyle factors in relation to incident dementia. As a part of lifestyle, coffee has been of considerable interest in relation to dementia because of its neurostimulatory effects.[12] The beneficial effect of coffee has been largely reported by epidemiological studies with short follow-up, whereas studies with a longer follow-up show no protective association.[13] Therefore, the protective effects of coffee on dementia remain debated. In **Chapter 3.1**, I studied coffee consumption and risk of dementia over both short and long-term follow-up periods. Extending these analyses, in **Chapter 3.2**, I studied associations of coffee consumption with cognitive function and MRI markers of brain volumes and brain pathology. In **Chapter 3.2**, I studied the role of cognitive reserve (operationalized by educational level) in protection against dementia after a stroke or TIA.

In **Chapter 4**, I studied serum biomarkers in relation to incident dementia and depression. In **Chapter 4.1**, I studied NT-proBNP, which is a marker of cardiovascular disease, in relation to incident dementia, Alzheimer's disease, and cognitive decline. In **Chapter 4.2**, I

studied many cardiovascular, metabolic, and renal biomarkers individually, and jointly, in relation to incident depression.

Finally, in **Chapter 5**, I discuss the main findings of these studies with certain methodological considerations, and provide a general outlook for future research.

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CHAPTER 2

PSYCHIATRIC DETERMINANTS OF NEUROLOGICAL OUTCOMES AND MORTALITY

CHAPTER 2.1

Depressive symptoms predict incident dementia during short but not long term follow-up period

Saira S. Mirza, Renée F.A.G de Bruijn, Nese Direk, Albert Hofman, Peter J. Koudstaal, M. Arfan Ikram, Henning Tiemeier

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ABSTRACT

Background: Whether depression is a long term risk factor for dementia or represents a prodrome of dementia is unclear. Therefore, we examined the relationship between depressive symptoms and dementia both during short and long follow-up in a population-based cohort.

Methods: In The Rotterdam Study 4,393 non-demented individuals were followed for incident dementia for 13.7 years by continuous monitoring. Cox proportional-hazards models for different time intervals were used to estimate the risk of incident dementia.

Results: 582 participants developed dementia during 13.7 years. Persons with depressive symptoms had an 8% increased risk of dementia compared to those without depressive symptoms during the over-all follow up. The risk was highest in the short and intermediate follow-up, particularly in men. We did not find an association in the follow-up period beyond 10 years.

Conclusions: Our results suggest that late-life depressive symptoms are part of a dementia prodrome rather than an independent risk factor of dementia.

INTRODUCTION

Dementia poses a high burden on society and health care, both in terms of financial costs as well as suffering for patients and care-givers. Current estimates indicate a prevalence of 35.6 million patients worldwide with another 7.7 million incident cases occurring annually.[1] In order to develop effective preventive and therapeutic strategies, it is crucial to unravel the multi-factorial etiology of dementia.

Depression and depressive symptoms are very common in the elderly and often co-occur with dementia.[2] Depression and dementia share many vascular risk factors[3] and various studies have shown that depression in late life is associated with a 2 to 5 fold increased risk of dementia.[4-8] Most studies investigated this association over a follow-up period of at most 7 years. In contrast, The Framingham Study studied a follow-up period of 17 years and reported a 70% greater risk of incident dementia in depressed individuals; however, the investigators did not distinguish the risk between short and long-term follow-up. Taken together, current data suggest a strong association between depression and incident dementia, but the question remains whether depression is a risk factor for dementia or merely a prodromal symptom of underlying dementia.[9] Given the long pre-clinical phase of dementia, it is conceivable that subclinical dementia causes depressive symptoms rather than depression being a true risk factor for dementia. One way to address this issue is to study the association of depression and dementia during a long follow-up and then explore the association over separate incremental periods of follow-up. The hypothesis to be tested is that there is a strong association between depression and dementia over a short follow-up period which attenuates with longer follow-up.

Also, some studies have suggested a difference between men and women in the association of depression with dementia, but data are still scarce.

Therefore, we studied the relationship of depressive symptoms and dementia both over long and short follow-up periods in a population based cohort. We further examined if the relationship between depression and dementia differs between men and women.

METHODS

Setting

This study was embedded in the Rotterdam Study; an ongoing population-based prospective study of the elderly that started 1990 and studies the incidence and determinants of chronic diseases in late life.[10] The Medical Ethics Committee of Erasmus Medical Center Rotterdam approved the study and written informed consent was obtained from all participants.

Every 3 to 4 years, all participants undergo an extensive home interview and a physical examination at the research center. In addition, all participants are continuously monitored for the occurrence of all major events during follow-up by linkage of the study database with medical files from general practitioners. The third examination round of Rotterdam study constituted the baseline of this study, because the data on depressive symptoms were complete and uniformly collected (using CES-D for the whole cohort).

Study population

Of the original cohort of 7,983 persons in 1990, 4,797 surviving persons participated in the third examination which took place from 1997-1999. From these 4,797 individuals, 4,602 (96%) completed the depression assessment questionnaire. Of these 4,602, 110 participants did not consent to undergo dementia screening and were excluded. We also excluded 92 participants who were demented at baseline and 7 who were lost to follow up. This yielded a total of 4,393 individuals (92% of total survivors) available for final analysis, who were followed for a maximum of 13.7 years (mean 8.7, SD 3.5 years) for incident dementia. Follow- up started from the day of depressive symptom screening to the date of incident dementia, date of death, or the censor date January 1st, 2011, whichever occurred first.

Assessment of depression

We used the validated Dutch version of the Center for Epidemiology Depression Scale (CES-D) for assessment of depressive symptoms at baseline. The CES-D comprises of 20 questions, each with a possible score of 0-3, and the score indicates clinically relevant depressive symptoms. Depressive symptom scores were used as a standardized continuous variable. Z scores were calculated as weighted individual score minus mean score, divided by the standard deviation. CES-D scores were weighted by missing values

only if missing values did not exceed 25%. For descriptive purposes however, a score of 16 or higher is considered suggestive of depressive symptoms.[11]

Assessment of incident dementia

Participants were screened for dementia at baseline and follow-up examinations using a three-step protocol.[12] Screening was done using the Mini Mental State Examination (MMSE) [13] and the Geriatric Mental Schedule (GMS) organic level.[14] Screen-positives (MMSE<26 or GMS organic level>0), subsequently underwent an examination and informant interview with the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX).[15] Participants who were suspected of having dementia, underwent, if necessary, further neuropsychological testing. Additionally, the total cohort was continuously monitored for dementia through computerized linkage between the study database and digitized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. In the end, a consensus panel, led by a neurologist, decided on the final diagnosis in accordance with standard criteria using the DSM-III-R criteria for dementia and the NINCDS-ADRDA for Alzheimer disease.[16] If required for differential diagnosis, neuro-imaging was used. Follow-up for incident dementia was virtually complete (98.63%) till January 1st, 2011.

Covariates

In addition to age and gender, education level, smoking, cognition level at baseline, hypertension, diabetes mellitus, prevalent stroke, and use of antidepressant medication were considered possible confounders. Smoking, hypertension, diabetes and stroke are well documented risk factors for all types of dementia. A low education level has been found to be associated with increased risk of dementia, especially in females. [17] In subsequent models, we adjusted for marital status, *APOE*-ε4 carrier status, cognitive complains at baseline, and psychotropic medication use.

Education level was assessed during the interview and people were classified into two categories; low level of education (primary only or primary and unfinished secondary) and intermediate to high (primary and secondary, vocational or university). Cognition was assessed by the MMSE at baseline.[13] Inquiring about smoking habits, participants were categorized into current, former and never smokers. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, or diastolic blood pressure ≥ 90 mmHg, or use of antihypertensive medication assessed by interview and pharmacy records.[18] Diabetes Mellitus type II was diagnosed as fasting blood glucose ≥ 126.13 mg/dl (multiply by factor 0.0555 to covert to mmol/l) or use of anti-diabetic medication evaluated by interview and

pharmacy records.[19] Previous stroke was determined by reported events on interview and confirmed by medical records. In addition, participants are continuously monitored for all major events through automated linkage of study database with GP files.[20] Information on use of antidepressants (ATC code n06) was obtained by interview, medical and pharmacy records.

Statistical analysis

We used Cox proportional-hazards model to assess the relationship between clinically relevant depressive symptoms and incident dementia (all-cause). We investigated not only the total follow-up period as a whole, but also separate 5-year time periods (0-5 years, 5-10 years and more than 10 years of follow up). This analysis was performed to study the timing of incident dementia in relation to the appearance of depressive symptoms. Several studies have used a follow-up time range of 1 to 5 years to study the short-term effect of depression on dementia incidence[5,6,21-24]; but there is no recommended cut-off of follow-up time to study depression as a dementia prodrome. In an alternative analysis, time periods were defined by ensuring equal number of 100 cases in each period (five periods 100 incident cases, the last period only counted 82 cases). This approach allows a more detailed assessment of risk ratio change over time and increases the power to detect changes. Hazard ratios of dementia for each time period of 100 cases were calculated separately as well as cumulatively. The cumulative time approach in both the main analysis and in the 100 case-analysis was a method carried out to ensure comparability with other studies, as most studies have examined the association of depressive symptoms and dementia using a Cox model and with using variable follow-up periods. Therefore, we first used a cumulative follow-up approach in which we examined the association between depression and dementia by increasing the years of follow-up by not changing the baseline. i.e. 0-5 years, 0-10 years, and 0-13.7 years. Similarly, for the 100-case analysis, we examined association of depression and dementia by increasing 100 cases in every subsequent step without changing our baseline. i.e. baseline to 100 cases, baseline to 200 cases, baseline to 300 cases and so on. As secondary analyses, we explored effect modification by gender and age (median age used as cut-off) using stratification and interaction terms. Additionally, we analyzed our data, using only Alzheimer's disease as outcome.

We ran an additional analysis to test sensitivity of our findings. Analysis was repeated excluding persons with clinically relevant depressive symptoms occurring prior to baseline. Depressive symptoms were also assessed, 4 years prior to baseline either with the CES-D (cut-off ≥ 16) in 48% of participants, or the Hospital Anxiety and Depression Scale, HADS-D

(cut-off ≥ 9) in 52% of participants, as part of a pilot.[25] In this sensitivity analysis, the effect of more chronic depressive symptoms, which are less likely to be an indicator of dementia prodrome, is reduced. If there were an effect of depression as part of a dementia prodrome only, our hazard ratios for the short follow-up would be expected to increase slightly if chronic cases are excluded.

Since, depressive symptoms in The Rotterdam study were re-measured at a follow-up round in 2002-2004, we also repeated our analysis using depressive symptoms assessed at this follow-up round as our baseline.

Although we have used depression as a standardized continuous variable in all analyses, we also performed our main analysis using CES-D score dichotomized at 16 points.

Results are presented as hazard ratios (HR) with 95 % confidence intervals (CI). All analyses were adjusted for age and gender in the first model and additionally for education, smoking, hypertension, diabetes, prevalent stroke, MMSE score and antidepressants' use in the second model. Data were analyzed using the Stata Software Version 12 (StataCorp, College Station, TX, USA).

RESULTS

Baseline characteristics of the study population are summarized in **Table 1**. The study included 4,393 individuals comprising 59% females (n=2,599). Mean age at baseline was 73 years (SD 7.3 years; range 61.1-105.8) and participants were followed for a maximum of 13.7 years (mean 8.7, SD 3.5 years). Applying the accepted cut-off of ≥ 16 for CES-D, seven percent (n=323) of the study population had clinically relevant depressive symptoms at baseline, however, we used continuous depression scores for all analyses.

Of the 4,393 individuals, 13% (n=582) developed dementia (all cause); 84% of those were Alzheimer's disease cases (n=489). Mean age of dementia diagnosis was 83 years (SD 6.3; range 65.5-102 years).

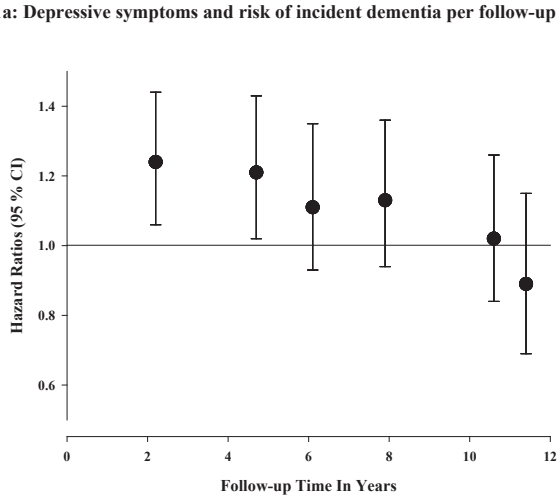
When investigating each time period with 100 subsequent cases separately, the risk of dementia decreased gradually from HR 1.24 (95% CI: 1.06, 1.44) in the first period, to HR 0.89 (95% CI: 0.69, 1.15) in the last period. Similar pattern was observed when we calculated hazards for cumulative time periods of 100 cases; HR 1.24 (95% CI: 1.06, 1.44) in the first period to HR 1.11 (95% CI: 1.03, 1.20) in the last period (**Figure 1**).

In the overall follow-up of 13.7 years, depressive symptoms were associated with a moderately increased risk of incident dementia **(Table 2)**.

Table 1. Baseline characteristics of the study population, N=4,393.

Characteristics	Descriptives
Age, years	72.7 (7.3)
Women	2,599 (59.2)
Body mass index, kg/m^2	26.9 (3.7)
Education	
Low	1,350 (31.2)
Intermediate to high	2,980 (68.8)
Smoking	
Never	1,520 (34.6)
Former	2,157 (49.1)
Current	716 (16.3)
Diabetes mellitus type 2	534 (12.2)
Stroke	338 (7.7)
Myocardial Infarction	427 (9.7)
Hypertension	2,990 (68.1)
Total cholesterol, mmol/L	5.82 (0.9)
HDL cholesterol, mmol/L	1.4 (0.4)
Antipsychotic use	642 (14.6)
Antidepressant use	135 (3.1)
Clinically Relevant Depressive Symptoms	323 (7.4)

Abbreviations: HDL High density lipoprotein.
Values are means (standard deviation) or counts (percentage).



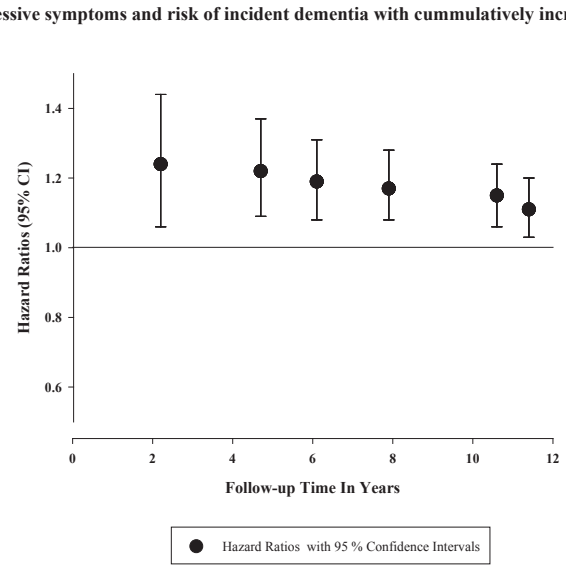


Figure 1. Depressive symptoms and risk of incident dementia-Effect estimates per 100 consecutive incident dementia cases.

Fully adjusted hazard ratios for risk of dementia at different time points in depressed individuals (N=4,393). Total number of dementia cases (N=582) were split into groups of 100 patients according to dementia incidence, and hazard ratios were calculated for each group of 100 cases. The circles represent the hazard ratios and the lines represent the 95 % confidence intervals.

Table 2. Clinically relevant depressive symptoms and risk of incident dementia-overall and gender stratified analysis, N=4,393.

Depressive symptoms	Follow-up time in years			
Total population (N=4,393)	**Overall follow-up**	**0-5 years**	**5-10 years**	**10-13.7 years**
	Hazard ratios (95% confidence intervals)			
Cases/N	582/4,393	222/4,393	238/3,529	122/2,554
Depressive score, (per SD)[a]	1.11 (1.03, 1.20)	1.19 (1.07, 1.33)	1.15 (1.03, 1.29)	0.84 (0.68, 1.05)
Depressive score, (per SD)[b]	1.08 (1.00, 1.17)	1.13 (1.01, 1.27)	1.14 (1.01, 1.29)	0.83 (0.66, 1.04)
Males (N=1,794)				
Cases/N	176/1,794	62/1,794	71/1,416	43/985
Depressive score, (per SD)[a]	1.20 (1.02, 1.41)	1.51 (1.23, 1.86)	1.20 (0.91, 1.58)	0.40 (0.17, 0.93)
Depressive score, (per SD)[b]	1.04 (0.88, 1.24)	1.31 (1.04, 1.66)	1.06 (0.79, 1.41)	0.38 (0.16, 0.93)
Females (N=2,599)				
Cases/N	406/2,599	160/2,599	167/2,113	79/1,569
Depressive score, (per SD)[a]	1.09 (1.01, 1.19)	1.12 (0.99, 1.27)	1.14 (1.01, 1.30)	0.94 (0.75, 1.18)
Depressive score, (per SD)[b]	1.09 (1.00, 1.18)	1.10 (0.96, 1.26)	1.15 (1.01, 1.31)	0.92 (0.73, 1.15)

Depression score is taken as a continuous standardized variable, and cases refer to incident dementia cases.

[a] Model 1: age and gender adjusted.

[b] Model 2: additionally adjusted for smoking, education, hypertension, diabetes, prevalent stroke, MMSE and anti-depressants use.

During a shorter follow-up time i.e. 5 years from baseline, depressive symptoms were associated with a high risk of incident dementia, fully adjusted HR 1.13 (95% CI: 1.01, 1.27). The same was true for the period of 5 to 10 years follow-up, HR 1.14 (95% CI: 1.01, 1.29). In contrast, we did not find any relationship in the third follow-up period in the fully adjusted model, HR 0.83 (95% CI: 0.66, 1.04).

After additional adjustments for marital status, *APOE*-ε4 carrier status, cognitive complaints at baseline and use of psychotropic drug use, our results remained unchanged.

In a secondary gender split analysis, we found a more pronounced effect in men than in women, though the overall pattern in both sexes was similar to the main analysis (**Table 2**). In depressed men however, the risk of dementia in the 10-13.7 year interval was reduced by 60%, HR 0.38 (95% CI: 0.16, 0.93). The interaction for gender was statistically significant ($p<0.001$). Interaction for age was not significant, hence not shown.

Repeating all analyses using Alzheimer's disease as outcome yielded similar results and patterns (**Supplement table 1**). Results using depressive symptoms as a dichotomized variable showed similar pattern and are shown in **Supplement table 2**.

In the sensitivity analysis, after excluding individuals who had been screened positive for clinically relevant depressive symptoms 4 years prior to baseline, the presence of depressive symptoms showed a 16 % higher risk of incident dementia in the 0-5 year follow-up, HR 1.16 (95% CI: 1.02, 1.32).

Using depressive symptoms assessed at the 2002-2004 examination round, we found a similar pattern of results as the main analysis (data not shown).

DISCUSSION

We found that persons with depressive symptoms had a higher risk of incident dementia, including Alzheimer disease. These associations were strongest for short follow-up time and attenuated with incrementally longer follow-up periods. Furthermore, the association was more pronounced in men than in women.

Prevalence of depressive symptoms is relatively low in this cohort. However it falls within the variable range of 2.8% to 35% reported in a review of depressive symptoms in the elderly.[26] In addition, the SHARE study has reported the Netherlands to be one of the lower depression prevalence countries in Europe.[27]

Strengths of the study include the large population-based cohort followed for over 13 years. However, certain methodological considerations need to be mentioned. First, we included clinically relevant depressive symptoms (assessed by CES-D) as the determinant rather than diagnosed depression. Therefore, we cannot be certain about the generalizability of our results to clinical depressive syndromes as well. Second, some residual confounding due to unknown or unmeasured confounders such as physical activity and diet cannot be completely ruled out. Third, there is a possibility that some selection through depressive symptoms and/or cognitive function in the previous examination round may have influenced the results. Fourth, depressive symptoms were related to death of participants in the first ten years of follow-up, so there is a possibility of some selection by death in the 10-13.7 year interval.

There are a few possible explanations for our observation that the association of depression with incident dementia was strongest with short follow-up and attenuated with longer follow-up. First, late-onset depressive symptoms preceding dementia could be merely a reactive phenomenon. It is possible that depression is a psychological response to the ongoing cognitive decline.[28] Second, late onset depressive symptoms may represent a prodrome of dementia. The prodrome can be defined as a pre-dementia syndrome in which the underlying subclinical dementing process manifests itself by depression or altered behavior, thus marking the onset of clinical dementia in the near future. Where a depression in adult life or a lifetime history of depression would be considered a risk factor for dementia, a very recent history of late-onset depressive symptoms may be an early clinical manifestation of the underlying neurodegenerative condition. This implies that both depression and dementia are the result of a common underlying process(es) but that symptoms of depression manifest earlier than dementia. It has been shown that patients experiencing cognitive decline together with late onset depression develop dementia within a few years after the onset of depression.[28] This prodromal hypothesis is in line with recent findings from large cohort studies suggesting that late-onset depression is a prodrome of dementia onset.[21,29] Studies have reported a positive association between depression and dementia over a shorter follow-up period of at most 5 years.[4-7] Previously in a subset of the Rotterdam Study, we reported a null association between depressive symptoms and dementia; however, differences in sample size, follow-up, age and cognition at baseline could explain the difference in results.[30]

Third, depression may increase the risk of dementia over a short term period only. Some individuals with depressive symptoms may be more vulnerable for incident dementia because of certain genetic or environmental risk factors although a shared etiology would typically convey a constant risk over time.[3,31] Several potential biological mechanisms

could be a common intermediate between depression and dementia such as hippocampal atrophy.[2] It has also been shown that depressed individuals have low levels of adrenaline[32] and serotonin[33], and deficits of these monoamines are also associated with increased severity of dementia. The underlying neurodegenerative process, might get accelerated due to depression, by the activation of hippocampal pituitary axis leading to increased cortisol levels, hence precipitating dementia.[28]

We also found that the association of depressive symptoms and dementia in the short term period was stronger in men than in women. This finding concurs with two prospective cohort studies which reported a stronger association of depressive symptoms and risk of incident dementia in men compared with women.[34,35] There is limited literature available in this context, and further epidemiological and etiological studies are highly recommended to better understand the gender differences in association between depression and dementia.

We observed a protective association between depression and dementia in the 10-13.7 year interval in men. However, since this period has a fewer number of cases (n=43) the estimates may not be very precise. It is also possible, that reduced effect estimates are a result of other competing risks in this period and deaths occurring due to other causes such as cardiovascular causes or cancers. Additionally, we speculate that perhaps depressed persons who survive ten years or more, become resilient against subsequent dementia.

In conclusion, late-onset depressive symptoms represent a part of the prodromal stage of dementia, rather than being a risk factor for dementia. Depressive symptoms posed a much higher risk of incident dementia in men compared with women in a short follow-up.

SUPPLEMENTARY INFORMATION

Supplementary Table 1. Clinically relevant depressive symptoms and risk of Alzheimer's disease, N=4,393.

Depressive symptoms	Follow-up time in years			
Total population (N-4,393)	Overall	0-5 years	5-10 years	10-13.7 years
	Hazard ratios (95% confidence intervals)			
Cases/N	489/4,393	194/4,393	199/3,529	96/2,554
Depressive score, (per SD)[a]	1.08 (0.99, 1.17)	1.16 (1.03, 1.31)	1.11 (0.98, 1.27)	0.79 (0.61, 1.03)
Depressive score, (per SD)[b]	1.05 (0.96, 1.15)	1.11 (0.98, 1.26)	1.09 (0.95, 1.25)	0.79 (0.60, 1.04)
Males (N=1,794)				
Cases/N	131/1,794	43/1,794	55/1,416	33/985
Depressive score, (per SD)[a]	1.16 (0.95, 1.41)	1.38 (1.04, 1.82)	1.30 (0.98, 1.73)	0.43 (0.17, 1.06)
Depressive score, (per SD)[b]	1.02 (0.83, 1.25)	1.16 (0.83, 1.60)	1.13 (0.83, 1.53)	0.48 (0.19, 1.22)
Females (N=2,599)				
Cases/N	358/2,599	151/2,599	144/2,113	63/1,569
Depressive score, (per SD)[a]	1.07 (0.97, 1.17)	1.13 (0.99, 1.28)	1.08 (0.93, 1.24)	1.08 (0.93, 1.24)
Depressive score, (per SD)[b]	1.06 (0.96, 1.16)	1.11 (0.97, 1.27)	1.07 (0.92, 1.25)	1.07 (0.92, 1.25)

Depression score is taken as a continuous standardized variable, and cases refer to incident Alzheimer's disease cases.

[a] Model 1: age and gender adjusted.

[b] Model 2: additionally adjusted for smoking, education, hypertension, diabetes, prevalent stroke, MMSE and anti-depressants use.

Supplementary Table 2. Clinically relevant depressive symptoms and risk of incident dementia, N=4,393.

Depressive symptoms	Follow-up time in years			
	Overall	0-5 years	5-10 years	10-13.7 years
	Hazard ratios (95% confidence intervals)			
Cases/N	582/4,393	222/4,393	238/3,529	122/2,554
Depressive symptoms[a]	1.46 (1.13, 1.89)	1.43 (0.96, 2.15)	1.94 (1.33, 2.82)	0.74 (0.34, 1.59)
Depressive symptoms[b]	1.38 (1.06, 1.80)	1.26 (0.83, 1.91)	1.94 (1.31, 2.87)	0.74 (0.34, 1.60)

Depression is used as dichotomized (cut-off CES-D score ≥ 16 taken as positive for depressive symptoms), and cases refer to incident dementia cases.

[a] Model 1: age and gender adjusted.

[b] Model 2: additionally adjusted for smoking, education, hypertension, diabetes, prevalent stroke, MMSE and anti-depressants use.

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CHAPTER 2.2

Ten year trajectories of depression and the risk of dementia. A population-based study

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Submitted

ABSTRACT

Background: Late life depressive symptoms have been extensively studied for their role in incident dementia, but were typically assessed at a single time-point. Such an approach neglects the course of depression, which, given its remitting and relapsing nature, might reveal more subtle associations with dementia.

Methods: In 5,300 dementia-free participants (mean age: 69.4 ±8, 58% women), we used depressive symptoms assessments at three examinations over an 11-year period (1993-2004), to identify trajectories of depression by latent class trajectory modelling. Dementia incidence by latent trajectory over a subsequent 10-year period (2004-2014) was computed using Cox proportional hazards models. We repeated the analyses censoring incident stroke, and for Alzheimer's disease only as outcome.

Results: We identified five trajectories characterized by low (75.1%), decreasing (10.1%), remitting (5.3%), increasing (7.8%), and high (1.8%) depressive symptoms. During 25,434 person-years, 417 participants had incident dementia. Only the trajectory with increasing depressive symptoms was associated with a higher risk of dementia, relative to the low depressive symptoms trajectory (reference): HR before censoring stroke 1.51 (95% CI:1.10,2.09), HR after censoring stroke 1.76 (95% CI: 1.27, 2.45) and Alzheimer's disease, HR 1.59 (95%CI: 1.12, 2.26).

Conclusions: Risk of dementia differed with different courses of depression which could not be captured by a single assessment of depressive symptoms. The higher risk of dementia only in the increasing trajectory suggests depression to be a prodrome of dementia.

INTRODUCTION

Clinical depression, particularly clinically-relevant depressive symptoms are not only highly prevalent in dementia but are also highly predictive of incident dementia.1

The course of depression and depressive symptoms over the lifetime varies across individuals.2 For instance, persons might suffer from clinically relevant depressive symptoms only transiently, followed by full remission. Others might have a consistent remitting and relapsing depression, whereas some may become chronically depressed. Such different courses may reflect different etiology and may consequently predict dementia risk differentially. For instance, depressive symptoms as a physiologic response to an adverse life event or diagnosis of a chronic illness may have different impact on the risk of dementia compared to depression with more severe biologic basis, such as brain pathology, or dysregulation of neurotransmitters. However, existing studies on the association of depression and dementia have assessed depression only once, which neglects the course of depression.3-5 Assessment of depressive symptoms in a remitting phase could lead to an underestimation of the risk of dementia associated with chronic depression. This remitting and relapsing nature of depression necessitates to study the course of depression in relation to risk of dementia.

Therefore, we used repeated measures of depressive symptoms over a 10-year period to study different trajectories of depression, and their subsequent risk of dementia in a population-based setting. This approach might also identify high risk groups from a population-based perspective to facilitate effective prevention and treatment targeted at the persons at risk.

METHODS

Setting

This study was embedded in the Rotterdam Study, a population-based study of adults aged ≥55 years, ongoing since 1990. Follow-up examinations including home interviews and physical exams at a research center take place every three to four years. In addition, the cohort is continuously monitored for all major events by a highly efficient data linkage system between the study database and the general practitioners (GPs).6 The Rotterdam Study has been approved by the medical ethics committee according to the Population

Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. Written informed consent was obtained from all participants.

Study population

This study utilized data on depressive symptoms from three examination rounds of the Rotterdam Study (1993-1995, 1997-1999, and 2002-2004). The examination round in 1993-1995 was defined as the baseline for this study because the assessment of depressive symptoms was introduced in the Rotterdam Study in this examination round. Incidence of dementia was investigated from last round of depressive symptoms assessment onward, i.e. from 2002-2004 until 2014 in those who were dementia-free or "at-risk" for dementia.

Identification of trajectories: At baseline (1993-1995), data for depressive symptoms were available for 4,597 dementia-free participants. In the examination rounds in 1997-1999 and 2002-2004, depressive symptoms were assessed in 4,294 and 3,251 participants respectively. In total, 5,300 participants had depressive symptoms data at at-least one examination round, 4,385 at more than one examination rounds, and 2,689 for all three examination rounds. 703 participants did not respond at the first round, but did so at later rounds. From 1993-1995 to 2002-2004, 512 participants had incident dementia. Any depressive symptoms scores available after the diagnosis of dementia (n=95 had depressive symptoms score available at one or two rounds) were not used to assign trajectories because of unreliability of these scores.

Survival cohort for dementia: From the study entry in 1993-1995 to the last round of depressive symptoms assessment in 2002-2004, 1,734 participants died. Of the 3,342 surviving persons who attended the examination round in 2002-2004, 139 had prevalent dementia and were excluded. Therefore, 3,203 (95.8%) dementia-free participants were included in the survival analysis. Follow-up for dementia was complete until January 2014. Survival analyses were performed beyond the date of last depressive symptoms assessment to avoid the problem of non-proportionality of hazards, and selective survival of participants during the period of depressive symptoms assessments. In addition, the concomitant events of incident dementia and deaths occurring during the period of depression assessments made it difficult to assess the true risks associated with each trajectory.

Assessment of depressive symptoms

In the first examination round, we measured depressive symptoms using two different instruments: the validated Dutch version of the Center for Epidemiology Depression Scale (CES-D),7 and the Hospital Anxiety and Depression Scale-Depression (HADS-D).8 At this round, a random half of participants received the CES-D questionnaire, while the other half received the HADS-D questionnaire. For all subsequent rounds, CES-D was used. The CES-D comprises 20 items, each with a possible score of 0-3, and the score ranges from 0-60.7 HADS-D comprises of 7 items each with a possible score of 0-3, and the score ranges from 0-21.8 Both scales have been validated for assessment of depression and can indicate clinically-relevant depressive symptoms. We checked for evidence whether this introduces information bias but we found the same percentage of screen positives (10%) using validated pre-defined cut-offs.9 For analyses, depressive symptoms scores were weighted for missing items, only if missing items did not exceed 25%. Scores with missing values exceeding 25% were excluded. Depressive symptoms scores were then used as a standardized continuous variable, calculated as weighted individual CES-D/HADS-D score minus mean score, divided by the standard deviation.

Assessment of dementia

Participants were screened for dementia at study entry and all follow-up examinations using a three-step protocol.10 First, screening was done using the Mini Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level. Second, screen-positives (MMSE<26 or GMS organic level>0) subsequently underwent an examination and informant interview with the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX). Participants who were suspected of having dementia, if necessary had further neuropsychological testing. Third, a consensus panel led by a neurologist, decided on the final diagnosis in accordance with the standards using the DSM-III-R criteria for dementia and the NINCDS-ADRDA for Alzheimer's disease. Additionally, the total cohort was continuously monitored for dementia through computerized linkage between the study database and digitized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. This data linkage system is highly efficient in the Dutch situation and the possibility of underestimation of cases is very low. In this setting, the GPs receive all medical information about their patients if they contact any medical care-giver or professional including specialists. We calculated the potential and observed person-years to calculate the completeness of dementia follow-up,11 which was 93.7% complete until January 1st, 2014.

Covariates

Age, sex, APOE-ε4 carrier status, educational level, body mass index (BMI), smoking habits, alcohol consumption, MMSE score, use of anti-depressant medication, and prevalent hypertension, diabetes mellitus 2, myocardial infarction (MI) and stroke were assessed at baseline (1993-1995) and considered potential confounders for the association between depression and dementia as they are associated with depression, and are independent risk factors for dementia.12,13 For APOE-ε4 carrier status, participants were classified into non-carriers of ε4 allele or carriers of one or two ε4 alleles. For educational level, participants were classified as having primary (primary only or unfinished secondary) or higher than primary education (secondary, vocational or university). BMI was calculated as weight in kilograms/height in meters squared. For smoking habits, participants were classified into never, past, or current smokers. Weekly reported alcohol consumption was categorized into beer, wine, liquor, and moderately strong alcohol types. These were converted to grams of alcohol by taking the average amount of ethanol in a drink. By adding the amount of ethanol in all four groups, total amount of alcohol in g/day was calculated.14 Information on the use of anti-depressants (ATC code n06) was obtained by interview. As this information was not present for the baseline round (1993-1995), we utilized information available from the previous examination round (1990-1992) and the next (1995-1997). Participants using antidepressants at any of these two rounds were labelled positive for anti-depressant use. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, or diastolic blood pressure ≥ 90 mmHg, or use of antihypertensive medication assessed by interview and pharmacy records. Diabetes mellitus 2 was diagnosed as fasting blood glucose ≥ 7.0 mmol/l, or use of anti-diabetic medication evaluated by interview and pharmacy records. Previous MI and strokes were determined by reported events on interview and confirmed by medical records.6

Statistical analyses

Identification of trajectories: We used latent class trajectory models (LCTM) to identify trajectories of depressive symptoms over time. This is a specialized form of finite mixture modelling, and is designed to identify trajectories, which are latent classes of individuals following similar progression of an outcome over time or with age.15 One of the key assumptions in this model is that, a population comprises multiple trajectories. We used the censored normal distribution (cnorm) in trajectory modelling which is intended for the analysis of repeatedly measured continuous scales. For every subject, the posterior probabilities for each trajectory were computed, taking into account their age, sex and educational level, and subjects were assigned post-hoc to the trajectory with the highest

probability. The final model was chosen, based on minimizing the Bayesian Information Criterion (BIC)16 Mean probabilities per trajectory ranged from 0.78 to 0.95. To facilitate interpretability, we assigned labels to the trajectories based on their graphical patterns.

Risk of dementia: We computed hazard ratios for dementia by assigned trajectory using Cox proportional hazards models. Adherence to proportional hazards assumption was tested by plotting smoothed Schoenfeld residuals against time; no violations of the assumption were identified. We also investigated the risk of dementia across trajectories after censoring incident strokes occurring during follow-up. For these analyses, all participants with prevalent stroke were excluded. Additionally, we investigated the risk of Alzheimer's disease across trajectories. To assess the possibility of reverse causality, we stratified the follow-up time arbitrarily at 3 years into a short-term stratum of 0-3 years and a long-term stratum of >3 years. We tested several cut-offs namely 2, 3, 4, and 5 years, but results for stratification at 3 years only are shown. Finally, to test the influence of mortality as a competing risk for dementia, we performed a competing risk analysis. For all analyses, two models were fitted. Model 1 was adjusted for age and sex only. Model 2 was additionally adjusted for APOE-ε4 carrier status, educational level, BMI, smoking, alcohol consumption, cognitive score at baseline, use of anti-depressant medication, and prevalent disease status at baseline, including hypertension, diabetes mellitus 2, MI, and stroke (where applicable).

All analyses were performed using the Stata Software Version 13 (StataCorp, College Station, TX, USA).

RESULTS

This study included 5,300 non-demented participants (mean±SD age: 69.4±8.0, 58% women) with up to 3 assessments of depressive symptoms during 11 years, to identify different trajectories of depressive symptoms. We identified five distinct trajectories of depressive symptoms (**Figure 1**): 75.1% of participants were in a trajectory defined by maintaining a low CES-D score throughout the follow-up (T1: low, n=3,826), 10.1% started moderately high but remitted (T2: decreasing, n=673), 5.3% started low, increased but remitted (T3: remitting, n=278), 7.8% started low on the CES-D but steadily increased throughout follow-up (T4: increasing, n=394), whereas 1.8% maintained a high score throughout (T5: high, n=129). Baseline characteristics of participants across trajectories are summarized in **Table 1**. As compared to persons in the other trajectories, T1 had equal proportions of men and women, and participants were likely to be younger and more educated.

Numbers of incident dementia cases and deaths per trajectory before the survival analyses are shown in **Supplement table 1**.

Of the 3,203 participants included in the survival analyses, 417 developed dementia during 25,435 person-years. Of these, 332 had Alzheimer's disease, and 25 had vascular dementia. Using T1 as the reference trajectory, we found that only those within the

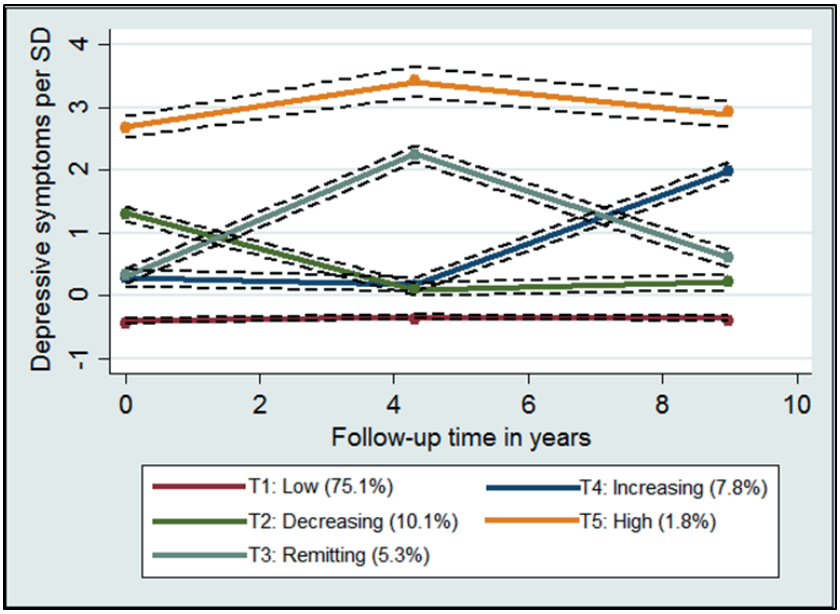

Figure 1. Trajectories of depressive symptoms, N=5,300.
The figure shows trajectories of standardized depressive symptoms scores over 11 years, using three measures of depressive symptoms. The plot uses mean follow-up time of 9 years.

increasing trajectory had a higher risk of dementia, HR 1.51 (95% CI: 1.10, 2.09) (**Table 2 and Figure 2**). Results remained consistent after censoring for incident stroke with only the increasing trajectory associated with a higher risk of dementia, HR 1.76 (95% CI: 1.27, 2.45) (**Table 3**). Both before and after censoring for incident stroke, estimates for the high trajectory were also suggestive of a higher incidence of dementia, although non-significant, uncensored HR 1.46 (95% CI: 0.80, 2.69). In the analyses for Alzheimer's disease as outcome, again, the increasing trajectory was the only trajectory associated with a higher risk of dementia, HR 1.59 (95% CI: 1.12, 2.26) (**Supplement table 2**). However, as opposed to the risk of all-cause dementia, risk estimates for the high trajectory were not suggestive of a higher risk of Alzheimer's disease, HR 1.00 (95% CI:

0.46, 2.18). The remitting trajectory was not associated with a higher risk of dementia, HR 1.04 (95% CI: 0.68, 1.60), or Alzheimer's disease, HR 1.08 (95% CI: 0.67, 1.75).

In the short-term follow-up of 0-3 years, estimates for the remitting trajectory, HR 1.50 (95% CI: 0.66, 3.33), and the increasing trajectory, HR 1.47 (95% CI: 0.74, 2.95) were suggestive of a higher risk of dementia (**Table 4**). However, after excluding the first 3 years

Table 1. Baseline characteristics of the study population between 1993 and 1995, N=5,300.

Characteristics	Low, n=3,826	Decreasing, n=673	Remitting, n=278	Increasing, n=394	High, n=129	P-value
Trajectory number on Figure 1	T1	T2	T3	T4	T5	
Age at baseline, years	68.3 (7.5)	72.2 (8.3)	70.4 (7.5)	73.5 (9.0)	72.8 (8.5)	<0.001
Women	2,006 (52.4)	445 (66.1)	213 (76.6)	328 (83.2)	98 (76.0)	<0.001
APOE-ε4 carrier status	1,021 (27.9)	177 (28.3)	63 (23.8)	95 (25.6)	37 (30.1)	0.49
Primary education	601 (15.7)	188 (30.0)	58 (21.0)	118 (30.0)	44 (34.1)	<0.001
Body mass index, kg/m^2	26.3 (3.4)	26.7 (3.8)	26.8 (3.6)	27.0 (3.7)	26.6 (3.6)	0.001
Current smoking	779 (20.4)	139 (20.6)	52 (18.7)	71 (18.0)	28 (21.7)	<0.001
MMSE, points	28.0 (1.6)	27.5 (1.9)	27.7 (1.5)	27.4 (1.8)	27.1 (1.8)	<0.001
Alcohol consumption, g/day	11.5 (14.3)	8.8 (14.3)	9.4 (13.3)	7.2 (11.7)	6.0 (8.9)	<0.001
Antidepressants' use	95 (2.5)	38 (5.6)	34 (12.2)	25 (6.3)	23 (17.8)	<0.001
Hypertension	2,611 (68.2)	500 (74.3)	183 (65.8)	293 (74.4)	97 (75.2)	0.001
Diabetes mellitus 2	200 (5.2)	50 (7.4)	22 (7.9)	25 (6.3)	12 (9.3)	0.03
Myocardial infarction	300 (7.8)	39 (6.0)	18 (6.5)	25 (6.3)	15 (11.6)	0.10
Stroke	113 (2.9)	43 (6.4)	15 (5.4)	29 (7.4)	12 (9.3)	<0.001

Abbreviations: MMSE Mini-Mental State Examination.
Values are means (standard deviation).or counts (percentage).
P-values are based on Analysis Of Variance (ANOVA) for continuous, and Chi2 test for categorical variables.

of follow-up, the remitting trajectory was no longer associated with a higher risk, but the increasing trajectory remained associated with a higher risk of dementia, HR 1.51 (95% CI: 1.05, 2.17). Estimates for the high trajectory were also suggestive of a higher risk, HR 1.42 (95% CI: 0.71, 2.86). In the competing risk analyses, the overall pattern of results remained consistent: the increasing trajectory showed a higher risk of dementia, HR 1.41 (95% CI: 1.02, 1.96) (Data not shown).

Of the 3,300 participants included in the survival analyses, 513 developed dementia during 25,469 person-years. Of the total 513, 417 had Alzheimer's disease, and 29 had vascular dementia. We found that only those within the increasing trajectory had a higher risk of dementia, HR 1.56 (95% CI: 1.17, 2.08) (**Table 2 and Figure 2**).

Table 2. Trajectories of depressive symptoms and risk of dementia, N=3,203.

Trajectories	Cases/n	Risk of dementia, hazard ratios (95% confidence intervals)			
		Model 1[a]	P-value	Model 2[b]	P-value
T1: Low	283/2,420	1.00 (ref)		1.00 (ref)	
T2: Decreasing	48/319	1.10 (0.81, 1.50)	0.53	1.04 (0.68, 1.62)	0.71
T3: Remitting	23/163	1.04 (0.68, 1.60)	0.85	1.51 (1.10, 2.09)	0.84
T4: Increasing	52/244	1.66 (1.23, 2.24)	0.001	1.20 (0.62, 2.32)	0.01
T5: High	11/57	1.46 (0.80, 2.69)	0.22	1.06 (0.77, 1.45)	0.58

[a] Model 1: adjusted for age (time varying covariate) and sex.
[b] Model 2: additionally adjusted for *APOE*-ε4 carrier status, educational level, BMI, smoking, alcohol consumption, cognition at baseline, use of anti-depressant medication, hypertension, diabetes type 2, prevalent stroke and myocardial infarction.

Results remained consistent after censoring for incident stroke with only the increasing trajectory associated with a higher risk of dementia, HR 1.76 (95% CI: 1.31, 2.38) (**Table 3**). After censoring for incident stroke, estimates for the high trajectory were also suggestive of a higher risk of dementia, although non-significant, HR 1.40 (95% CI: 0.75, 2.62). In the analyses for Alzheimer's disease as outcome, again, the increasing trajectory was the only trajectory associated with a higher risk of dementia, HR 1.58 (95% CI: 1.15, 2.16) (**Supplement table 1**). However, as opposed to the risk of all-cause dementia, risk estimates for the high trajectory were not suggestive of a higher risk of Alzheimer's disease, HR 1.00 (95% CI: 0.48, 2.04). The remitting trajectory was not associated with a higher risk of dementia, HR 1.29 (95% CI: 0.90, 1.86), or Alzheimer's disease, HR 1.18 (95% CI: 0.77, 1.80).

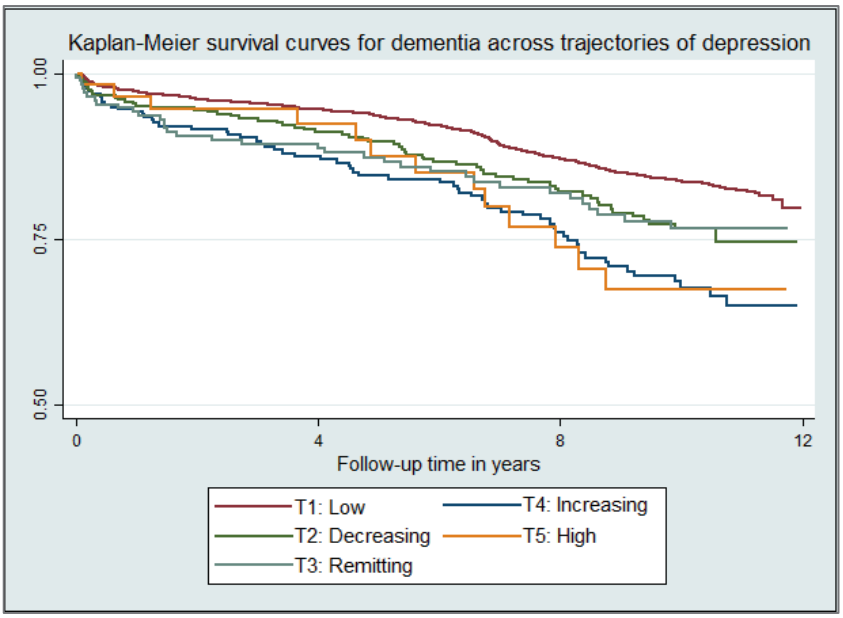

Figure 2. Figure 2. Dementia-free survival across different trajectories of depresive symptoms, N=3,300.

The figure shows Kaplan-Meier survival curves for dementia for different trajectories of standardized depressive symptoms'scores.

Table 3. Trajectories of depressive symptoms and risk of dementia after censoring incident stroke, N=3,028.

Trajectories	Cases/n	Risk of dementia, hazard ratios (95% confidence intervals)			
		Model 1[a]	P-value	Model 2[b]	P-value
T1: Low	257/2,290	1.00 (ref)		1.00 (ref)	
T2: Decreasing	45/306	1.12 (0.81, 1.54)	0.49	1.08 (0.78, 1.50)	0.64
T3: Remitting	22/156	1.08 (0.70, 1.68)	0.72	1.12 (0.72, 1.76)	0.60
T4: Increasing	50/226	1.84 (1.34, 2.51)	<0.001	1.76 (1.27, 2.44)	0.001
T5: High	10/50	1.47 (0.78, 2.77)	0.24	1.29 (0.64, 2.58)	0.47

175 cases of prevalent stroke were excluded at baseline; 234 participants suffered an incident stroke during follow-up.

[a]Model 1: adjusted for age (time varying covariate) and sex.

[b]Model 2: additionally adjusted for *APOE*-ε4 carrier status, educational level, BMI, smoking, alcohol consumption, cognition at baseline, use of anti-depressant medication, hypertension, diabetes type 2, and myocardial infarction.

In the short-term follow-up of 0-3 years, the highest risk of dementia was observed in persons within the remitting trajectory, HR 1.91 (95% CI: 1.13, 3.22), followed by those within the increasing trajectory, HR 1.59 (95% CI: 0.99, 2.55) which was borderline significant (**Table 4**). However, after excluding the first 3 years of follow-up, the remitting trajectory was no longer associated with a higher risk, but the increasing trajectory remained associated with a higher risk of dementia, HR 1.53 (95% CI: 1.07, 2.21). Estimates for the high trajectory were also suggestive of a higher risk, HR 1.46 (95% CI: 0.73, 2.94). In the competing risk analyses, although the risk estimates attenuated slightly, the overall pattern of results remained consistent; the increasing trajectory showed a higher risk of dementia, HR 1.52 (95% CI: 1.13, 2.05) (Data not shown).

Number of incident dementia cases and deaths per trajectory before the survival analyses is shown in **Supplement table 2**.

Table 4. Trajectories of depressive symptoms and risk of dementia with follow-up time stratified at 3 years.

Trajectories	Risk of dementia, hazard ratios (95% confidence intervals)					
	0-3 years of follow-up			>3 years of follow-up		
	Cases/n	Fully adjusted model	P-value	Cases/n	Fully adjusted model	P-value
T1: Low	53/2,420	1.00 (ref)		230/2,154	1.00 (ref)	
T2: Decreasing	8/319	0.71 (0.32, 1.58)	0.40	40/277	1.15 (0.81, 1.62)	0.42
T3: Remitting	7/163	1.48 (0.66, 3.33)	0.34	16/142	0.91 (0.54, 1.53)	0.73
T4: Increasing	12/244	1.47 (0.73, 2.95)	0.27	40/194	1.51 (1.05, 2.17)	0.03
T5: High	1/57	0.47 (0.06, 3.48)	0.46	10/44	1.42 (0.71, 2.86)	0.32

Hazard ratios are adjusted for age as a time varying covariate, sex, *APOE*-ε4 carrier status, educational level, BMI, smoking, alcohol consumption, cognition at baseline, use of anti-depressant medication, hypertension, diabetes type 2, prevalent stroke, and myocardial infarction.

DISCUSSION

In this study of community-dwelling older adults, we identified 5 distinct trajectories of depressive symptoms, characterized by low, decreasing, remitting, increasing and high depressive symptoms. The trajectory with increasing depressive symptoms was consistently associated with a higher risk of dementia. The trajectory typical of remitting depression was only associated with a higher risk of dementia in the short term.

We found that persons with steadily increasing symptoms of depression had a significantly higher incidence of dementia. This finding is consistent with the prodromal hypothesis, which suggests that depressive symptoms in older age possibly represent a prodrome or an early stage of dementia.[1,17,18] This implies that depressive symptoms appear as a "reaction" to the underlying subclinical cognitive impairment, and lie in a continuum between subclinical cognitive impairment and overt dementia. Where the available literature supporting the prodromal hypothesis is largely based on a single assessment of depression, we studied the course of depressive symptoms, and could demonstrate the gradual escalation of symptoms which started several years before the onset of clinical dementia.

These findings suggest that depression and dementia both are manifestations of a common etiology, where symptoms of depression precede the onset of clinical dementia. On a molecular level, the biological underpinnings of depression and neurodegenerative diseases overlap considerably, including insufficient antioxidant defense and neurogenesis, increased apoptosis, and immune system dysregulation.[19] Several potential biological mechanisms or their interplay can account for the observed association. First, vascular disease, is implicated in the development of depression, formulating the "vascular depression hypothesis",[20] as well as in the development of dementia, including Alzheimer's disease.[21,22] Second, studies suggest that hippocampal atrophy might also give rise to symptoms of depression, besides resulting in cognitive dysfunction.[1,23] Third, dysregulation of neurotransmitters could be the common pathway underlying the observed association. Studies have shown that altered serotonin activity and low adrenergic activity is observed in both depression and cognitive impairment.[24-26] Recently, lower levels of melatonin, which itself a serotonin derivative, have been implicated in cognitive impairment and depression in population-based settings.[27] Fourth, in recent studies low serum folate levels are suggested to be associated with both depression and dementia syndromes.[28] Finally, inflammation has been suggested as a possible link between depression and cognitive decline.[29]

Risk estimates for the trajectory with sustained high depressive symptoms in our study were also suggestive of a higher risk of dementia, but lack of sufficient power in this group might explain why these results did not reach statistical significance. However, the high trajectory was not associated with a higher risk of Alzheimer's disease. It can be speculated that the risk in this group was largely explained by vascular dementia, as persons within this trajectory had the highest prevalence of cardiovascular risk factors and events.

In our study, although the remitting trajectory was substantially high on depressive symptoms at around the second round of assessment, persons in this trajectory did not have a higher risk of dementia than those with no depressive symptoms. On one hand, this finding might suggest that having severe symptoms of depression at one point in time does not have any lasting influence, and does not imply a higher risk of dementia. It is likely that this peak of symptoms merely represent a normal reaction to adverse life events, such as loss of a partner or a loved one, or diagnoses of chronic debilitating illnesses, which might cause a transitory increase in depressive symptoms. On the other hand, we observed that the remitting trajectory showed a higher risk of dementia during the short-term. It is possible that the remitting trajectory is a reflection of the increasing trajectory except for the time lag between their peaks, and the rise of depressive symptoms in the remitting trajectory also represents a prodrome of incident dementia which occurred in the first few years of follow-up.

The main strengths of this study are the use of repeated measures of depressive symptoms, and the trajectory approach that allowed to study different courses of depression in relation to incidence of dementia. Other strengths include a population-based setting, large sample size, long follow-up, robust dementia follow-up, and adjustment for pertinent covariates. There are some limitations as well. Our analyses were fit based on assigned trajectories, and do not take into account the uncertainty in class-membership of each individual. As such, our confidence intervals may be anti-conservative. However, given that the posterior probabilities of class-membership were universally high (mean=, maximum=), and the robustness of our findings for the increasing symptoms trajectory across several analyses, it is unlikely that this would affect the general conclusions. There is a possibility of selection on health. Since depression is related to a higher risk of mortality, participants with most severe depression might have died before the survival analyses. This might have led to an underestimation of results, and is particularly true for the high trajectory, in which 49.8% of participants died before the survival analyses. Further, estimates for the high symptoms trajectory might not be precise because of insufficient power. Incidence of dementia during the trajectories (i.e.

before the start of the at-risk period for dementia used in survival analysis) was highest in the remitting, followed by the decreasing and high trajectories, possibly leading to an underestimation of risks due to selection. Additionally, we had insufficient power to compare the risks of vascular dementia.

In conclusion, different trajectories of depression, identified by repeated measures of depressive symptoms, were associated with different risks of dementia. Increasing depressive symptoms were associated with a higher risk of dementia. Future studies are warranted to unravel the biological underpinnings of these associations.

SUPPLEMENTARY INFORMATION

Supplementary Table 1. Trajectories of depressive symptoms and risk of Alzheimer's disease, N=3,203.

Trajectories	Cases/n	Risk of dementia, hazard ratios (95% confidence intervals)			
		Model 1[a]	P-value	Model 2[b]	p-value
T1: Low	283/2,420	1.00 (ref)		1.00 (ref)	
T2: Decreasing	48/319	1.10 (0.78, 1.55)	0.59	1.05 (0.74, 1.50)	0.77
T3: Remitting	23/163	1.06 (0.66, 1.70)	0.80	1.08 (0.67, 1.74)	0.75
T4: Increasing	52/244	1.69 (1.21, 2.35)	0.002	1.59 (1.12, 2.26)	0.009
T5: High	11/57	1.12 (0.53, 2.40)	0.76	1.00 (0.46, 2.18)	0.99

[a] Model 1: adjusted for age (time varying covariate) and sex.
[b] Model 2: additionally adjusted for *APOE*-ε4 carrier status, educational level, BMI, smoking, alcohol consumption, cognition at baseline, use of anti-depressant medication, hypertension, diabetes type 2, prevalent stroke, and myocardial infarction.

Supplementary Table 2. Incident dementia and deaths before the survival analysis.

Trajectory of depressive symptoms	Total number of participants	Dementia	Deaths
T1: Low	3,826	310 (8.1)	1,145 (30)
T2: Decreasing	673	92 (13.7)	307 (45.6)
T3: Remitting	278	46 (16.6)	91 (32.7)
T4: Increasing	394	47 (11.9)	128 (35.5)
T5: High	129	17 (13.2)	63 (49.0)

Values are counts (percentage).

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CHAPTER 2.3

17-year trajectories of depressive symptoms in community-dwelling older adults and the risk of mortality over 14 years

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Submitted

ABSTRACT

Background: Clinical depression and depressive symptoms have consistently been shown to be associated with excess mortality. However, populations of depressed persons are typically comprised of individuals that follow different courses of depression and thus might carry different risks of mortality.

Methods: In the population-based Rotterdam Study, we examined depressive symptoms trajectories of 5,754 participants (aged ≥ 55) over 17 years, and related them to the risk of mortality. Depressive symptoms were assessed by the Center for Epidemiological Studies Depression scale (CES-D), and follow-up for mortality was complete until March 2015 (mean follow-up 14±6 years, max=21.7 years). Joint-latent-class trajectory modelling was used to identify trajectories of depressive symptoms over time, and model mortality risk over time across trajectories, adjusting for relevant confounders.

Results: Six trajectories of depressive symptoms were identified, characterized by low, chronic-mild, decreasing, remitting, increasing, and high depressive symptoms. Trajectories with increasing or chronic high depressive symptoms had a high risk of mortality (>30% by six years), while the trajectories with chronic-mild and remitting depressive symptoms had a low risk (<10% by six years).

Conclusions: The chronicity and course of depressive symptoms predicted mortality better than the severity at one time point. Chronic high or consistently increasing symptoms were strongly related to mortality, whereas persons with a trajectory typical of remitting depression did not show excess mortality. Our results open avenues for etiological and prognostic research to focus upon risk factors key to a particular trajectory.

INTRODUCTION

Clinical depression or clinically relevant depressive symptoms are highly prevalent among community-dwelling older adults.[1] Clinically relevant depressive symptoms, which gauge sub-threshold depression, are not only more prevalent but are associated with impairment similar to major depression.[2] Both clinical depression and depressive symptoms have been repeatedly shown to be associated with a higher risk of mortality in patient and community samples.[3,4]

Depressive symptoms may root from diverse biologic and psychosocial factors including genetic predisposition and gene-environment interactions, chronic diseases or disability, life events and daily hassles, social disadvantage, personality attributes, and bereavement.[2] In every individual with depressive symptoms, depression follows a unique course dependent upon a combination of such risk and perpetuating factors, which typically interact. In large populations, these individuals can be combined into unique groups with distinct trajectories of depressive symptoms course.[5] For instance, persons with clinically relevant depressive symptoms may experience them transiently and undergo full remission. Other persons may undergo partial remission only. Yet other persons may experience several repeated recurrences of symptoms throughout life with intermittent periods of health, while a minority may become chronically depressed. However, it remains largely unknown how these different courses of depressive symptoms relate to mortality, as the existing studies mainly focus on prevalent depression or depressive symptoms. Thus the course of depression is largely neglected, which is an important gap considering the relapsing and remitting nature of depression.[6-10] This issue can be addressed by utilizing repeated measures of depressive symptoms to study the course of depression, identify different trajectories of depression in a population, and then study the risk of mortality across these trajectories. Such an approach can help unravel high risk groups, and "filter out" the effects of any group in which depressive symptoms may have little or no effect on long-term health outcomes. Understanding the possibly differential risks associated with the course of depressive symptoms upon mortality and other outcomes opens avenues for efficient preventive and therapeutic strategies.

The aims of this study were to identify different trajectories of depressive symptoms in community-dwelling older adults using repeated measures of depressive symptoms, and study the risk of mortality across these trajectories over a maximum follow-up of 22 years. We were especially interested to investigate how the remitting and relapsing course of

depressive symptoms relate to mortality, as many persons suffer from depression during the life time, but revert back to the non-depressed state.

METHODS

Setting

This study was embedded in a large population-based cohort of persons aged 55 and older, ongoing since 1990. Follow-up examinations including home interviews and physical exams at a research center take place every three to four years. In addition, the cohort is continuously monitored for all major events by a highly efficient data linkage system between the study database and the general practitioners.[11] This study is approved by the medical ethics committee according to the Population Study Act, executed by the Ministry of Health of the Netherlands. A written informed consent was obtained from all participants.

Study population

This study utilized depressive symptoms data from four examination rounds between 1993 and 2011**,** and included 5,754 participants with at least one assessment of depressive symptoms at any of the four rounds. Number of participants that had depressive symptoms data available at more than one examination round was 4,385, and 1,639 participants had depressive symptoms data for all four examination rounds. At baseline (1993-1995), depressive symptoms data were available for 4,874 participants. In the subsequent rounds in 1997-1999, 2002-2004, and 2009-2011, data for depressive symptoms were available for 4,500, 3,434, and 2,107 participants respectively.

Assessment of depressive symptoms

In the first examination round, we measured depressive symptoms using two different instruments: the validated Dutch version of the Center for Epidemiological Studies Depression Scale (CES-D),[12] and the Hospital Anxiety and Depression Scale-Depression (HADS-D).[13] In this round, assessment of depressive symptoms was introduced in the study protocol, and as a pilot project, a random half of participants received the CES-D questionnaire, while the other half received the HADS-D questionnaire. For all subsequent rounds, only CES-D was used. The CES-D comprises 20 items, each with a possible score of 0-3, and the score ranges from 0-60.[12] HADS-D comprises of 7 items each with a possible score of 0-3, and the score ranges from 0-21.[13] Both scales are valid and reliable

instruments for assessment of depression and can indicate clinically relevant depressive symptoms. We checked for evidence whether this introduces information bias but we found the same percentage of screen positives (10%) using validated, pre-defined cut-offs.[14] For analyses, depressive symptoms scores were used as a standardized variable, calculated as weighted individual score minus mean score, divided by the standard deviation. Scores were weighted for missing items only if missing items did not exceed 25%; scores with missing items >25% were excluded.

Vital Status

Information on vital status was obtained continuously via computerized linkages from municipal authorities. Mortality follow-up was complete until March 2015.

Other measurements

Covariates

All covariates were measured at baseline, and were considered potential confounders because they are associated with depression and are independent predictors of mortality.

Socio-demographic: Age, sex, educational level,[15] partner status,[16] and loss of a partner were considered potential confounders. Educational level was categorized as primary (primary only or unfinished secondary) or higher than primary education (secondary, vocational or university). For partner-status, participants were classified as living or not living with a partner. Participants were inquired if they had experienced loss of a partner and categorized into two groups accordingly.

Health-indicators: Body mass index (BMI) was calculated as weight in kilograms/height in meters.[2] Smoking habits were inquired during the home interview and participants were classified into never, past, and current smokers. Cognition [17] was assessed by the Mini-Mental State examination (MMSE).[18] Disability index was derived from the Activities of Daily Living from the Stanford Health Assessment Questionnaire Disability Index (HAQ-DI).[19]

Prevalent disease and medication: Hypertension was defined as systolic blood pressure ≥ 140 mmHg, or diastolic blood pressure ≥ 90 mmHg, or use of antihypertensive medication assessed by interview and pharmacy records.[20] Diabetes Mellitus type 2 (DM) was diagnosed as fasting blood glucose ≥ 7.0 mmol/l, or use of anti-diabetic medication evaluated by interview and pharmacy records.[21] Previous stroke, myocardial infarction (MI), and hip fractures were determined by reported events on interview and confirmed

by medical records.[11] For cancers, linkage of the regional pathology databases to the Rotterdam Study identified cancer patients in more than 95% of persons. Use of anti-depressant medication was assessed by interview and confirmed by pharmacy records.[22]

Dementia

Dementia was diagnosed according to DSM-III-R criteria for dementia, NINCDS-ADRDA for Alzheimer's disease, NINCDS-AIREN criteria for vascular dementia following a standard protocol, and through continuous monitoring as described previously.[23]

Statistical analyses

Main analyses

Participants entered the study at the examination round in 1993-1995 and were followed for all-cause mortality until 2015. We used the joint latent class mixed model (Jointlcmm-R-package) [24] to study the effect of longitudinal course of depressive symptoms over time on the risk of mortality. This model, which is a form of linear mixed model, identifies distinct trajectories, which are latent classes of individuals following similar progression of an outcome over time or with age.[25-27] For every subject, the posterior probabilities to belong to each trajectory were computed, taking into account their age, sex and educational level, and subjects were assigned to the trajectory with the highest probability. We tested different numbers and forms of possible trajectories starting with 1 trajectory, till the Bayesian Information Criterion (BIC) minimized.[26] The criteria for the final number of trajectories were based on minimizing the BIC while maintaining the posterior probabilities by class (>0.70), and class size (at least 2% of the population). Analyses were repeated using different random starting values to prevent convergence to a local maximum. We included random intercepts and random slopes over time. The joint survival model was defined by a two parameter Weibull distribution with a class-specific baseline risk function for mortality. In a basic survival model (Model 1), we adjusted for age and sex only, and in an extended model (Model 2), we additionally adjusted for educational level, partner-status, loss of partner, BMI, smoking, cognitive score, disability index, prevalent disease at baseline, including hypertension, DM, MI, stroke, cancer, hip fractures, and use of anti-depressant medication. Survival curves were plotted for the mean of the covariates. Analyses were conducted using the lcmm package in R version 3.1.2.

During the period of depressive symptoms assessments (1993-2011), 814 participants were diagnosed of incident dementia. In a sensitivity analyses, we did not use the

depressive symptoms scores after the diagnoses of dementia, however, since the results did not change meaningfully, we used all the depressive symptoms assessments available.

Alternative statistical approach

We performed the latent-class trajectory modelling using the Stata Software Version 13,[28] which uses the same methodology to assign trajectories as the Jointlcmm. However, it has the limitation of unavailability of a joint survival model with trajectory classification. Detailed methods for this analysis are provided in the supplement text (Supplement methods). In a further step, we used the trajectory classifications as indicator variables to calculate hazard ratios for mortality using Cox proportional hazards (PH) models, using the low depressive symptoms trajectory as reference. Adherence to the PH assumption was tested by plotting smoothed Schoenfeld residuals against time. For these analyses also, two models were fitted. Model 1 was adjusted for age and sex, and Model 2 was adjusted for all aforementioned covariates. Analyses were performed using the Stata Software Version 13 (StataCorp, College Station, TX, USA).

Finally, for comparability with other studies, we also tested the association of clinically relevant depressive symptoms measured continuously at baseline with mortality, using Cox PH models.

Differences in baseline characteristics across trajectories

To test whether baseline characteristics of persons differ across trajectories, we used Analysis of Variance (ANOVA) test with post-hoc Bonferroni for continuous variables, and logistic regression for categorical variables. This was performed for both main and alternative statistical analyses.

RESULTS

Main analyses

5,754 participants (mean age 70±8.1 years, 59% women), who were dementia-free at baseline, were followed for a mean of 14±6 years (maximum follow-up= 21.7 years), during which 3,716 participants died. For the trajectory classification, the model with six latent classes of depressive symptoms had the best fit with the lowest BIC (**Table 1**). Mean posterior probabilities of class-membership were at least 0.71 (lowest average of any class). In contrast, the seven-class model had lower mean posterior probabilities for class-

membership (0.57). Further, this model only added a small class (n=111) with a pattern largely overlapping the low symptoms trajectory in the six-class model, thus added little extra information.

The six trajectories of depressive symptoms are shown in **Figure 1A**. They were named according to their graphical pattern to facilitate interpretability. 76% of participants were assigned to a trajectory which had low depressive symptoms throughout the study period (*Low*, n=4,386). The more symptomatic classes were characterized by the following trajectories: mild symptoms throughout the follow-up with little fluctuation up till 19 years (*Chronic-mild*, n=298, 5.2%); high depressive symptoms at baseline, remitted and then remained low (*Decreasing*, n=247, 4.3%); a transient increase in depressive symptoms but remitted (*Remitting*, n=293, 5.1%); increasing symptoms throughout the follow-up (*Increasing*, n=395, 6.9%); and chronic high depressive symptoms throughout (*High*, n=135, 2.3%).

Table 2 shows a comparison of baseline characteristics of participants by trajectories. Those assigned to the increasing or high depressive symptoms trajectory had a higher prevalence of chronic disease and health events.

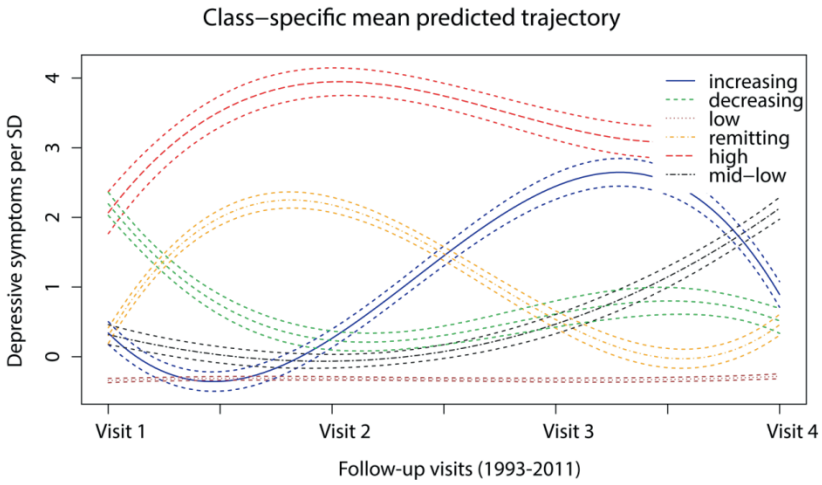
In the survival model adjusted for age and sex only (**Model 1**), those in the *high* depressive symptoms trajectory had a high cumulative incidence of mortality (predicted risk of death over 35% by 6 years and 80% by 12 years) (**Table 3, Figure 1B**). Those in the *increasing* trajectory had a similar high mortality (predicted risk of death 33% by 6 years and over 75% by 12 years). These risks were substantially higher than the risks in the *chronic-mild* trajectory (predicted risk of death 0.6% by 6 years and 12% by 12 years). The *low, decreasing*, and *remitting* trajectories of depressive symptoms, all had similar risks of mortality (e.g. ~30% by 12 years).

Table 1. Summary of joint model fits.

N classes	Log-likelihood	N parameters	BIC	Score test (p-value)	Latent class proportion (%)
1 Class	-33056.31	16	66251.14	76.71 (0)	100
2 Classes	-32228.41	26	64681.91	29.51 (0)	93.97, 6.03
3 Classes	-31678.28	36	63668.25	22.98 (0)	88.58, 6.78, 4.64
4 Classes	-31330.15	46	63058.56	17.86 (<0.001)	5.72, 82.55, 6.71, 5.02
5 Classes	-30971.22	56	62427.27	12.84 (0.002)	7.7, 7.79, 76.82, 5.11, 2.59
6 Classes	-30735.53	66	62042.46	10.43 (0.005)	6.86, 4.29, 76.23, 5.09, 2.35, 5.18
7 Classes	-30687.38	76	62032.75	10.16 (0.006)	6.73, 4.17, 73.71, 5.13, 2.29, 6.05, 1.93

Abbreviations: N = number, BIC = Bayesian information criterion.
Minimum posterior probability for six-class model was 0.71
Minimum posterior probability for 7 class model was 0.57; the seventh class in this model comprised 111 participants with a pattern largely overlapping the low symptoms trajectory.

1A



1B

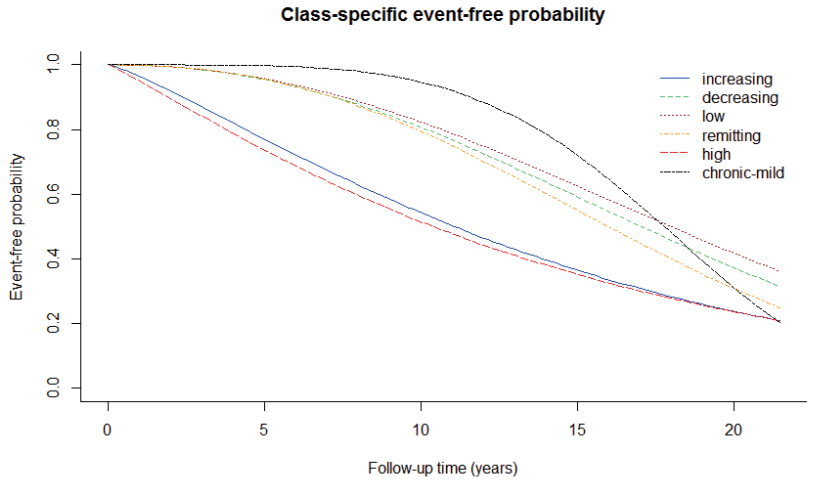


Figure 1. Trajectories of depressive symptoms and risk of mortality. **1A**: Predicted evolution of depressive symptoms over time. Trajectory classification is adjusted for age, sex and education. **1B**: Class-specific survival. Survival curves adjusted for age and sex.

Table 2. Baseline characteristics of study population between 1993-1995, N=5,754.

Characteristics	Increasing N=395	Decreasing N=247	Low N=4368	Remitting N=293	High N=135	Chronic-mild N=298
Socio-demographics						
Age	71.5 (7.9)	69.6 (8.4)	69.4 (8.2)[a]	69.9 (7.8)	71.1 (8.0)	69.1 (7.5)[a]
Women	248 (62.8)	154 (62.3)	2503 (57.1)[a]	212 (72.3)[a, b, c]	81 (60.0)[d]	204 (68.5)[c]
Primary education	84 (21.3)	57 (23.1)	835 (19.0)	54 (18.4)	36 (26.7)[c]	60 (20.1)
Living with a partner	245 (62.7)	146 (59.8)	2958 (68.3)[a, b]	191 (65.4)	76 (58.0)[c]	196 (66.0)
Loss of a partner	30 (10.1)	32 (16.7)[a]	283 (9.8)[b]	22 (10.4)	16 (14.0)	24 (11.6)
Health indicators						
Body mass index	26.8 (3.6)	26.6 (3.4)	26.4 (3.3)	26.5 (3.4)	26.3 (3.4)	26.9 (3.5)
Current smoking	108 (27.3)	56 (22.7)	84 (19.3)[a]	52 (17.7)[a]	39 (28.9)[c, d]	42 (14.1)[a, b, c, e]
MMSE score	27.5 (1.7)	27.5 (2.1)	27.9 (1.6)	27.7 (1.5)	27.2 (2.0)	27.9 (1.5)
Disability index	0.5 (0.6)	0.4 (0.5)	0.3 (0.4)[a, b]	0.4 (0.5)[c]	0.7 (0.7)[a, b, c, d]	0.4 (0.5)[a, c, e]
Medical history						
Hypertension	312 (79.0)	163 (66.0)[a]	3138 (71.5)[a]	197 (67.2)[a]	98 (72.6)	209 (70.1)[a]
Diabetes Mellitus 2	31 (7.8)	15 (6.1)	242 (5.5)	23 (7.8)	13 (9.6)[c]	14 (4.7)[e]
Myocardial infarction	42 (10.6)	15 (6.1)[a]	323 (7.4)[a]	23 (7.8)	15 (11.1)	14 (4.7)[a, e]
Stroke	33 (8.3)	9 (3.6)[a]	150 (3.4)[a]	15 (5.1)	15 (11.1)[b, c, d]	5 (1.7)[a, d, e]
Cancer	36 (9.1)	10 (4.0)[a]	217 (4.9)[a]	15 (5.1)[a]	12 (8.9)[c]	8 (2.7)[a, e]
Hip fractures	10 (2.5)	0	40 (0.9)[a]	0	3 (2.2)	1 (0.3)a
Anti-depressants use	11 (3.4)	7 (3.5)	58 (2.0)[a]	16 (6.8)[c]	15 (12.6)[a, b, c, d]	10 (4.7)[c, e]

Values are counts (percentage) or means (standard deviation).

[a] If a trajectory is different from *increasing* Trajectory; [b] If a trajectory is different from *decreasing* trajectory; [c] If a trajectory is different from *low* trajectory; [d] If a trajectory is different from *remitting* trajectory; [e] If a trajectory is different from *high* trajectory.

Comparisons are based on Analysis of Variance (ANOVA) for continuous and logistic regression for categorical variables.

In the fully adjusted survival model (Model 2), somewhat different results were obtained suggesting that covariates accounted for some of the observed effects. The *increasing* trajectory was still characterized by a very high cumulative incidence of mortality (predicted risk of death 85% by 6 years), like the *decreasing* depressive symptoms trajectory (predicted risk of death 59% by 6 years) (**Figure 2**). The *high, low* and *remitting* trajectories only showed a moderate risk in this model (predicted risk of death <25% by 6 years). The *chronic-mild* trajectory again showed low risk of <2 % by 6 years.

Alternative statistical approach

Results for these analyses are shown as a supplement (**Supplement Figure 1 & 2, Supplement Table 1**). The main setback of this approach was that the hazard ratios (HRs) were calculated without accounting for the uncertainty in class-membership and does not fit a joint survival model of depressive symptoms trajectories and mortality. However, the probabilities of the class-membership in these analyses ranged from 0.7 to 0.9, and both the obtained classes and the association with mortality were largely similar to those obtained in the joint survival model. The differences observed were that we identified two trajectories with a *remitting* pattern in this analysis, and did not find the *chronic-mild* trajectory. Additionally, the HRs suggested a much higher risk of mortality in those with *high* depressive symptoms, than observed in the joint survival model. We show these trajectories and HRs for illustration only, but can only be very cautiously interpreted. This was also reflected in the non-proportionality of hazards for the *remitting* and the *increasing* trajectories in these analysis.

When we tested the association of clinically relevant depressive symptoms at baseline for comparability with other studies, depression was associated with a higher risk of mortality in the basic model, HR per SD 1.15 (95% CI: 1.05, 1.25), but this attenuated after adjusting for all confounders, HR per SD 1.03 (95% CI: 0.99, 1.07).

Table 3. Cumulative incidences of death for trajectories of depressive symptoms.

Follow-up	Cumulative incidences of mortality per class, (%)					
	Increasing n=395	Decreasing n=247	Low n=4,386	Remitting n=293	High n=135	Chronic-mild n=298
3 years	14.1	1.6	1.5	1.4	17.5	0.03
6 years	33.1	7.1	6.6	7.1	37.9	0.6
9 years	54.4	17.4	15.9	18.3	59.7	3.6
12 years	75.9	31.6	28.5	34.7	80.7	11.9

Cumulative incidence adjusted for age and sex.

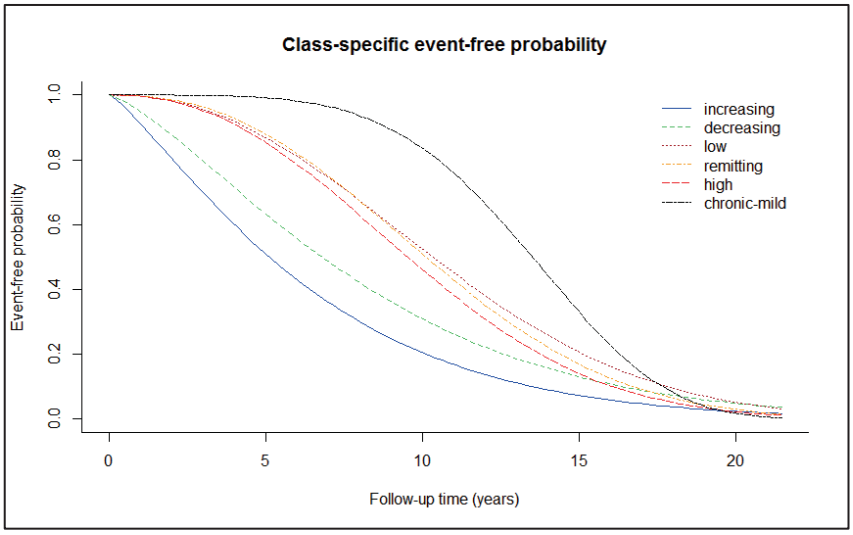


Figure 2. Survival across different trajectories of depressive symptoms.
Survival curves are adjusted for age, sex educational-level, partner-status, smoking status, body mass index, cognitive score, disability index, prevalent disease (hypertension, diabetes mellitus type 2, hip fractures, myocardial infarction, stroke, and cancer), and use of anti-depressant medication.

DISCUSSION

This population-based prospective study of 5754 older adults suggests that different trajectories of depressive symptoms have different risks of mortality during a long-follow-up. Incidence of mortality was most substantial in persons with increasing, decreasing, or high depressive symptoms trajectories. In contrast, persons with low or remitting depressive symptoms had a low incidence of mortality.

Individuals in the high and increasing depression trajectories in our study were more likely to be older, single or widowed, less educated, or have a higher prevalence of physical disability and chronic diseases. These findings concur with the well-documented evidence suggesting that depression is associated with increasing age, lower education, having no partner, and chronic diseases including diabetes mellitus, coronary heart disease, stroke, cancers, and fractures, particularly of the hip joint.[29-33]

Previously, three distinct patterns of depressive symptoms severity in relation to differential mortality risk have been reported over a 12-month period in primary-care older adults.[5] In our study, the trajectories with *increasing* and *high* depressive symptoms were associated with a higher risk of mortality. This suggests that increasing or chronic symptoms predict mortality, and not high symptoms at only one point in time. However, the excess mortality in persons in the chronic *high* symptoms trajectory could be largely explained by pre-existing comorbidities, such as hypertension, diabetes, myocardial infarction, and stroke. In the other trajectories, such as with *increasing* and *decreasing* symptoms, arguably, more subclinical physiologic mechanisms underlie the association with mortality rather than clinically apparent disorders. For example, the hypothalamic-pituitary-axis (HPA), which is often hyperactive in depressed patients,[34] hyperactivity of the autonomic nervous system and enhanced platelet aggregation in depressed patients, all are common causes of cardiovascular disease and thus may contribute to mortality.[35,36] Also, lifestyle factors, such as non-compliance to medication, unhealthy life-style with poor diet or lack of exercise could underlie our observations.

Interestingly, the *remitting* trajectory, although being high on the depressive symptoms scale at at-least one point, did not have excess mortality. Perhaps high depressive symptoms in these persons indicate a “normal reaction” to life stressors followed by full recovery and had no lasting adverse influence on health. It is also possible that after getting diagnosed with chronic illnesses such as diabetes or hypertension, individuals react with a transient increase in depressive symptoms, but at the same time become more conscious about health and thus might have a better survival. These results suggest

that a remitting course of depressive symptoms has *no worse* survival than a trajectory characterized by few or no depressive symptoms. In some persons, the poor prognosis of depressive symptoms would certainly have to be modified if depressive symptoms were measured repeatedly. Hence, other studies observing a higher mortality risk associated with prevalent depression [6-10] might be under-estimating the risk of those with more chronic depression, and at the same time over-estimating the risk for those with remitting symptoms.

An interesting finding consistent across both analyses, is the higher risk of mortality observed for the *decreasing* trajectory, although the remission after round 2 was quite stable. One reason could be the partner loss at baseline. It is known that lack of partner support and social engagement can adversely affect health in general,[16,29,37] which might explain this finding. Additionally, the chronicity of symptoms prior to the study observation period might explain this higher risk, but, we did not have that information.

Finally, depression assessment at baseline only modestly predicted mortality. Perhaps in our study, more depressive symptoms at a single assessment possibly did little effect and the association was largely explained by the presence of unfavorable socio-demographic factors, poor health, and chronic diseases.

The novelty of this study is to study different trajectories of depressive symptoms over 17 years in relation to mortality. Other strengths include a large population-based sample, a long and thorough mortality follow-up, and consideration of several potential confounders. The main limitation of the study is that we could not calculate confidence intervals for the survival model which a limitation of the statistical software. Although we calculated hazard ratios with confidence intervals in the alternative approach (Stata), they might not be precise as the uncertainty associated with class-membership could not be accounted for in this method. Further in this method, the proportionality of hazard assumption was not met for two trajectories. Moreover, we did not exclude participants with incident dementia during trajectory assignment, and they remained at-risk for mortality in our analyses, as excluding them could have introduced selection bias. We also repeated analyses by excluding the depressive symptoms scores after dementia diagnosis, as it might have introduced some information bias. However, the trajectories were largely similar, and thus all available scores were used. Another limitation is that while we could model the course of depressive symptoms over a 17 year period, we did not have information on how depressed the participants were previously. Finally, although we assessed the association between depressive symptoms trajectories and mortality in a number of ways, each analysis had inherent limitations that restrict causal interpretation.

Nonetheless, the current analyses represent an important first step toward describing the potential role of chronicity and late-life course of depressive symptoms in excess mortality. Future research may build upon these observed patterns to address refined etiological questions.

SUPPLEMENTARY INFORMATION

Methods

Identification of trajectories using Stata Software Version 13 (StataCorp, College Station, TX, USA):

We used the Traj Stata command for the latent class trajectory modelling of depressive symptoms data over time. This is a specialized form of finite mixture modelling, and is designed to identify distinct trajectories, which are latent classes of individuals following similar progression of an outcome over time or with age.(Jones BL, 2013) One of the key assumptions of the model is that a population comprises multiple trajectories. We used the censored normal distribution (cnorm) in trajectory modelling which is intended for the analysis of repeatedly measured continuous scales. For every subject, the posterior probabilities for each trajectory were computed, taking into account their age, sex and educational level, and subjects were assigned to the trajectory with the highest probability. We tested different numbers and forms of possible trajectories starting with 1 trajectory, till the Bayesian Information Criterion (BIC) minimized. Thus the final number of trajectories was determined using the BIC(Proust-Lima C, 2015) and by assessing the mean posterior probabilities of belonging to each trajectory according to final classification. After identification, we assigned names to the trajectories based on their graphical patterns to facilitate comprehension and interpretation of results.

Assessment of risk of mortality:

Subsequently, we studied the risk of mortality in the identified trajectories of depressive symptoms using Cox proportional hazards model. Adherence to the proportional hazards (PH) assumption was tested by plotting smoothed Schoenfeld residuals against time. The trajectory classifications were used as indicator variables only, to calculate hazard ratios of depressive symptoms for mortality in survival analyses. Two models were fitted: Model 1 was adjusted for age and sex only, and Model 2 was additionally adjusted for educational level, partner-status, loss of partner, BMI, smoking, cognitive score, disability index, prevalent disease at baseline, including hypertension, DM, MI, stroke, cancer, hip fractures, and use of anti-depressant medication.

Supplementary Table 1. Risk of mortality across trajectories of depressive symptoms.

Trajectories	Deaths/N	Risk of mortality, Hazard ratios (95% Confidence intervals			
		Model 1[a]	P-value	Model 2[b]	P-value
T1: Low	2,226/3,812	1.00 (ref)		1.00 (ref)	
T2: Decreasing	444/580	1.46 (1.32, 1.62)	<0.001	1.24 (1.11, 1.37)	<0.001
T3: Remitting I	219/310	1.11 (0.97, 1.28)	0.13	0.94 (0.81, 1.08)	0.36
T4: Remitting II[c]	104/164	0.98 (0.80, 1.19)	0.83	0.78 (0.64, 0.95)	0.01
T5: Increasing[c]	622/762	1.30 (1.18, 1.43)	<0.001	1.15 (1.05, 1.27)	0.003
T6: High	101/126	1.90 (1.55, 2.32)	<0.001	1.38 (1.12, 1.69)	0.002

[a] Model 1: adjusted for age and sex.
[b] Model 2: additionally adjusted for educational-level, partner-status, smoking status, body mass index, cognitive score, disability index, prevalent disease (hypertension, diabetes mellitus type 2, hip fractures, myocardial infarction, stroke, and cancer), and use of anti-depressant medication.
[c] Proportional hazards assumption not met for these trajectories.

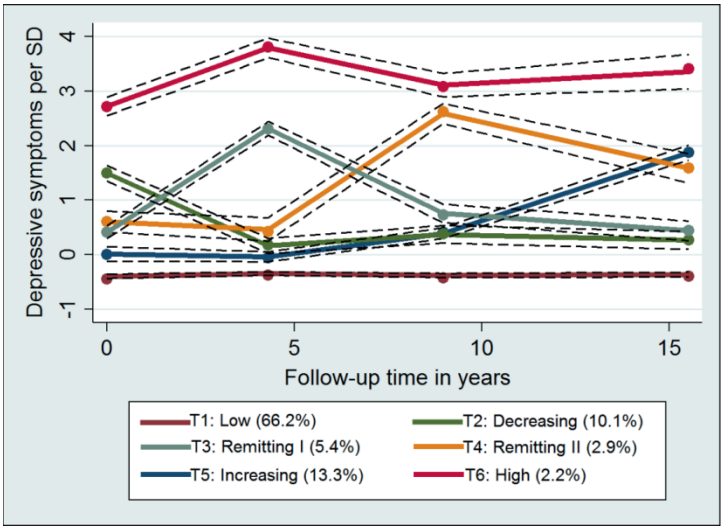


Figure 1. Trajectories of depressive symptoms over 17 years, N=5,754.
The figure shows distinct trajectories of standardized depressive symptoms scores over 17 years of follow-up.

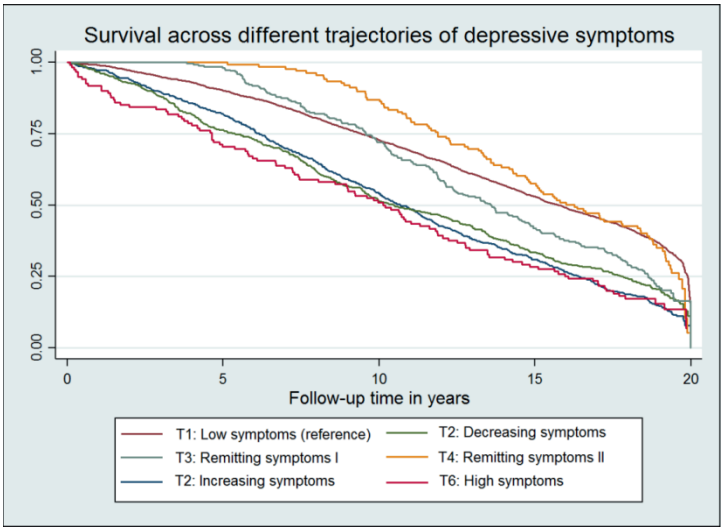


Figure 2. Survival across different trajectories of depressive symptoms.
The figure shows Kaplan-Meier survival curves for different trajectories of standardized depressive symptoms scores over 20 years of follow-up.

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CHAPTER 2.4

Anxiety is not associated with the risk of dementia or cognitive decline: the Rotterdam Study

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ABSTRACT

Background: Anxiety and depression frequently co-occur in the elderly and in patients with dementia. Prior research has shown that depression is related to the risk of dementia, but the effect of anxiety on dementia remains unclear. We studied whether anxiety symptoms and anxiety disorders are associated with the risk of dementia and cognition. In the population-based Rotterdam Study.

Methods: In 1993-1995, anxiety symptoms were assessed in 2,708 non-demented participants with the Hospital Anxiety and Depression Scale (HADS) (sample I). In 2002-2004, anxiety disorders were assessed using the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, in 3,069 non-demented participants (sample II). In both study samples, participants were continuously monitored for dementia until January 1, 2011. Cognition was tested in 2002-2004 and at a follow-up visit in 2009-2011 in sample II only.

Results: In sample I, 358 persons developed dementia and in sample II, 248 persons developed dementia. We did not find an association with the risk of dementia for anxiety symptoms (hazard ratio (HR) 1.05, 95%-confidence interval (CI) 0.77; 1.43, Wald-statistic 0.08, p-value 0.77, degrees of freedom (df) 1) or for anxiety disorders (HR 0.92, 95%-CI: 0.58; 1.45, Wald-statistic 0.14, p-value 0.71, df 1). We could demonstrate an association of anxiety disorders with poor cognition cross-sectionally, but this attenuated after additional adjustments.

Conclusions: Our findings do not offer evidence for an association between anxiety symptoms or anxiety disorders with the risk of dementia or with cognition. This suggests that anxiety is not a risk factor nor a prodrome of dementia in an elderly, community-dwelling population.

INTRODUCTION

Psychiatric disorders, such as anxiety and depression, are common co-manifestations in dementia.[1-4] Not only are anxiety and depression often diagnosed in demented persons, but anxiety and depression may be one of the presenting symptoms of dementia.[2] Studies demonstrating a longitudinal association between depression and incident dementia suggest that depression might be a risk factor of dementia or alternatively an early clinical marker of incipient dementia.[5,6] Since anxiety shares both symptoms and risk factors with depression,[7] it is often thought that anxiety is also associated with the risk of dementia. Here too, the underlying hypothesis is based on either a shared etiology between anxiety and dementia, or on anxiety as early symptom of incipient and yet to be diagnosed dementia.[7-9]

Previous studies examining the association between anxiety and dementia or cognition remain inconclusive.[8-23] Methodological differences, such as assessment of anxiety or selection of study participants might explain inconsistencies. Moreover, in some studies reporting an association between anxiety and increased risk of dementia, the influence of depression on these associations remains unclear.[8,23]

In the prospective, population-based Rotterdam Study we studied the association of anxiety symptoms and anxiety disorders with the risk of incident dementia. To further explore the effect of anxiety, we also related anxiety disorders to cognition, both cross-sectionally and longitudinally, in persons without dementia.

METHODS

Setting

The Rotterdam Study is a prospective, population-based cohort that started in 1990 and is conducted among inhabitants, aged 55 years and older, of Ommoord, a district of Rotterdam, The Netherlands. Of the 10,215 invited inhabitants, 7,983 (78%) agreed to participate in the baseline examination. Up until 2013, there have been 5 examination rounds. Details of the study have been described elsewhere.[24] For this study, two baselines were chosen because information on anxiety symptoms and anxiety disorders was collected at different examination rounds.

Anxiety symptoms were assessed in 1993-1995 and this examination was constituted as baseline for sample I. Of the 6,315 subjects that participated at baseline, a random half (N=3,060) was invited to undergo screening for anxiety symptoms using the Hospital Anxiety and Depression Scale (HADS). Of these, 83 participants were excluded because they were not sufficiently screened for anxiety symptoms. Furthermore, 44 persons were excluded because they had prevalent dementia, 215 because they did not agree to undergo screening for dementia, and 10 participants were excluded due to lack of follow-up data. Eventually, sample I comprised 2,708 non-demented subjects that underwent both screening for anxiety symptoms and had follow-up information on dementia diagnosis.

Anxiety disorders were assessed in 2002-2004 and this examination was constituted as baseline for sample II. Of the 3,550 participants that were eligible, a total of 3,259 persons underwent screening for anxiety disorders. Of this sample, 124 persons were excluded because they were prevalent demented, 56 because they did not agree to undergo screening for dementia, and 10 participants were excluded for lack of follow-up data. Finally, sample II comprised 3,069 non-demented subjects that underwent screening for anxiety disorders and had follow-up information on dementia diagnosis. Of the 3,069 subjects that were included in sample II, 1,506 subjects were also included in sample I. Follow-up for dementia for both samples was complete until January 1, 2011 (for 98.3% of potential person-years in sample I and for 96.9% of potential persons-years in sample II). Sample II was also used to assess the association between anxiety disorders and cognition. Cognition was assessed at baseline in 2002-2004 and at follow-up in 2009-2011. Of the 3,069 subjects in sample II, 2,351 subjects had information on cognitive performance cross-sectionally and 1,115 subjects had information on cognitive decline longitudinally.

The medical ethics committee at Erasmus University of Rotterdam approved the study and written informed consent was obtained from all subjects.

Anxiety symptoms

Anxiety symptoms were assessed in 1993-1995 in a random half of the study population using the HADS.[25] The HADS is a brief questionnaire that is used often in the Netherlands for the assessment of anxiety symptoms and depressive symptoms. It consists of two subscales, the HADS-Depression (HADS-D) and HADS-Anxiety (HADS-A), each including 7-items. Total scores range from 0-21 with higher scores indicating more symptoms of depression or anxiety. A score of 8 or higher on the HADS-A scale was considered positive for anxiety symptoms.

Anxiety disorders

Anxiety disorders were diagnosed in 2002-2004 using a slightly adapted version of the Munich version of the Composite International Diagnostic Interview (M-CIDI).[26] We assessed the 12-month prevalence of anxiety disorders. The following anxiety disorders were assessed with a computerized diagnostic algorithm according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria: generalized anxiety disorder, panic disorder, agoraphobia, social phobia, and specific phobia.[27] These results were converted into a binary variable that stated whether a participant had at least one of the above-mentioned anxiety disorders or was free of any anxiety disorders. This variable was used in the analysis.

Dementia

Participants were screened for dementia at every examination round using a three-step protocol. Screening was done using the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level.[28,29] Screen-positives (MMSE <26 or GMS organic level >0) subsequently underwent an examination and informant interview with the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX).[30] Participants who were suspected of having dementia, underwent, if necessary, further neuropsychological testing. Additionally, the total cohort was continuously monitored for dementia through computerized linkage between the study database and digitized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. When information on neuro-imaging was required and available, it was used for decision making on the diagnosis. In the end, a consensus panel, led by a neurologist, decided on the final diagnosis in accordance with standard criteria for

dementia (Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised (DSM-III-R)) and Alzheimer Disease (National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA)).[31,32]

Cognition

In sample II, cognitive performance was assessed at baseline in 2002-2004 and at follow-up in 2009-2011 with a cognitive test battery comprising Letter-Digit Substitution Task (LDST)[33]. Stroop test,[34] Verbal Fluency Test (VFT),[35] and 15-Word verbal Learning Test based on Rey's recall of words (15-WLT).[36] These tests tap into several cognitive domains: executive function, information processing, and memory function.[37] Higher test scores indicate better cognitive performance in all tests, except for the Stroop test, in which lower test scores indicate better cognitive performance. To calculate cognitive decline, we subtracted the test scores at the baseline examination from the test scores at the follow-up examination.

Other measurements

In sample I, depressive symptoms were assessed at baseline using the HADS-D subscale of the HADS.[25] A score of 9 or higher on the HADS-D scale was considered positive for depressive symptoms.[38] In sample II, depressive disorders were assessed at baseline in two steps. First, all participants were screened for depressive symptoms using the Center for Epidemiological Studies-Depression scale (CES-D).[39] Second, the participants with clinically significant depressive symptoms (CES-D ≥16) were invited for a semi-structured interview using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN).[40] Subsequently, depression was defined according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR).[41] The category of clinical depressive disorders included the DSM-IV-TR-defined major depressive disorder, dysthymia, and depressive disorder not otherwise specified (including the former category of minor depression). Low educational level was defined as less than 12 years of education. Information on *apolipoprotein E (APOE)* genotype was obtained using polymerase chain reaction on coded DNA samples without knowledge of the dementia diagnosis.[42] Missing values in covariates (for every variable less than 6.5%) were imputed based on age and sex.

Statistical analyses

We used Cox proportional hazards models to assess the associations between anxiety symptoms and anxiety disorders with incident dementia. We also investigated the relation between the various subtypes of anxiety disorders and incident dementia. Plotting the Kaplan Meier curves did not reveal any overt violations of the proportional hazards assumption (see supplementary figures I and II, Supplemental Digital Content 1, which show the dementia free survival curves of persons with and without anxiety symptoms or disorders). The underlying time-scale in these models was the follow-up time. Follow-up time for both analyses was defined from time at assessment of anxiety symptoms or anxiety disorders until January 1, 2011. Subjects were censored within this time period when they were diagnosed with dementia, died, or decided to terminate their participation in the study. All models were adjusted for age and sex (basic model). In sample I, we adjusted subsequently for low educational level, *APOE*-ε4 carrier status, and depressive symptoms (fully adjusted model) for being potential confounders. In sample II we adjusted for the same potential confounders but instead of depressive symptoms now adjusted for depressive disorders. The same set of analyses was repeated for Alzheimer disease separately. To compare the results of samples I and II, we conducted a sensitivity analysis stratifying the follow-up time of sample I. A cut-off of 5.8 years was chosen because this was the mean follow-up time of sample II. We used linear regression models to examine the effect of anxiety disorders on cognition. These models were adjusted for age and sex (basic model), and subsequently for low educational level, *APOE*-ε4 carrier status, and depressive disorders (fully adjusted model). In the analyses with cognitive decline, all models were additionally adjusted for time between the baseline and follow-up assessment of cognition.

All analyses were performed using IBM SPSS statistics for Windows, Version 20.0 (IBM Corp, Armonk, NY).

RESULTS

The baseline characteristics of our study samples are presented in **Table 1**. In sample I, 361 participants had anxiety symptoms. The mean follow-up in sample I was 11.8 years (standard deviation (SD) 5.0, total follow-up 32,047 person-years) during which 358 persons were diagnosed with incident dementia, of whom 291 with incident Alzheimer disease. In sample II, 258 participants had anxiety disorders. Of these, 9 persons had panic disorder, 51 specific phobia, 27 social phobia, 80 generalized anxiety disorder, and 127

persons suffered from agoraphobia. The mean follow-up in sample II was 5.8 years (SD 1.9, total follow-up 17,778 person-years) during which 248 persons were diagnosed with incident dementia, of whom 207 with incident Alzheimer disease.

Table 1. Population characteristics.

	Sample I, Anxiety symptoms	Sample II, Anxiety disorders
	N=2,708	N=3,069
Age, years	68.6 (8.5)	75.5 (6.2)
Women	1,495 (55.2)	1,810 (59.0)
APOE-ε4 carrier	712 (28.1)	786 (26.8)
Low educational level	797 (29.4)	850 (27.7)
Anxiety symptoms	361 (13.3)	NA
Anxiety disorders	NA	258 (8.4)
Depressive symptoms	236 (8.7)	NA
Depressive disorders	NA	81 (2.6)

Abbreviations: N number of persons in sample, SD standard deviation, *APOE apolipoprotein E*, NA not applicable
Data are presented as means (standard deviation) or counts (percentage).
Percentages are calculated without missing data. For all reported variables, missing numbers occurred in 6.5% or less of all participants.

We did not find an association between anxiety symptoms and incident dementia (**Table 2**). The HADS-anxiety and HADS-depression subscales were moderately correlated within our population (Spearman's rank correlation coefficient 0.62, p-value <0.001). However, additional adjustments for depressive symptoms and other potential confounders only slightly altered the associations.

Table 2. Associations between anxiety and incident dementia.

Anxiety		Dementia				Alzheimer's disease		
	n/N	Hazard Ratio (95% CI)	Wald-statistic	p-value	n/N	Hazard Ratio (95% CI)	Wald-statistic	p-value
Anxiety symptoms								
Basic model[a]	358/2,708	1.05 (0.77, 1.43)	0.08	0.77	291/2,708	1.01 (0.71, 1.43)	0.00	0.97
Fully adjusted model[b]	358/2,708	0.99 (0.69, 1.41)	0.01	0.94	291/2708	1.07 (0.73, 1.59)	0.13	0.72
Anxiety disorders								
Basic model[a]	248/3,069	0.92 (0.58, 1.45)	0.14	0.71	207/3,069	0.98 (0.60, 1.59)	0.01	0.92
Fully adjusted model[b]	248/3,069	0.81 (0.50, 1.30)	0.80	0.37	207/3,069	0.87 (0.53, 1.45)	0.28	0.60

Abbreviations: n number of cases, N number of persons at risk, CI confidence interval.
Comparisons between groups are based on Wald tests, degrees of freedom=1.
[a] Basic model: Adjusted for age and sex.
[b] Fully adjusted model: Adjusted for age, sex, low educational level, *apolipoprotein E*-ε4 carrier status, and depressive symptoms in anxiety symptoms or depressive disorders in anxiety disorders.

Persons with anxiety disorders did not have an increased risk of dementia (**Table 2**). Consistent with these findings, no associations were found between subtypes of anxiety disorders and incident dementia. The corresponding hazard ratios (HR) were: generalized anxiety disorder (HR 0.46, 95%-confidence interval (CI): 0.15, 1.44, Wald-statistic 1.78, p-value 0.18, degrees of freedom (df) 1), agoraphobia (HR 0.81, 95%-CI: 0.41, 1.58, Wald-statistic 0.39, p-value 0.53, df 1), and specific phobia (HR 1.20, 95%-CI: 0.50, 2.92, Wald-statistic 0.17, p-value 0.68, df 1). Associations remained stable after additional adjustments. Unfortunately, small sample size prevented us to investigate the relation between panic disorder or social phobia and dementia. Results were similar for Alzheimer disease (**Table 2**) and after stratification of follow-up time (**Table 3**).

Participants with anxiety disorders performed poorer at baseline on the LDST (difference in cognitive performance -1.21, 95%-CI -2.14; -0.29, t-statistic -2.57, df 2,347, p-value 0.01) and 15-WLT delayed recall (difference in cognitive performance -0.43, 95%-CI: -0.83, -0.04, t-statistic -2.18, df 2,347, p-value 0.03). These associations attenuated after adjusting for low educational level, *APOE*-ε4 carrier status, and depressive disorders (**Table 4**). We did not observe any associations between anxiety disorders and cognitive decline, except for the interference subtask of the Stroop test (fully adjusted difference in cognitive decline 5.05; 95% CI: 0.98, 9.11, t-statistic 2.43, df 1,107). However, the p-value of this association (p-value 0.02) would not have survived correction for multiple testing for seven cognitive tests. Additional information on mean cognitive test scores and mean cognitive decline is provided as supplementary material (see supplementary information, which provides the mean test scores and mean cognitive decline of our study sample).

Table 3. Associations between anxiety symptoms and incident dementia with stratified follow-up time.

	Dementia					
	Follow-up ≤ 5.8 years n/N=103/2,708			Follow-up > 5.8 years n/N=255/2,243		
	HR (95% CI)	Wald-statistic	p-value	HR (95% CI)	Wald-statistic	p-value
Basic model[a]	1.08 (0.63, 1.85)	0.09	0.77	1.02 (0.70, 1.50)	0.02	0.90
Fully adjusted model[b]	0.95 (0.52, 1.74)	0.03	0.87	1.00 (0.64, 1.55)	0.00	0.99
	Alzheimer's disease					
	Follow-up ≤ 5.8 years n/N=80/2,708			Follow-up > 5.8 years n/N=211/2,243		
	HR (95% CI)	Wald-statistic	p-value	HR (95% CI)	Wald-statistic	p-value
Basic model[a]	1.02 (0.55, 1.89)	0.00	0.95	1.00 (0.66, 1.52)	0.00	0.99
Fully adjusted model[b]	1.01 (0.51, 1.99)	0.00	0.99	1.11 (0.69, 1.79)	0.19	0.66

Abbreviations: n=number of cases, N=number of persons at risk, HR=hazard ratio, CI=confidence interval
Comparisons between groups are based on Wald tests, degrees of freedom=1.
[a] Basic model: Adjusted for age and sex
[b] Fully adjusted model: Additionally adjusted for age, sex, low educational level, *apolipoprotein E*-ε4 carrier status, and depressive symptoms

Table 4. Anxiety disorders and cognition.

	LDST (correct answers)	Stroop 1 (seconds)	Stroop 2 (seconds)	Stroop 3 (seconds)	VFT (animal names)	Immediate recall (correct answers)	Delayed recall (correct answers)
Cross-sectional (N=2,351)							
Basic model[a]	-1.21 (-2.14, -0.29)	0.41 (-0.20, 1.02)	0.53 (-0.20, 1.25)	1.76 (-1.31, 4.84)	-0.73 (-1.47, 0.01)	-0.83 (-1.67, 0.01)	-0.43 (-0.83, -0.04)
Fully adjusted model[b]	-0.66 (-1.57, 0.25)	0.37 (-0.24, 0.99)	0.43 (-0.30, 1.17)	0.97 (-2.14, 4.09)	-0.57 (-1.32, 0.17)	-0.44 (-1.28, 0.40)	-0.30 (-0.69, 0.10)
Longitudinal (N=1,115)							
Basic model[a]	-0.17 (-1.16, 0.83)	0.48 (-0.29, 1.25)	0.73 (-0.04, 1.50)	4.74 (0.73, 8.76)	0.26 (-0.76, 1.29)	-0.28 (-1.47, 0.91)	0.00 (-0.53, 0.53)
Fully adjusted model[b]	-0.27 (-1.27, 0.74)	0.45 (-0.34, 1.23)	0.74 (-0.05, 1.52)	5.05 (0.98, 9.11)	0.33 (-0.71, 1.37)	-0.36 (-1.56, 0.85)	-0.05 (-0.59, 0.49)

Abbreviations: LDST Letter-Digit Substitution Task, Stroop 1 reading subtask of Stroop test, Stroop 2 color naming subtask of Stroop test, Stroop 3 interference subtask of Stroop test, VFT Verbal Fluency Test, Immediate recall 15-Word Learning Test immediate recall, Delayed recall 15-Word Learning Test delayed recall, N number of persons included in analysis

Values are the differences in cognitive performance (95% confidence interval) between persons with anxiety disorders and those without anxiety disorders. Comparisons between groups are based on t-tests. For cross-sectional analyses the values are differences in test scores. Degrees of freedom in the cross-sectional analyses: 2,347 in the basic model and 2,344 in the fully adjusted model. For longitudinal analyses, the values are differences in change of test scores between the between baseline and follow-up assessment of cognition. Degrees of freedom in the longitudinal analyses: 1,110 in the basic model and 1,107 in the fully adjusted model. Higher test scores indicate better cognitive performance in all tests, except for the Stroop test.

[a] Basic model: Adjusted for age, sex, and time between measurements (if applicable).

[b] Fully adjusted model: Adjusted for age, sex, low educational level, *apolipoprotein E* -ε4 carrier status, depressive disorders, and time between measurements (if applicable).

DISCUSSION

In this study, we found that persons with anxiety symptoms or anxiety disorders did not have an increased risk of dementia. Persons with anxiety disorders performed poorer in several cognitive tests at baseline, but these associations attenuated after additional adjustments. Moreover, we did not find an association between anxiety disorders and cognitive decline.

Strengths of this study are its prospective, population-based design and nearly complete dementia case finding. We thoroughly examined the association between anxiety and dementia using both anxiety symptoms and anxiety disorders. Furthermore, we assessed the association between anxiety disorders with cognitive performance cross-sectionally and cognitive decline longitudinally. This study also has limitations. Since we used two samples with different baselines, the subjects of sample I were younger than those of sample II, which may limit direct comparison of results. Moreover, there might be some survivor effect, because only the more healthy subjects survived up until the baseline of sample II. This effect was especially present when we examined the association between anxiety disorders and cognition, as a relatively small sample underwent the complete cognitive test battery. Unfortunately, we did not have information on duration of anxiety disorders and were not able to study the importance of duration of anxiety disorders on the risk of dementia. Furthermore, small sample size prevented us to examine the associations between some of the subtypes of anxiety disorders and dementia, which would be an interesting topic for further research. Finally, we studied a relatively homogeneous sample mainly of white, middle class persons. This limits generalizability of our results to other populations.

The results of this study do not provide evidence for an association between anxiety symptoms or anxiety disorders and incident dementia. This suggests that anxiety is not a risk factor of dementia. Given that anxiety often occurs in dementia, there is a possibility that anxiety presents as a very early symptom, or prodrome, of dementia. However, when we stratified follow-up time in the association between anxiety symptoms and dementia, results remained similar. An explanation for our findings could be that anxiety occurs in a later phase of the dementia syndrome as a reaction to declining cognitive abilities. As for cognition, we only found marginal effects of anxiety disorders on cognitive performance at baseline, which attenuated after additional adjustments. For longitudinal cognitive performance, we found an association between anxiety disorders and decline in performance on the interference subtask of the Stroop test. This effect estimate was also relatively small (only one third of the SD of the study sample, please see supplementary

table I) and the association was not statistically significant after correction for multiple testing. However, it is possible that anxiety disorders are associated with cognitive decline on a specific domain, such as executive function.[43-45] Further studies are needed to clarify this association.

Several studies have reported on the association between anxiety and dementia or cognitive decline, but results have been inconclusive.[8-23] There are various explanations for these inconsistent findings. Firstly, there was a large variation across studies regarding the assessment of anxiety; different questionnaires were used and anxiety was defined in various ways. Although we found similar results when we examined the association between anxiety symptoms and anxiety disorders with dementia, it is likely that some discrepancies between studies are due to methodological variability.

Secondly, there was also variability in selection of study participants. Whereas our study sample was a community-dwelling population, several other studies selected participants with mild cognitive impairment (MCI). One study found that among subjects with MCI, anxiety was a predictor for conversion to dementia, while for cognitively healthy subjects it was not.[17] Since MCI is an intermediate phase between normal aging and dementia, there is a possibility that anxiety appears as a presenting symptom in subjects, who are already on the verge of developing dementia. It is conceivable that persons become more anxious as they notice a decline in cognitive performance and everyday functioning. Support for this hypothesis comes from a study, which found that among MCI patients anxiety symptoms increased with increasing cognitive and functional impairment according to the Clinical Dementia Rating score (CDR).[9] Another study found that MCI patients with anxiety had more abnormal cerebrospinal fluid (CSF) concentrations of amyloid-β42 and tau compared to MCI patients without anxiety symptoms.[46] These results could imply a shared etiology between anxiety and dementia. Although there was no difference in cognitive performance at baseline between the two groups of MCI subjects, this was a cross-sectional study and unable to detect cognitive decline. Therefore, it is possible that the MCI subjects with abnormal CSF concentrations were on the verge of developing dementia and were anxious because they had been noticing a decline in their cognitive performance.

Thirdly, when selecting participants from a memory clinic, there might be referral bias. For instance, persons with an anxious personality are often more worried and might be more prone to visit a memory clinic when they notice only the slightest memory complaint. These persons may be less likely to convert to dementia compared to non-anxious persons who might visit a memory clinic only after experiencing more advanced memory

complaints.[14] This could explain why some studies found a protective effect of anxiety on the risk of dementia in participants with MCI.[14,22] Finally, an important consideration when examining the effect of anxiety on dementia is the overlap with depression.[7] One study, that assessed the association between several facets of neuroticism and dementia, observed an association between anxiety and increased risk of dementia.[23] However, there was a moderate correlation with depression (correlation=0.68) for which models were not adjusted. Another large population-based study also found anxiety symptoms to be associated with dementia and Cognitive Impairment No Dementia (CIND) over a follow-up of 17 years.[8] However, models were only adjusted for psychological distress, and not specifically for depressive symptoms or depressive disorder. In both studies it therefore remained unclear to what extent the associations were independent of depression.

In conclusion, our data do not offer evidence for an association between anxiety symptoms or anxiety disorders and dementia. Persons with anxiety disorders had a worse cognitive performance at baseline, but these associations attenuated after additional adjustments. Moreover, anxiety disorders were not related to cognitive decline. These results suggest that anxiety is not a risk factor nor a prodrome of dementia in an elderly, community-dwelling population.

SUPPLEMENTARY INFORMATION

Supplementary Table 1. Cross-sectional test scores and longitudinal decline in test scores.

Cognitive tests	Cross-sectional test scores	Longitudinal decline in test scores
	N=2,351	N=1,115
LDST, correct answers, mean (SD)	25.93 (6.70)	-2.82 (4.41)
Stroop 1, seconds, mean (SD)	19.00 (4.29)	0.49 (3.42)
Stroop 2, seconds, mean (SD)	25.07 (5.09)	1.60 (3.43)
Stroop 3, seconds, mean (SD)	59.41 (22.45)	7.00 (17.91)
VFT, animal names, mean (SD)	20.60 (5.15)	-1.46 (4.56)
Immediate recall, correct answers, mean (SD)	20.29 (5.89)	-1.15 (5.26)
Delayed recall, correct answers, mean (SD)	6.43 (2.71)	-0.55 (2.35)

Abbreviations: N number of persons, LDST Letter-Digit Substitution Task, SD standard deviation, Stroop 1 reading subtask of Stroop test, Stroop 2 color naming subtask of Stroop test, Stroop 3 interference subtask of Stroop test, VFT Verbal Fluency Test, Immediate recall 15-Word Learning Test immediate recall, Delayed recall 15-Word Learning Test delayed recall.
Data represent means (standard deviations). Decline in test scores was the change of test scores between the between baseline and follow-up assessment of cognition. Higher test scores indicate better cognitive performance in all tests, except for the Stroop test.

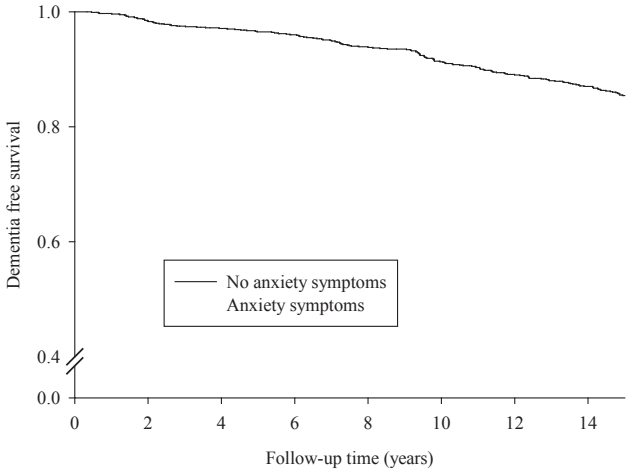

Figure 1. Dementia free survival of anxiety symptoms versus no anxiety symptoms.
The figure shows Kaplan-Meier curve of dementia free survival of anxiety symptoms versus no anxiety symptoms.

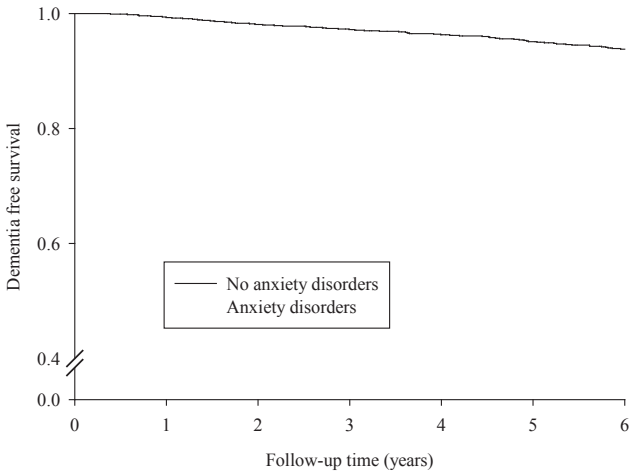

Figure 2. Dementia free survival of anxiety disorders versus no anxiety disorders.
The figure shows Kaplan-Meier curve of dementia free survival of anxiety disorders versus no anxiety disorders.

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CHAPTER 2.5

Anxiety does not predict mortality. A population-based study

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ABSTRACT

Background: The association of anxiety and excess mortality in community-dwelling persons is debated.

Methods: In the population-based Rotterdam Study, we followed 2,716 non-demented participants assessed for anxiety symptoms in 1993-1995 (Sample I), and 3,079 non-demented participants assessed for anxiety disorders in 2002-2004 (Sample II), for all-cause mortality till 2013. We assessed anxiety symptoms by the Hospital Anxiety and Depression Scale, and anxiety disorders by the composite International Diagnostic Interview. Anxiety disorders were diagnosed according to the DSM-IV TR criteria. Cox proportional hazards models were used to study associations between anxiety and mortality.

Results: In sample I, 1,451 participants died during 19.3 years, whereas in sample II, 1,138 participants died during 11.3 years. Anxiety symptoms but not disorders showed an increased risk of mortality (HR 1.10, 95% CI: 1.02, 1.14) which attenuated after adjusting for cardiovascular confounders (HR 1.04, 95% CI: 1.00, 1.10). Neither anxiety symptoms (HR 0.99, 95% CI: 0.92, 1.07), nor anxiety disorders (HR 0.99, 95% CI: 0.77, 1.29) were associated with an increased risk of mortality after additionally adjusting for depression. No gender-differences were observed.

Conclusions: Anxiety is not independently associated with excess mortality in a community-dwelling elderly population; any observed association was largely explained by cardiovascular risk factors or depression.

INTRODUCTION

Anxiety and depression frequently co-occur with a high degree of overlap of both symptoms and diagnosed disorders.[1] The association between depressive symptoms and diagnosed depressive disorders with increased risk of mortality is well documented, particularly in the elderly.[2,3] However, literature on association between anxiety and excess mortality is relatively sparse and inconsistent. In a review in 2004, Dewey et al concluded that anxiety was not associated with a significant increase in mortality.[4] Since then, some studies have shown an excess mortality in persons with anxiety,[5-9] but others have failed to confirm this association.[10,11] One study reported a lower risk of mortality in people with anxiety aged 80 or older only,[12] while another observed a lower risk of mortality only in those with co-morbid depression.[10,13] The HUNT study reported a U-shaped association between anxiety and mortality during 6 years of follow-up.[13]

Most of these studies studied anxiety symptoms as risk factor for mortality[6,8,12-15], few tested diagnosed anxiety disorders,[10,11,16,17] and only one study tested both symptoms and disorders in relation to mortality[5] Although the significant overlap of symptoms between anxiety and depression makes it imperative to examine confounding or effect modification by depression, not all studies have considered depression as a covariate in their study of mortality. In addition, most studies followed participants for short periods only, and rarely did the follow-up exceed 10 years. In a short follow-up, the issue of reverse causality cannot be completely ruled out.

There are a few studies reporting gender-specific associations between anxiety and mortality. Recent findings from the ESPRIT study suggested an association of anxiety and mortality only in women and that anxiety disorders, but not symptoms, were associated with excess mortality.[5] Previously, Denolet et al also reported an association in middle-age Dutch women,[6] but data from the LASA study suggested that anxiety disorders predict higher mortality only in men.[17] These inconsistencies in results can partly be attributed to methodological variation including use of varying instruments for anxiety assessment, difference in sample sizes, age of participants, number of deaths, follow-up time, and controlling for confounders, particularly depression.

This paper aims to investigate the association of anxiety with risk of all-cause mortality in a large population-based setting. Importantly, we investigated both anxiety symptoms and DSM-anxiety disorders and examined the difference in association of anxiety and mortality between men and women.

METHODS

Setting

This study was embedded in the Rotterdam Study, an ongoing population-based prospective study of the elderly that started in a suburb of Rotterdam (Ommoord) in 1990 and studies the incidence and determinants of chronic diseases in late life.[18] In 1990, 7,983 participants were enrolled in the Rotterdam Study, and every 3 to 4 years, all participants undergo an extensive home interview and a physical examination at the research center. In addition, all participants are continuously monitored for the occurrence of all major events during follow-up by linkage of the study database with medical files from general practitioners. The second (1993-1995) and fourth (2002-2004) examination rounds of Rotterdam study formed the two baselines of this study. The Rotterdam Study is approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. A written informed consent was obtained from all participants.

Study Population

We defined two samples in the Rotterdam Study; Sample I which was assessed for anxiety symptoms', and sample II which was assessed for DSM-anxiety disorders.

Sample I-Anxiety symptoms

In the second examination round of the Rotterdam Study in 1993-1995, we assessed anxiety symptoms in 2,977 individuals by the Hospital Anxiety and Depression Scale (HADS), which is a valid and reliable instrument for anxiety and depression assessment.[19] HADS incorporates two subscales with 7 items for assessment of each anxiety (HADS-A) and depression (HADS-D). Score ranges from 0-21 for each HADS-A and HADS-D; more score indicating higher anxiety. Anxiety scores were weighted by missing values only if missing values did not exceed 25%. For analysis, anxiety symptoms' score was used as a standardized continuous variable. Z-scores were calculated as (anxiety scores-mean anxiety score) / standard deviation of anxiety score. After excluding 259 participants with prevalent dementia, and 2 individuals who were lost to follow-up, a total of 2,716 individuals were available for analysis.

Sample II-DSM-Anxiety disorders

In the fourth examination round of the Rotterdam Study in 2002-2004, assessment of DSM-anxiety disorders was added to the data collection protocol in the Rotterdam Study.

In 3,430 individuals, as part of the home interview, an adapted version of the Munich version of the Composite International Diagnostic Interview (M-CIDI)[20] was administered to assess 1-year prevalence of the following anxiety disorders according to the DSM-IV-TR criteria.[21] generalized anxiety disorder, panic disorder, agoraphobia, social phobia, and specific phobia as described previously by Hek et al.[22] For analysis, we categorized participants into two groups on basis of being free of any anxiety disorder or having at least one of the above mentioned anxiety disorders. After excluding 348 participants with prevalent dementia, and 3 who were lost to follow-up, a total of 3,079 individuals were available for analysis.

Of these 3,079, 1512 (49.1 %) participants were also included in the analyses of anxiety symptoms. Persons with prevalent dementia were excluded to ensure the reliability of anxiety assessments. Follow-up for mortality for both anxiety symptoms and anxiety disorders was complete till January 2013.

Vital Status

Information on vital status was obtained continuously via computerized linkages from municipal authorities in Rotterdam, notifications from the general practitioners (GPs) or nursing homes, or notification from family. Cardiovascular deaths were ascertained by a research physician by weighing all available clinical information in each potential cardiovascular death according to predefined criteria[23] to adjudicate the underlying cause of death as being cardiovascular or non-cardiovascular. All cases were verified by a physician specializing in cardiology, whose judgment was considered final.

Covariates

Education level was assessed during the interview and people were classified into low (primary only or primary and unfinished secondary), intermediate (secondary or vocational) or high education (higher vocational or university). Participants were categorized into current, former and never-smokers based on interview. Diabetes mellitus type 2 was diagnosed as fasting blood glucose ≥ 126.13 mg/dl, or use of anti-diabetic medication evaluated by interview and pharmacy records.[24] Myocardial Infarction (MI) was confirmed by interview, and hospital, GP and medical specialists' records. Previous stroke was determined by reported events on interview and confirmed by medical records. In addition, participants were continuously monitored for all major events through automated linkage of study database with GP files. A low level of education,[25] smoking, prevalent diabetes, MI, and stroke are well established risk factors for mortality.

Cognition was assessed by the Mini-Mental State Examination (MMSE) at baseline[26] as cognition might influence the response of participants to the anxiety assessment questionnaires. Living situation, which might influence the level of anxiety in participants, was defined as living independently or in a nursing home. Beer, wine, liquor and other strong alcohol consumption was converted to grams of alcohol by taking the average amount of ethanol in a drink. By summing the amount of ethanol in all four groups, total amount of alcohol in g/day was calculated.[27] All covariates were measured at the time of anxiety symptoms' assessment or anxiety disorders' assessment.

Depression as a covariate

In the cohort with assessment of anxiety symptoms, depressive symptoms were assessed by the HADS-D questionnaire.[19] In the cohort with assessment of DSM-anxiety disorders, the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) interview,[28] a semi-standardized clinical interview was administered to assess the depressive disorders according to the DSM-IV-TR criteria.[21]

Statistical analysis

We used Cox proportional-hazards models to evaluate the relation of anxiety symptoms and DSM-anxiety disorders with risk of all-cause mortality. The underlying time-scale in these analyses was the follow-up time. For both samples, effect modification by gender was explored using stratification by gender and use of interaction terms. Anxiety symptoms' score was used as a standardized continuous variable. A quadratic term for anxiety score was also included to explore a non-linear relation between anxiety symptom scores and mortality. Three models were fitted for each analysis. Model 1 was adjusted only for age and sex. Model 2 was additionally adjusted for smoking, education, cognition status at baseline, living situation, alcohol intake, and prevalent diabetes, myocardial infarction, and stroke. Model 3 was additionally adjusted for depression. In the cohort with assessment of anxiety symptoms, we adjusted for depressive symptoms, and in the cohort with assessment of anxiety disorders, we adjusted for depressive disorders.

We repeated our analyses using cardiovascular mortality as outcome instead of all-cause mortality. We also repeated our analyses excluding the positively-worded items from HADS-Anxiety scale in the anxiety symptom analyses, and excluding specific phobias from anxiety disorder definition, in the anxiety disorder analyses. As studies with short follow-up[8,14] have reported that anxiety is associated with excess mortality, we also investigated the association of anxiety symptoms and disorders with risk of mortality in shorter follow-up periods of 3 and 5 years.

Results are presented as hazard ratios (HR) with 95 % confidence intervals (CI). Data were analyzed using the Stata Software Version 13 (StataCorp, College Station, TX, USA).

RESULTS

Baseline characteristics of all persons in the study are described in **Table 1**. In sample I with assessment of anxiety symptoms, 1,451 participants died during 19.3 years (mean 13.2 years, SD 5.5). Pearson correlation coefficient for anxiety and depression scores as assessed by HADS was 0.66 and did not differ between men and women.

Anxiety symptoms showed an increased risk of mortality in models adjusted for age and gender, HR 1.10 (95% CI: 1.02, 1.14, Score-test 2.91, p-value 0.004) which attenuated to marginal significance after adjusting for education, cardiovascular risk factors, baseline cognition and prevalent disease, HR 1.04 (95% CI: 1.00, 1.10, Score-test 1.54, p-value 0.12), and further attenuated to non-significant, after additional adjustment for depressive symptoms, HR 0.99 (95% CI: 0.92, 1.07, Score-test -0.25, p-value 0.80) (**Table 2**). Men but not women with anxiety, had a higher risk of mortality in multivariable model, however the association became non-significant after additionally adjusting for depressive symptoms.

Table 1. Baseline characteristics of cohorts with anxiety symptoms' and anxiety disorders' assessments followed till 2013 for mortality.

Characteristics	Anxiety symptoms, N=2,716 (1993-1995)	Anxiety disorders, N=3,079 (2002-2004)
Women	1,502 (55.3)	1,820 (59.1)
Age, years	68.7 (8.5)	75.5 (6.2)
Body mass index, kg/m^2	26.3 (3.6)	27.5 (4.1)
Education		
Low	799 (29.8)	856 (28.1)
Intermediate to high	1,882 (70.2)	2,189 (71.9)
Smoking		
Never	885 (32.6)	929 (30.2)
Former	1,245 (45.8)	1,707 (55.4)
Current	586 (21.6)	443 (14.4)
Hypertension	1,212 (44.6)	2,006 (65.1)
Diabetes mellitus 2	136 (5.1)	360 (11.7)
Myocardial infarction	188 (7.0)	304 (9.9)
Stroke	79 (3.0)	160 (5.2)

Values are means (standard deviation), or counts (percentage).

Table 2. Anxiety symptoms and risk of mortality from 1993-2013.

Anxiety score (HADS-A)	Deaths/N	Risk of Mortality, hazard Ratios (95 % confidence intervals)		
		Model 1[a]	Model 2[b]	Model 3[c]
All participants				
Anxiety symptoms, per SD	1,451/2,716	1.10 (1.02, 1.14)	1.04 (1.00, 1.10)	0.99 (0.92, 1.07)
Men				
Anxiety symptoms, per SD	702/1,214	1.13 (1.04, 1.22)	1.09 (1.00, 1.19)	1.03 (0.91, 1.16)
Women				
Anxiety symptoms, per SD	749/1,502	1.04 (0.98, 1.11)	1.01 (0.94, 1.09)	0.97 (0.88, 1.06)

Abbreviations: HADS-A Hospital Anxiety and Depression Scale-Anxiety; SD standard deviation.
[a] Model 1: adjusted for age and sex (if applicable).
[b] Model 2: additionally adjusted for education, smoking, living situation, cognition at baseline, alcohol intake, and prevalent diabetes mellitus 2, myocardial infarction and stroke.
[c] Model 3: additionally adjusted for depressive symptoms.

We did not observe any evidence of effect modification by gender through testing interaction between anxiety symptoms and gender (P-value=0.23). There was no indication of a curvilinear relation between anxiety and mortality (P-value=0.29).

In sample II assessed for anxiety disorders,1,138 participants died during 11.3 years (mean 7.4 years, SD 2.5). Anxiety disorders were not associated with the risk of mortality, HR 0.99 (95% CI: 0.77-1.29, Score-test -0.03, p-value 0.98) in the fully adjusted model (**Table 3**). Stratification by gender also showed no association between anxiety disorders and risk of mortality.

Analyses of anxiety symptoms and anxiety disorders in relation to cardiovascular mortality, showed similar results as all-cause mortality (**Supplement table 1**). Repeating the analyses with anxiety symptoms after excluding the positively-worded items from HADS-A, results remained unchanged, HR 1.00 (95% CI: 0.98, 1.03, Score-test 0.37, p-value 0.71) in fully adjusted model. Results also remained unchanged when we repeated the anxiety disorder analyses after excluding specific phobias from the anxiety definition, HR 1.04 (95% CI: 0.79, 1.37, Score-test 0.31, p-value 0.76) in fully adjusted model. There was some evidence of effect of anxiety symptoms on short-term mortality (**Supplement table 2**). Men but not women with anxiety symptoms at baseline showed a significantly higher risk of mortality only during first three years of follow-up in fully adjusted models. In the analyses with anxiety disorders and risk of mortality during short follow-up, again men with anxiety disorders showed a higher risk of mortality than controls during 3 years only, however non-significant.

Table 3. DSM-Anxiety disorders and risk of mortality from 2002-2013.

Anxiety disorders	Deaths/N	Risk of Mortality, hazard Ratios (95 % confidence intervals)		
		Model 1[a]	Model 2[b]	Model 3[c]
All participants				
Anxiety disorders	1,138/3,079	0.98 (0.78, 1.23)	1.03 (0.81, 1.33)	0.99 (0.77, 1.29)
Men				
Anxiety disorders	548/1,259	1.23 (0.81, 1.87)	1.11 (0.69, 1.79)	1.09 (0.68, 1.77)
Women				
Anxiety disorders	590/1,820	0.91 (0.70, 1.18)	0.98 (0.73, 1.32)	0.96 (0.70, 1.30)

[a] Model 1: adjusted for age and sex (if applicable).
[b] Model 2: additionally adjusted for education, smoking, living situation, cognition at baseline, alcohol intake, and prevalent diabetes mellitus 2, myocardial infarction and stroke.
[c] Model 3: additionally adjusted for depressive disorders.

DISCUSSION

In this population-based study with a long follow-up, we examined the association of anxiety symptoms and DSM-anxiety disorders with risk of mortality. Anxiety disorders were not associated with excess mortality. Anxiety symptoms showed a marginally increased risk of mortality which disappeared after adjustment for cardiovascular risk factors, co-morbidity, and depressive symptoms.

Strengths of the study include the population-based design, large sample size, assessment of both anxiety symptoms and anxiety disorders, and a long follow-up. A methodological consideration is that the anxiety symptoms and anxiety disorders were not assessed at the same time, as anxiety disorder assessment was added in the fourth examination round in the Rotterdam Study. In addition, he possibility of some survivor bias, with a selective survival of individuals at the fourth examination round, cannot be ruled out.

Anxiety symptoms were associated with a marginally increased risk of mortality particularly in men. However, this association was largely explained by the presence of cardiovascular risk factors, prevalent stroke, MI, and diabetes, and comorbid depressive symptoms. This finding is in line with recent findings from the ESPRIT Study. However, this study also reported that anxiety disorders but not symptoms are related to excess mortality, only in women even after adjustment for depressive symptomatology.[5] It is plausible that persons with chronic disease can develop anxiety or have more severe anxiety. In our study, many individuals who were depressed and therefore had a high risk of mortality, also exhibited symptoms of anxiety; correlation between HADS sub-scales for

anxiety and depression was 0.66. Given the considerable comorbidity between anxiety and depression, a substantial correlation is expected on the symptom level. Of the 362 (13.3 %) participants who had anxiety symptoms, and the 238 (8.8 %) who had depressive symptoms, 151 (5.6%) participants had comorbid depression and anxiety, applying the accepted cut-offs.[19] This suggests that the items on the anxiety sub-scale inherently tap depressive symptoms. In addition, as the symptoms of anxiety and depression overlap, it is difficult to distinguish between the two mood disorders by questionnaires. Moreover, such a substantial correlation may not only reflect the symptoms shared between anxiety and depression, but also point towards a common disorder. Nevertheless, others may argue that, given this overlap of symptomatology, there is a possibility of overcorrection of the actual association between anxiety and mortality, when we additionally adjusted for depressive symptoms. However, after adjusting for cardiovascular risk factors and prevalent disease in the second model, the association was left only marginally significant.

When we investigated the association between anxiety symptoms and mortality during shorter follow-up of 3 years, a significantly increased risk was observed in men. This may indicate reverse causality in our study in a short follow-up. In this case, the underlying cause of death was not anxiety; rather anxiety was possibly secondary to existent morbidities which caused mortality in a short follow-up.

Our analyses of DSM-anxiety disorders and risk of mortality are very consistent with those of anxiety symptoms and mortality, although the overlap between anxiety disorders and depressive disorders was small. Of the 252 (8.2 %) participants with anxiety disorders and 81 (2.6 %) with depressive disorders, only 36 (1.2 %) had both anxiety and depression. This low co-morbidity reflects the fact that interviews of depressive disorders and those to diagnose anxiety disorders were conducted with different instruments, SCAN and CIDI and on different days, but less than one month apart. We did not find an association in univariable or multivariable analyses, and no difference between men and women. Similar results have been previously reported.[10,11]

Conclusively, anxiety symptoms or anxiety disorders were not associated with excess mortality. Observed associations between anxiety symptoms and increased mortality were largely explained by the presence of cardiovascular risk factors, prevalent disease, and comorbid depression. This reflects the substantial overlap in symptoms between anxiety and depression. In addition, the observed association between anxiety symptoms and short-term mortality was probably explained by existing morbidity.

SUPPLEMENTARY INFORMATION

Supplementary Table 1. Anxiety and risk of cardiovascular mortality.

Anxiety measure	Deaths/N	Risk of mortality, hazard ratios (95% confidence intervals)		
		Model 1[a]	Model 2[b]	Model 3[c]
Anxiety symptoms, per SD	458/2,716	1.13 (1.03, 1.23)	1.07 (0.98, 1.18)	1.01 (0.88, 1.14)
DSM-Anxiety disorders	313/3,079	0.83 (0.52, 1.33)	0.81 (0.47, 1.40)	0.76 (0.44, 1.34)

Abbreviation: SD standard deviation.
Mortality follow-up complete until 2013.
[a] Model 1: adjusted for age and sex (if applicable).
[b] Model 2: additionally adjusted for education, smoking, living situation, cognition at baseline, alcohol intake, and prevalent diabetes mellitus 2, myocardial infarction and stroke.
[c] Model 3: additionally adjusted for depression (adjusted for depressive symptoms in cohort assessed for anxiety symptoms, and adjusted for depressive disorders in cohort assessed for anxiety disorders).

Supplement Table 2. Anxiety and risk of mortality in short follow-up of 3 years.

Anxiety score (HADS-A)	Deaths/N	Risk of mortality, hazard ratios (95% confidence intervals)		
		Model 1[a]	Model 2[b]	Model 3[c]
All participants				
Anxiety symptoms, per SD	181/2,716	1.21 (1.06, 1.38)	1.16 (0.99, 1.36)	1.12 (0.90, 1.39)
Men				
Anxiety symptoms, per SD	92/1,214	1.55 (1.31, 1.85)	1.61 (1.32, 1.96)	1.77 (1.28, 2.44)
Women				
Anxiety symptoms, per SD	89/1,502	0.96 (0.79, 1.16)	0.83 (0.65, 1.07)	0.79 (0.57, 1.09)
DSM-Anxiety disorders	**Deaths/N**	**Risk of mortality, hazard ratios (95% confidence intervals)**		
		Model 1[a]	Model 2[b]	Model 3[c]
All participants				
Anxiety disorders	259/3,079	1.01 (0.61, 1.66)	0.98 (0.53, 1.81)	0.80 (0.42, 1.52)
Men				
Anxiety disorders	148/1,259	2.26 (1.22, 4.17)	1.57 (0.68, 3.62)	1.37 (0.59, 3.21)
Women				
Anxiety disorders	111/1,820	0.49 (0.21, 1.11)	0.67 (0.27, 1.67)	0.54 (0.21, 1.41)

Abbreviations: HADS-A Hospital Anxiety and Depression Scale-Anxiety; SD standard deviation.
[a] Model 1: adjusted for age and sex (if applicable).
[b] Model 2: additionally adjusted for education, smoking, living situation, cognition at baseline, alcohol intake, and prevalent diabetes mellitus 2, myocardial infarction and stroke.
[c] Model 3: additionally adjusted for depression (adjusted for depressive symptoms in cohort assessed for anxiety symptoms, and adjusted for depressive disorders in cohort assessed for anxiety disorders).

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CHAPTER 2.6

Mild cognitive impairment and risk of depression and anxiety: a population-based study

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Submitted

ABSTRACT

Background: Many people with Mild Cognitive Impairment (MCI), a transitional stage between healthy aging and dementia, suffer from concomitant depression or anxiety disorders. Whether MCI also increases the risk of future depression or anxiety is not known.

Methods: MCI was assessed in 4,198 participants (mean age 72 years; women 58%) between 2002 and 2005, in the population-based Rotterdam Study. Criteria for MCI assessment included presence of subjective memory complaints and objective cognitive impairment, and absence of dementia. Depressive and anxiety disorders were assessed at baseline, and reassessed between 2009 and 2012, using Schedules for Clinical Assessment of Neuropsychiatry interviews, and the Composite International Diagnostic Interview respectively. Associations of MCI with prevalent and incident DSM depressive and anxiety disorders were assessed by multiple logistic regression.

Results: Of the 4,168 participants eligible for analyses, 413 had MCI at baseline, of which 22 had comorbid depression, and 46 had comorbid anxiety. Participants with MCI were more likely to have depressive disorders, OR 1.94 (95% CI:1.20,3.15) as well as anxiety disorders, OR 1.70 (95% CI:1.19,2.42). During the study period, 6 non-depressed/anxious participants with MCI at baseline developed incident depression, and 11 developed incident anxiety. Persons with MCI had a higher risk of developing both depressive, OR 3.13 (95% CI:1.26,7.77) and anxiety disorders, OR 2.59 (95% CI:1.31,5.12).

Conclusions: MCI is not only a risk factor for dementia but also a harbinger of depressive and anxiety disorders. Our results suggest that common pathological pathways underlie cognitive and psychiatric outcomes, thus opening avenues for joint etiological research.

INTRODUCTION

Dementia poses a high burden on society and health care, both in terms of suffering for patients and care-givers as well as financial costs.[1] Since brain pathology is thought to accumulate for years before the onset of dementia, much research has been dedicated to study this preclinical phase of dementia. In this context, mild cognitive impairment (MCI) has been conceptualized as a transitional stage between normal cognition and dementia, and serves as a clinical construct in which meaningful interventions are possible.[2]

Another important manifestation thought to be a part of dementia prodrome is the occurrence of affective disorders, namely depression and anxiety.[3] Indeed, there is ample evidence suggesting that depression in late life is associated with a 2-fold increased risk of dementia.[4,5] Extending these findings, some recent studies have also shown that depression is a risk factor for MCI.[6-8] These studies imply that depression precedes MCI in the chronological order of events. However, given that both MCI and affective symptoms are considered to manifest during the preclinical stage of dementia, it is also not unlikely that MCI precedes depression and anxiety. However, the association of MCI in relation to risk of depression or anxiety has never been investigated. Therefore, we investigated the cross-sectional and longitudinal associations of MCI with depression and anxiety in a population-based cohort of older adults.

METHODS

Setting

This study was part of the Rotterdam Study, a population-based cohort ongoing since 1990 in Ommoord, a district of Rotterdam.[9] In 1990, 7,983 participants aged 55 years or older were enrolled. In 2000, the original cohort was expanded by additionally enrolling 3,011 participants who had become 55 years of age or moved to the district since the start of the study. Follow-up examinations including home interviews and physical exams at a research center take place every 3 to 4 years. The Rotterdam Study is approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the “Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)”. A written informed consent was obtained from all participants.

Study population

Between 2002 and 2005, the original cohort and the expanded cohort were re-examined, and an extensive neuropsychological test battery was implemented. Given that extensive neuropsychological testing is required to determine MCI, 2002-2005 was set as baseline for MCI screening in our study. Of the 6,061 study participants who underwent examinations between 2002 and 2005, 192 participants were excluded because they were demented, 67 because they were not sufficiently screened for dementia, and another 250 participants because they did not answer the questions regarding subjective cognitive complaints. An additional 1,354 participants were excluded because they missed one or more cognitive test scores or had unreliable test scores. Consequently, MCI was validly assessed in 4,198 participants.

Between 2002 and 2005, depression and anxiety disorders were assessed in the Rotterdam Study. Of the 4,198 participants with available MCI data, depression data were available for 4,168 participants, while anxiety data were available for 4,060 participants. At baseline, 125 participants fulfilled the criteria for depressive disorders, while 330 participants met the criteria for anxiety disorders.

Between 2009 and 2012, depressive disorders were reassessed in 3,117 participants of the 3,370 participants attending the examination round (798 participants died during follow-up, 4,168-798=3,370). After excluding 125 depression cases at baseline, and 25 persons who were diagnosed of incident dementia during the study period, depressive disorders data were available for 2,967 persons for the analyses of MCI and incident depressive disorders (response rate= 92%).

Between 2009-2012, anxiety disorders were reassessed in 2,714 participants of the 3,293 participants attending the examination round (767 participants died during follow-up, 4,060-767=3,239). We excluded 330 anxiety cases at baseline, and 9 persons who were diagnosed with incident dementia during the study period. Therefore, 2,375 participants were available for the analyses of MCI and incident anxiety disorders (response rate=82%.).

Assessment of MCI

MCI was assessed using the following criteria: 1) presence of subjective memory complaints, 2) presence of objective cognitive impairment, and 3) absence of dementia.[10]

Subjective memory complaints were assessed by interview, which included three questions on memory (difficulty remembering, forgetting what one had planned to, and difficulty finding words), and three questions on everyday functioning (difficulty managing financing, problems using a telephone, and difficulty getting dressed). Persons answering "yes" to at least one of these questions were scored positive on subjective memory complaints. Objective cognitive impairment was assessed using a cognitive test battery that comprised Letter-digit substitution task, Stroop test, Verbal fluency test, and 15-Word verbal learning test based on Rey's recall of words. To obtain more robust measures, we calculated different compound scores for various cognitive domains including memory function, information processing speed, and executive function. Briefly, compound score for memory was calculated as the mean Z-score for the immediate and delayed recall of the 15-Word verbal learning test. For information processing speed, average Z-scores for the Stroop reading and Stroop colour-naming sub tasks and the Letter-digit substitution task were used. For calculating compound score of executive function, Z-scores of Stroop interference subtask, the Letter-digit substitution task, and the verbal fluency task were used. Persons were classified as cognitively impaired if they scored below 1.5 standard deviations of the age and education adjusted means of the study population.

Subsequently, we sub-classified MCI into amnestic and non-amnestic MCI. Amnestic MCI was defined as persons with MCI who had an impaired test score on memory irrespective of other domains. Non-amnestic MCI was defined as persons with MCI having normal memory function, but an impaired test score on executive function or information processing speed.

Assessment of depressive disorders

Depressive disorders were diagnosed during home interview.[11] Participants were screened for symptoms of depression with the Center for Epidemiological Studies Depression (CES-D) scale. Screen-positive persons (CES-D-score≥16) were invited for a semi-structured clinical interview with the Schedules for Clinical Assessment of Neuropsychiatry (SCAN).[12] This interview was conducted by a trained clinician at the participant's home one week to two months (median time interval: three weeks) after the screening procedure and the anxiety interview done simultaneously. We were able to use the SCAN in this population-based setting, because depression can be screened for with high sensitivity.[13] With a computerized DSM-IV based diagnostic algorithm, major depression, minor depression and dysthymia during the past month were diagnosed.

Assessment of anxiety disorders

During the home interview, an adapted version of the Munich version of the Composite International Diagnostic Interview (M-CIDI) [14] was administered to all participants, to assess 1-year prevalence of the following anxiety disorders according to the DSM-IV-TR criteria:[15] generalized anxiety disorder, panic disorder, agoraphobia, social phobia, and specific phobia as described previously.[16] Participants were classified as positive for anxiety disorders if they had at least one of the above mentioned anxiety disorders. The M-CIDI was specifically designed to obtain DSM-IV diagnoses of mental disorders, and the test–retest reliability for the anxiety disorders is satisfactory.[14]

Other assessments

Assessment of dementia for exclusion during the study period

Participants were first screened for dementia at baseline and follow-up examinations using a three-step protocol.[17] Screening was done using the Mini Mental State Examination (MMSE)[18] and the Geriatric Mental Schedule (GMS) organic level.[19] Second, screen-positives (MMSE<26 or GMS organic level>0) subsequently underwent an examination and informant interview with the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX).[20] Participants who were suspected of having dementia, if necessary, had further neuropsychological testing. Additionally, the total cohort was continuously monitored for dementia through computerized linkage between the study database and digitized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. Third, a consensus panel led by a neurologist, decided on the final diagnosis in accordance with the standards using the DSM-III-R criteria for dementia and the NINCDS-ADRDA for Alzheimer's disease.[21]

Covariates

Age, sex, educational level, body mass index, smoking status, total serum cholesterol, high-density lipoprotein (HDL) cholesterol, prevalent disease including hypertension and diabetes mellitus type 2, and cardiovascular events including myocardial infarction and stroke were considered potential confounders for the association of MCI with depression and anxiety. Education, vascular factors and cardiovascular events are implicated as risk factors for MCI and are also associated with depression and anxiety.

Education was assessed during the home interview and participants were classified into three groups: low educational level (primary or unfinished secondary, or lower vocational

training), intermediate (secondary, or intermediate or higher vocational training), and high (completed college or university). Body mass index was calculated as weight in kilograms/height in meters squared. We defined smokers as never, former or current. Serum total cholesterol and HDL cholesterol (mmol/L) were measured by an automated enzymatic procedure (Boehringer Mannheim System). Hypertension was defined as a blood pressure ≥140/90mmHg or use of blood pressure lowering medication, prescribed for the indication of hypertension.[22] Diabetes mellitus was defined as a fasting serum glucose level ≥7.0 mmol/L, non-fasting serum glucose level ≥11.1 mmol/L, or use of anti-diabetic medication.[23] At study entry, history of myocardial infarction and stroke was assessed using home interviews and confirmed by reviewing medical records.[24,25]

Statistical Analyses

We first assessed the cross-sectional associations of MCI with depressive disorders and anxiety disorders using logistic regression. In secondary analyses, we also assessed the association of subtypes of MCI (amnestic and non-amnestic) with depressive and anxiety disorders.

Subsequently, we examined the longitudinal associations of MCI with depressive and anxiety disorders using logistic regression. For these analyses, cases of depression at baseline were excluded for the depression analyses, and similarly, cases of anxiety at baseline were excluded before performing the anxiety analyses. We also excluded persons with incident dementia diagnosed during the study period. Further, we examined the longitudinal associations of subtypes of MCI with incident depressive and anxiety disorders.

For all analyses, two models were fitted. In both cross-sectional and longitudinal analyses, model 1 was adjusted for age and sex only. Model 2 was additionally adjusted for educational level, body mass index, smoking status, serum total cholesterol, HDL cholesterol, hypertension, diabetes mellitus 2, myocardial infarction, stroke, and cohort. The longitudinal analyses were additionally adjusted for time interval between the two assessments of depression and anxiety.

The following covariates had missing values, which were dealt with using multiple imputations and chained equations (ICE command Stata): education level (1.5%), BMI (1.5%), total cholesterol (1.7%), HDL cholesterol (1.7%), and myocardial infarction (0.6%).

For all tests, a significance level of 0.05 was used. All analyses were performed using Stata Software Version 13 (Stata Corp, College Station, TX, USA).

RESULTS

Characteristics of participants included in the analyses of depressive disorders are summarized in **Table 1**. Numbers for the analyses of anxiety disorders were similar but slightly less, as shown in the **Supplement Table**.

Table 1. Baseline characteristics of the study population included in the analyses of MCI and depressive disorders.

Characteristics	Cross-sectional analysis of depressive disorders, N=4,168		Longitudinal analysis of depressive disorders, N=2,796	
	No MCI n=3,755	MCI n=413	No MCI n=2,755	MCI n=212
Age, years	71.4 (7.1)	73.3 (7.5)	70.0 (6.4)	70.0 (6.2)
Women	2,183 (58.1)	215 (52.1)	1,608 (58.4)	108 (50.9)
Body mass index, kg/m^2	27.6 (4.1)	27.7 (4.1)	27.7 (4.0)	27.7 (4.0)
Educational level				
Low	341 (9.2)	71 (17.3)	197 (7.3)	30 (14.3)
Intermediate	2,826 (76.5)	284 (69.3)	2,100 (77.5)	147 (70.0)
High	528 (14.3)	55 (13.4)	412 (15.2)	33 (15.7)
Smoking				
Never	1120 (29.8)	111 (26.9)	838 (30.4)	58 (27.4)
Former	2,067 (55.0)	230 (55.7)	1,536 (55.7)	126 (59.4)
Current	568 (15.1)	72 (17.4)	381 (13.8)	28 (13.2)
Total, cholesterol, mmol/L	5.6 (1.0)	5.4 (0.9)	5.7 (1.0)	5.5 (1.0)
HDL cholesterol, mmol/L	1.5 (0.4)	1.4 (0.4)	1.5 (0.4)	1.4 (0.4)
Hypertension	3,024 (80.5)	344 (83.3)	2,156 (78.3)	161 (75.9)
Diabetes mellitus	508 (13.5)	74 (17.9)	316 (11.5)	31 (14.6)
Myocardial infarction	252 (6.7)	50 (12.3)	157 (5.7)	22 (10.5)
Stroke	112 (3.0)	22 (5.3)	53 (1.9)	11 (5.2)

Values are means (standard deviation) or counts (percentage).

Table 2 presents the results for the cross-sectional associations of MCI and its subtypes, with depressive and anxiety disorders. Participants with MCI were more likely to suffer from depressive disorders than persons without MCI, OR 1.94 (95% CI: 1.20, 3.15). In secondary analyses, we found similar associations for both amnestic and non-amnestic MCI with depressive disorders, compared to those without MCI: amnestic, OR 2.02 (95% CI: 0.95, 4.29), and non-amnestic, OR 1.90 (95% CI: 1.05, 3.40).

In the cross-sectional analyses of MCI and anxiety disorders, participants with MCI were more likely to suffer from anxiety disorders than persons without MCI, OR 1.70 (95% CI:

1.19, 2.42). In analyses with subtypes of MCI, participants with non-amnestic MCI were more likely to have an anxiety disorder, OR 2.00 (95% CI: 1.30, 3.04), but not those with amnestic MCI, OR 1.28 (95% CI: 0.69, 2.38).

Results for the longitudinal analyses of MCI with depressive and anxiety disorders are presented in **Table 3**. For the analyses of MCI and incident depression, we found that MCI at baseline was associated with a higher relative risk of incident depressive disorders, OR 3.13 (95% CI: 1.26, 7.77). In secondary analyses, we found that non-amnestic MCI was associated with a higher relative risk of depressive disorders, OR 3.77 (95% CI: 1.40, 10.13).

MCI at baseline was also associated with a higher relative risk of anxiety disorders, OR 2.59 (95% CI: 1.31, 5.12). Both amnestic MCI, OR 3.22 (95% CI: 1.21, 8.55), and non-amnestic MCI, OR 2.24 (95% CI: 0.90, 5.56), predicted the risk of anxiety after adjusting for all potential confounders.

Table 2. Cross-sectional association of MCI with depressive and anxiety disorders.

MCI	Analyses of depressive disorders, N=4,168					Analyses of anxiety disorders, N=4,060				
	Cases/N[a]	Odds ratios (95% confidence intervals)				Cases/N[a]	Odds ratios (95% confidence intervals)			
		Model 1[b]	p	Model 2[c]	p		Model 1[b]	p	Model 2[c]	p
No MCI	103/3,755	Reference		Reference		284/3,662	Reference		Reference	
MCI	22/413	2.02 (1.25, 3.26)	0.004	1.94 (1.20, 3.15)	0.007	46/398	1.74 (1.24, 2.44)	0.001	1.70 (1.19,2.42)	0.004
Amnestic	8/161	2.11 (1.00, 4.44)	0.05	2.02 (0.95, 4.29)	0.07	14/160	1.35 (0.76, 2.39)	0.30	1.28 (0.69,2.38)	0.42
Non-Amnestic	14/252	1.97 (1.10, 3.53)	0.02	1.90 (1.05, 3.40)	0.03	32/238	2.00 (1.34, 2.98)	0.001	2.00 (1.30,3.04)	0.001

[a] Cases/N: number of depression or anxiety cases/total number of participants in respective groups; p: p-values.
[b] Model 1: adjusted for age and sex.
[c] Model 2: additionally adjusted for educational level, BMI, smoking, total cholesterol, HDL cholesterol, hypertension, diabetes mellitus 2, myocardial infarction, stroke, and cohort.

Table 3. Longitudinal association of MCI with incident depressive and anxiety disorders.

MCI	Analyses of depressive disorders, N=2,967					Analyses of anxiety disorders, N=2,375				
	Cases/N[a]	Odds ratios (95% confidence intervals)				Cases/N[a]	Odds ratios (95% confidence intervals)			
		Model 1[b]	p	Model 2[c]	p		Model 1[b]	p	Model 2[c]	p
No MCI	29/2,755	Reference		Reference		75/2,224	Reference		Reference	
MCI	6/212	2.96 (1.21,7.25)	0.02	3.13 (1.26,7.77)	0.01	11/151	2.54 (1.31,4.94)	0.006	2.59 (1.31,5.12)	0.006
Amnestic	1/80	1.58 (0.21,11.9)	0.66	1.79 (0.23,13.70)	0.57	5/67	2.92 (1.12,7.62)	0.03	3.22 (1.21,8.55)	0.02
Non-Amnestic	5/132	3.66 (1.39,9.65)	0.009	3.77 (1.40,10.13)	0.008	6/84	2.31 (0.97,5.52)	0.06	2.24 (0.90,5.56)	0.08

For these analyses, cases of dementia which occurred during the study period were excluded. These were 25 cases in the analyses of depressive disorders, and 9 for the analyses of anxiety disorders.

The odds ratios represent the relative risk of any depressive disorder (major depressive disorder, MDD, dysthymia, or minor depression), or anxiety disorder (generalized anxiety disorder, GAD, panic disorder, or phobias) over 9 years.

[a] Cases/N: number of depression or anxiety cases/total number of participants in respective groups; p: p-values.

[b] Model 1: adjusted for age and sex.

[c] Model 2: additionally adjusted for educational level, BMI, smoking, total cholesterol, HDL cholesterol, hypertension, diabetes mellitus 2, myocardial infarction, stroke, cohort, and time interval between two assessments.

DISCUSSION

In this population-based study, persons with MCI were more likely to have prevalent depressive and anxiety disorders as compared to those without MCI. Moreover, MCI was associated with a higher relative risk of incidence of each depressive and anxiety disorder. Non-amnestic MCI was associated with a higher relative risk of incident depressive disorders, whereas both subtypes of MCI were associated with a higher relative risk of incident anxiety disorders.

We found that persons with MCI had a higher prevalence of depressive and anxiety disorders compared to those without MCI, which is in line with the existing literature.[26-30] There are no prospective studies to investigate the association of MCI with depressive or anxiety disorders. The only evidence about an association of MCI with depression and anxiety is either derived from cross-sectional studies, or a few short follow-up studies which reported depression a risk factor for MCI, i.e. depression precedes the development of MCI.[6,7,29]

We found that MCI increased the risk of incident depression and anxiety. A lack of longitudinal studies on this subject hampered comparison of our results. However, several potential explanations gave rise to our hypothesis and can account for the observed associations. First, both depression and anxiety could occur as a "reactive" response to the underlying cognitive impairment, and symptoms could worsen with the increasing cognitive impairment. Experiencing forgetfulness, gradual inability to perform everyday tasks, and a fear of developing dementia could be intimidating enough to trigger severe symptoms of anxiety or depression in vulnerable individuals with MCI. Possibly, psychiatric symptoms would affect those persons with MCI more, who are more "aware" of their condition, and are able to quantitate the decline in their cognitive abilities. This might be particularly true for symptoms of anxiety, and could explain the finding of the prominent association between non-amnestic MCI with prevalent anxiety disorders in our study. Previously, an equally high prevalence of anxiety in both subtypes of MCI has been reported, but only in small clinical sample.[29] Nevertheless, the results regarding subtypes of MCI in our study should be interpreted with caution, as these comparative analyses lacked sufficient power. Second, depressive or anxiety disorders might affect those individuals who are genetically or environmentally more vulnerable to develop these conditions. Perhaps in vulnerable persons, depressive or anxiety disorders precipitate earlier, or their risk is amplified by having MCI. Third, the incidence of depression or anxiety in persons with MCI might represent an early stage of dementia. This implies that depression or anxiety appear as an intermediate stage between MCI and dementia.

Studies have shown that depression in late life is a prodrome of dementia.[31] Fourth, both MCI and psychiatric symptoms can result from a common etiological factor where cognitive symptoms precede emotional symptoms**.** One such pathway could be the vascular damage.[32] In this regard, the vascular depression hypothesis had been put forward, which implicates vascular pathology as a link between cognitive disorders, namely dementia, and depression, as the two entities frequently co-occur.[32] Some studies have also suggested that the atrophy of hippocampus results in both cognitive impairment and depression.[33] There is limited evidence as to the pathophysiology of anxiety disorders in context of early stages of neurodegeneration, but some studies have highlighted the role of caudate nucleus pathology as a possible link between MCI and anxiety.[29] The caudate nucleus plays a vital role in executive function, and shows typical pathological features in early stages of dementia,[34] and has been implicated in anxiety disorders.[35,36] In Alzheimer's disease patients with comorbid anxiety, the role of bilateral entorhinal cortex, amygdala, anterior parahippocampal gyri, left superior temporal gyrus, and insula has been implicated.[37,38] However, pathological changes in both the hippocampus and the caudate nucleus could very well be the consequences of underlying vascular pathology. Another common pathway could be the dysregulation of neurotransmitters. Both altered serotonergic activity observed in anxiety and depression, and the low adrenergic activity observed in depression, are also associated with cognitive disorders.[39-41] Further, inflammation has also been implicated by some studies as a common pathway between cognitive impairment and depression.[42]

Studies have also argued that MCI is a psychiatric entity. This hypothesis proposes that in MCI, although the underlying pathology is neurodegenerative, the manifestation is largely in the form of psychiatric symptoms.[43,44] Persons experiencing a decline in their cognitive abilities and being aware of this decline are likely to react with an alteration in mood or behavior, and sleeping or eating habits.

There are several strengths of this study. To our knowledge, this is the first study to investigate MCI in relation to incident anxiety and depression. Other strengths include a large population-based sample, adjusting for several potential confounders, and a robust continuous monitoring of dementia which enabled to exclude incident cases occurring during the study period. However, there are certain limitations. We did not measure visuospatial ability, and therefore could not include this component in our diagnostic criteria for MCI. We did not have sufficient number of cases to test the association between amnestic MCI and incident depression and therefore the estimates might not be precise. Finally, residual confounding due to unknown or unmeasured confounders might be present.

In conclusion, MCI is a possible precursor of psychiatric outcomes, such as depressive and anxiety disorders. Therefore, MCI should not just be regarded as a potential transition stage between normal aging and dementia, but a forerunner of both cognitive and psychiatric outcomes. Our results suggest a shared etiology between neurodegenerative and psychiatric disorders and opens avenues for etiological research to unravel the common biological pathways underlying cognitive and psychiatric disorders.

SUPPLEMENTARY INFORMATION

Supplementary Table. Baseline characteristics of the study population included in the analyses of MCI and anxiety disorders.

Characteristics	Cross-sectional analysis of anxiety disorders, N=4,060		Longitudinal analysis of anxiety disorders, N=2,384	
	No MCI n=3,662	MCI n=398	No MCI n=2,230	MCI n=154
Age, years	71.4 (7.1)	73.3 (7.5)	69.4 (6..2)	69.1 (5.8)
Women	2,339 (57.7)	202 (50.9)	1,261 (56.6)	67 (43.5)
Body mass index, kg/m^2	27.6 (4.1)	27.6 (4.1)	27.7 (4.0)	27.5 (4.0)
Educational level				
Low	375 (9.4)	69 (17.5)	142 (6.5)	17 (11.2)
Intermediate	3,028 (75.9)	271 (68.8)	1,701 (77.8)	106 (69.7)
High	585 (14.7)	54 (13.7)	344 (15.7)	29 (19.1)
Smoking				
Never	1,214 (29.9)	105 (26.4)	680 (30.5)	38 (24.7)
Former	2,207 (54.4)	221 (55.7)	1,238 (55.5)	96 (62.3)
Current	634 (15.6)	71 (17.9)	312 (14.0)	20 (13.0)
Total, cholesterol, mmol/L	5.6 (1.0)	5.4 (1.0)	5.7 (0.9)	5.5 (1.0)
HDL cholesterol, mmol/L	1.4 (0.4)	1.4 (0.4)	1.5 (0.4)	1.4 (0.4)
Hypertension	3,254 (80.2)	330 (83.1)	1,715 (77.0)	109 (70.8)
Diabetes mellitus	559 (13.8)	71 (17.9)	253 (11.3)	22 (14.3)
Myocardial infarction	286 (7.1)	49 (12.6)	119 (5.3)	14 (9.2)
Stroke	120 (3.0)	22 (5.5)	37 (1.7)	9 (5.8)

Values are means (standard deviation) or counts (percentage).

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CHAPTER 3

LIFESTYLE FACTORS AND NEUROLOGICAL OUTCOMES

CHAPTER 3.1

Coffee consumption and incident dementia

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ABSTRACT

Background: Coffee consumption has been frequently reported for its protective association with incident dementia. However, this association has mostly been reported in studies with short follow-up periods, and it remains unclear to what extent reverse causality influences this association. Studying the long-term effect of coffee consumption on dementia with stratified follow-up time may help resolve this issue.

Methods: In the population-based Rotterdam Study, coffee consumption was assessed in 1989-1991 (N=5,408), and reassessed in 1997-1999 (N=4,368). Follow-up for dementia was complete until 2011. We investigated the association of coffee consumption and incident dementia for the two examination rounds separately using flexible parametric survival models. We studied the entire follow-up period as well as stratified follow-up time at 4 years.

Results: For both examination rounds, we did not find an association between coffee consumption and dementia over the entire follow-up. In contrast, for both examination rounds, a protective association was observed only in the follow-up stratum of 0 to 4 years.

Conclusions: Our data suggest that coffee consumption is not associated with incident dementia during long-term. The protective association observed in the short-term might be driven by reverse causality.

INTRODUCTION

Coffee is one of the most popular beverages throughout the world and has been frequently reported for its protective association with incident dementia[1-3] and cognitive decline.[4-6] Coffee contains a diverse group of biologically active compounds namely caffeine, phytochemicals, and minerals, which have considerable metabolic, physiological, cellular, and molecular influence.[7] The beneficial effect of coffee on dementia has been attributed mainly to caffeine, a neuro-stimulatory compound.[8] Coffee also prevents endothelial damage[9] by reducing the risk of hypertension and diabetes mellitus type 2[1] which is partly attributed to its anti-oxidant properties. Therefore coffee-intake might also reduce the risk of dementia indirectly.

To date, most studies reporting a protective association between coffee and dementia have examined the association over relatively short follow-up periods, mostly ranging from 1 to 5 years.[3,6,10,11] In contrast, studies with longer follow-up time have produced inconsistent results.[1,5,12,13] An advantage of studies with short follow-up, particularly those with younger populations, is that less attrition occurs and therefore there is less opportunity for bias from differential attrition. However, in short follow-up studies, it is challenging to disentangle the independent influence of coffee consumption on dementia from the effects of lifestyle factors which may indicate general well-being of individuals. Thus, protective associations observed in short follow-ups might represent reverse causality. Stratification of follow-up time into short and long follow-up may elucidate this issue, but was not performed in previous studies.

In a population-based cohort study with repeated assessments of coffee consumption, we investigated the association of coffee with dementia. Specifically, we examined this association over the entire follow-up as well as stratified follow-up time to address reverse causality.

METHODS

Setting

This study was embedded in the Rotterdam Study, an ongoing population-based cohort of the elderly that started in 1989-1990 and studies the incidence and determinants of chronic diseases in late life.[14] The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. A written informed consent was obtained from all participants.

Every 3 to 4 years, all participants undergo an extensive home interview and a physical examination at the research center. In addition, all participants are continuously monitored for the occurrence of all major events by linkage of the study database with medical files from general practitioners. Coffee consumption was assessed in 1989-1991 and in1997-1999. Follow-up started from the day of study entry, till the date of incident dementia, date of death, or the censor date January 1st, 2011, whichever occurred first.

Study population

Of the original cohort of 7,983 participants, 482 with prevalent dementia, and 455 who were not sufficiently screened for dementia, were excluded. Of the remaining 7,046 participants, 1,638 who did not have coffee consumption data available at baseline were also excluded. This yielded a total of 5,408 participants available for analysis, followed for a maximum of 20.7 years, (mean 13.2, standard deviation (SD) 5.4years) for incident dementia. The missing data for coffee consumption was completely at random , and occurred mainly because the food frequency questionnaires were not offered to all participants due to unavailability of trained interviewers at certain times. It was not related to any participant characteristics and therefore, was not likely to be a source of bias in our study.

From the 5,408 persons assessed in 1989-1991, 4,797 individuals participated in the follow-up examination round in 1997-1999 (major lost to follow-up was because of deaths). We excluded 347 participants with prevalent dementia, 7 who were lost to follow-up, and 75 for whom coffee data were not available at this repeated assessment. This yielded a total of 4,368 individuals for analysis, followed for a maximum of 13.8 years, (mean 8.7, (SD) 3.5 years) for incident dementia.

Assessment of coffee consumption

Daily coffee consumption was assessed as part of the home interview. In addition, types of coffee consumed, such as regular, decaffeinated, instant, or a combination of these was also inquired. Coffee consumption was categorized into three categories for main analyses; no coffee to low coffee consumption (0-1 cup/day) which was used as the reference category, moderate consumption (>1-3 cups/day) and high coffee consumption (>3 cups/day). Similar cut-offs have been used in studies investigating the association between coffee consumption and dementia.[1,13] Coffee consumption was not dichotomized as non-drinkers and drinkers, as the percentage of non-drinkers in our sample was very small (<3.1%).

Assessment of dementia

Participants were screened for dementia at baseline and follow-up examinations using a three-step protocol.[15] Screening was done using the Mini Mental State Examination (MMSE) [16] and the Geriatric Mental Schedule (GMS) organic level.[17] Screen-positives (MMSE<26 or GMS organic level>0) subsequently underwent an examination and informant interview with the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX).[18] Participants who were suspected of having dementia, if necessary, had further neuropsychological testing. Additionally, the total cohort was continuously monitored for dementia through computerized linkage between the study database and digitized medical records from general practitioners (GPs)and the Regional Institute for Outpatient Mental Health Care. This data linkage system is highly efficient in the Dutch situation, where the GPs receive all medical information about the patients if they contact any medical care-giver or professional including specialists. In case of death of a patient, GPs also receive information about the cause of death. Finally, a consensus panel led by a neurologist, decided on the final diagnosis in accordance with the standards using the DSM-III-R criteria for dementia and the NINCDS-ADRDA for Alzheimer's disease[19] If required for differential diagnosis, neuro-imaging was used. We calculated the potential and observed person-years to calculate the completeness of follow-up.[20] Follow-up for incident dementia was complete (98.2 % for cohort followed from baseline and 97.6% for cohort followed from repeated assessment of coffee consumption) till January 1st, 2011.

Covariates

In addition to age and gender, education level, family history of dementia, working status, body mass index (BMI), smoking, diabetes, hypertension, and alcohol use were considered as potential confounders. Smoking, hypertension and diabetes are well documented risk

factors for dementia. Low education level has been found to be associated with increased risk of dementia.[21] BMI was used as a covariate as a measure overall dietary intake. Working status was used as a measure of social engagement in participants. Smoking and alcohol intake are important lifestyle factors as is coffee consumption.

Level of education was assessed during the interview and participants were classified into eight categories; primary education , primary with unfinished secondary education, lower vocational, lower secondary, intermediate vocational, general secondary, higher vocational, and university education. BMI was calculated as weight in kilograms/height in meters-squared. For family history, participants were asked if their parents, siblings or children suffer from dementia. Participants were asked if they are engaged in any paid or volunteer work versus retired or unemployed. Smoking habits were also assessed during the interview and participants were classified as current, former or never smokers. Diabetes mellitus type 2 was defined as non-fasting serum glucose level exceeding 198 mg/dl or the use of anti-diabetic medication or insulin.[22] Blood pressure was measured twice at the right arm in sitting position at the research center; average of two blood pressure readings was used. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or use of antihypertensive medication assessed by interview and pharmacy records.[23] Alcohol consumption was assessed as part of the home interview. Weekly reported alcohol consumption was categorized into beer, wine, liquor, and moderately strong alcohol types. These were converted to grams of alcohol by taking the average amount of ethanol in a drink. “A drink” was defined as 200 ml of beer (8.0 g ethanol), 100 ml of wine (10.0 g ethanol), 50 ml of liquor (14.0 g ethanol), or 75 ml of moderately strong alcohol (10.5 g ethanol). By adding the amount of ethanol in all four groups, total amount of alcohol in g/day was calculated.[24]

Statistical analyses

Coffee consumption was assessed at two examination rounds (1989-1991 and 1997-1999), which formed the baseline for two respective analyses. Using these baselines, we assessed coffee consumption and risk of dementia using flexible parametric models for survival analyses. We studied the entire follow-up period, and subsequently stratified follow-up time at 4 years into a stratum of short follow-up of 0-4 years, and a longer follow-up of >4 years.

In order to obtain more robust effect estimates, we meta-analyzed the hazard ratios from the 0-4 year intervals from both analyses, based on the martingale properties of survival data, which state that the time increments in a survival analysis are independent of each

other. Since the hazard at any given time instant is not influenced by the hazards at the previous instant of time in a survival data of the same persons, it is justified to pool the hazard ratios from these two sets of same persons, assessed at different time points, assuming that they are independent.[25] We performed simulations to further verify the independence of the hazard ratios of the two subsequent time epochs.

Several sensitivity analyses were performed. The 4-year cut-off was arbitrary and was used to ensure comparability with other studies. Therefore, analyses were repeated examining intervals of 2, 3 and 5 years. Analyses were also repeated using 0-2 cups of coffee per day as reference: 0-2 cups/day, >2-3 cups/day, and >3 cups/day. In addition, all analyses were repeated after excluding persons drinking decaffeinated coffee only (n= 660 from baseline, and n=429 from repeated assessment of coffee), excluding non-coffee-drinkers (n=156 from baseline, and n=134 from repeated assessment of coffee), and by using Alzheimer's disease as outcome instead of all-cause dementia. Effect modification of the association between high coffee consumption and dementia by smoking and diabetes mellitus 2 was assessed by stratification and use of interaction terms. We also repeated our analyses stratified on baseline age. Finally, change in coffee consumption from baseline to repeated assessment was investigated in relation to incident dementia.

Results are presented as hazard ratios (HR) with 95 % confidence intervals (CI). All analyses were adjusted for age, sex, and additionally for education, family history of dementia, working status, BMI, smoking, diabetes mellitus 2, hypertension, and alcohol intake. Data were analyzed using the Stata Software Version 13 (StataCorp, College Station, TX, USA).

RESULTS

In our data, persons in the >3 cups category at both coffee consumption assessments were younger, more involved in work, more educated, and had a lower prevalence of hypertension and diabetes. However, they had more prevalence of current smoking and lower HDL cholesterol as compared to those in the lower coffee consumption categories. **Table 1** summarizes the baseline characteristics of persons in the analyses.

From 1989-1991 to end of follow-up, 814 persons developed incident dementia during 71,629 person-years. From 1997-1999 onwards, 578 persons developed dementia during 38,002 person-years. We found no evidence of a protective association between higher coffee consumption and incidence of dementia over the entire follow-up for both

Table 1. Characteristics of participants at baseline exam in 1989-1991 and at repeated exam in 1997-1999.

	At Baseline, N=5,408				At Follow-up, N=4,368			
Characteristics	**0-1 cup/day**	**>1-3 cups/day**	**>3 cups/day**	**P-value**	**0-1 cup/day**	**>1-3 cups/day**	**>3 cups/day**	**P-value**
Age, years	70.3 (8.6)	69.5 (7.8)	66.3 (7.3)	<0.001	75.1 (8.0)	74.3 (7.5)	70.9(6.5)	<0.001
Women	230 (63.9)	1,160 (64.7)	1,802 (55.3)	<0.001	323 (65.6)	1,090 (64.2)	1,164 (53.4)	<0.001
Education Level								
Low	133 (37.0)	631 (35.4)	1,104 (34.1)	0.02	164 (34.2)	569 (34.0)	613 (28.5)	0.002
Intermediate	182 (50.7)	991 (55.6)	1,876 (57.9)		262 (54.6)	948 (56.7)	1,317 (61.3)	
High	44 (12.3)	161 (9.0)	259 (8.0)		54 (11.2)	156 (9.3)	218 (10.1)	
Family history of dementia	72 (20.1)	426 (23.8)	813 (25.0)	0.10	96 (20.2)	400 (24.1)	511 (23.9)	0.18
Working (volunteering incl.)	42 (11.7)	235(13.2)	559 (17.3)	<0.001	93 (18.9)	386 (22.7)	541 (24.8)	0.014
Smoking								
Never	174 (48.5)	731 (41.1)	909 (28.1)	<0.001	220 (44.7)	654 (38.5)	633 (29.0)	<0.001
Former	153 (42.6)	774 (43.5)	1,378 (42.6)		234 (47.6)	840 (49.5)	1,069 (49.1)	
Current	32 (8.9)	275 (15.4)	950 (29.4)		38 (7.7)	204 (12.0)	476 (21.9)	
Body mass index, kg/m^2	25.9 (3.8)	26.1 (3.6)	26.5 (3.6)	<0.001	26.7 (3.7)	26.8 (3.6)	26.9 (3.8)	0.2
Hypertension	218 (60.6)	1,065 (59.4)	1,662 (51.0)	<0.001	354 (71.9)	1,201 (70.7)	1,415 (64.6)	<0.001
Diabetes mellitus 2	33 (9.2)	189 (10.5)	283 (8.7)	0.09	143 (29.1)	478 (28.1)	466 (21.3)	<0.001
Total cholesterol, mg/100 ml	255.2(46.4)	259.1(46.4)	259.1 (46.4)	0.1	224.3 (39.4)	228.1 (38.2)	224.3 (34.8)	0.06
HDL cholesterol, mg/100 ml	50.3 (14.3)	54.1 (13.9)	50.3 (15.5)	0.01	54.5 (18.6)	54.5 (15.5)	50.3 (15.5)	<0.001

Abbreviations: HDL High Density Lipoprotein.
Values are means (standard deviation) or counts (percentage).
P-values indicate differences in characteristics across coffee consumption categories, as estimated by T-tests for continuous variables and Chi-square test for categorical variable.

analyses: HR 1.09 (95% CI: 0.94, 1.25) for the first coffee assessment, and HR 1.11 (95% CI: 0.94, 1.32) for the second assessment (**Table 2**).

Table 2. Hazard ratios (95% confidence intervals) for incident dementia during the entire follow-up period.

Coffee consumption	Risk of dementia, hazard ratios (95% confidence intervals)		
	n/N	Model 1[a]	Model 2[b]
Coffee consumption at baseline exam (1989-1991)			
Continuous	814/5,408	1.02 (0.98, 1.07)	1.02 (0.98, 1.06)
Categorical			
0-1 cup/day	67/360	1 (ref)	1 (ref)
>1-3 cups/day	296/1,792	0.92 (0.70, 1.20)	0.88 (0.67, 1.16)
>3 cups/day	451/3,256	1.04 (0.80, 1.35)	1.00 (0.76, 1.30)
Coffee consumption at repeated exam (1997-1999)			
Continuous	578/4,368	1.04 (0.99, 1.10)	1.05 (0.99, 1.11)
Categorical			
0-1 cup/day	80/492	1 (ref)	1 (ref)
>1-3 cups/day	235/1,698	0.87 (0.67, 1.12)	0.86 (0.66, 1.12)
>3 cups/day	263/2,178	1.01 (0.78, 1.31)	1.03 (0.79, 1.34)

[a] Model 1: Hazard ratios adjusted for age and sex.
[b] Model 2: Hazard ratios additionally adjusted for education level, body mass index, smoking, hypertension, diabetes mellitus 2, alcohol use, family history of dementia, and working status.

Table 3 shows hazard ratios after stratifying follow-up time at 4 years. At both assessment rounds of coffee consumption, we found a protective association between higher coffee consumption and incident dementia during the first 4 years of follow-up, comparing individuals drinking 3 or more cups of coffee per day with individuals drinking no coffee or up to 1 cup per day. Meta-analysis of hazard ratios for >3 cups category in the 0-4 year interval from the two assessment rounds showed a statistically significant association between coffee consumption of >3 cups/day and dementia, HR 0.70 (95% CI: 0.51, 0.96).

In contrast, during the follow-up period from 4 years onwards, we found an increased risk of incident dementia in persons drinking 3 or more cups of coffee per day compared with individuals drinking no coffee or up to 1 cup per day (**Table 3**).

Table 3. Hazard ratios (95% confidence intervals) for incident dementia after stratifying follow-up time at 4 years.

Coffee consumption	Risk of dementia, hazard ratios (95% confidence intervals)					
	Stratum 0-4 years			Stratum >4 years		
	n/N[a]	Model 1[b]	Model 2[c]	n/N	Model 1[b]	Model 2[c]
Coffee consumption at baseline exam (1989-1991)						
Continuous	112/5,408	0.94 (0.83, 1.05)	0.94 (0.83, 1.06)	702/4,935	1.04 (0.99, 1.08)	1.04 (0.99, 1.08)
Categorical						
0-1 cup/day	13/360	1 (ref)	1 (ref)	54/318	1 (ref)	1 (ref)
>1-3 cups/day	50/1,792	0.92 (0.50, 1.70)	0.88 (0.47, 1.63)	246/1,596	0.92 (0.69, 1.24)	0.90 (0.67, 1.21)
>3 cups/day	49/3,256	0.75 (0.40, 1.40)	0.74 (0.39, 1.39)	402/3,021	1.10 (0.83, 1.47)	1.05 (0.78, 1.41)
Coffee consumption at repeated exam (1997-1999)						
Continuous	152/4,368	0.97 (0.83, 1.08)	0.96 (0.86, 1.07)	426/3,727	1.06 (1.02, 1.13)	1.07 (1.01, 1.15)
Categorical						
0-1 cup/day	27/492	1 (ref)	1 (ref)	53/387	1 (ref)	1 (ref)
>1-3 cups/day	75/1,698	0.87 (0.56, 1.34)	0.87 (0.55, 1.37)	160/1,405	0.88 (0.64, 1.20)	0.85 (0.62, 1.17)
>3 cups/day	50/2,178	0.74 (0.46, 1.19)	0.69 (0.42, 1.15)	213/1,935	1.13 (0.83, 1.53)	1.14 (0.83, 1.56)

[a] n/N: dementia cases/number of persons at risk for dementia.
[b] Model 1: adjusted for age and sex.
[c] Model 2: additionally adjusted for education level, body mass index, smoking, hypertension, diabetes, mellitus 2, alcohol use, family history of dementia, and working status.

Results did not change meaningfully on repeating our analysis using 2, 3 and 5 years as cut-offs of follow-up (**Supplement table 1**), and using 0-2 cups of coffee consumption as reference (data not shown). Results also did not change after excluding persons drinking decaffeinated coffee, excluding non-coffee-drinkers, and using Alzheimer's disease as outcome (data not shown).

Interaction terms were non-significant for both smoking (p-value 0.185) and diabetes (p-value 0.697). In addition, no significant change in results was observed after stratification on smoking and diabetes (data not shown). Results also remained unchanged after stratification on 75 years of age and are shown in **supplement table 2**.

Finally, we investigated change in coffee consumption from baseline assessment to reassessment of coffee consumption in relation with dementia. Persons who maintained drinking >3 cups/day (1,755) showed a statistically significantly lower risk of incident dementia over 4 years of follow-up compared to participants who reduced their intake, HR 0.59 (95% CI: 0.35, 0.98).

DISCUSSION

In this population-based study using coffee assessment of the same persons at two time points, we found no protective association between coffee consumption and the risk of dementia during the entire follow-up period. Instead, we found a protective association between coffee consumption and incident dementia with short follow-up, which reversed with long follow-up.

Strengths of the study include a population-based design, the long follow-up period including stratification, repeated assessment of coffee consumption, and intensive dementia case-ascertainment. There are certain methodological considerations which should be discussed. Coffee consumption was self-reported in our study. The methods of brewing coffee as filtering or boiling were not registered. Possibility of some residual confounding due to unknown or unmeasured confounders, such as physical activity, depression, and dietary patterns cannot be completely ruled out. We however adjusted for many potential confounders and the results remained unchanged. Another methodological consideration is that we assume no interaction with time and aging in our study.

Coffee drinking, similar to other lifestyle factors, is associated with a number of socio-demographic variables and health in general,[13] but it did not independently predict dementia in our study during long follow-up. The Finnish twin study[13] and the Honolulu-Asia Aging Study[12] also reported similar findings; the former used cognitive performance as outcome, measured by a telephonic interview and the latter included only men. In contrast, the CAIDE study reported a protective association of midlife coffee consumption and dementia over 21 years in 1409 participants. It is possible that a long-term protective association is present only for continued coffee consumption from midlife onwards, but note that the study included relatively young individuals which led to relatively small number of incident dementia cases.[1]

The novelty of our study is that we stratified follow-up time at 4 years, and found a protective association between coffee consumption and incident dementia during the short follow-up of 0-4 years. Importantly, this observation was made for both coffee assessments.

A possible explanation for our finding of a short-term protective association is reverse causality. Elderly drinking more cups of coffee per day are likely to be engaged in physical and social activities and are healthier in general. Coffee drinking therefore, like alcohol drinking or socializing, or any other lifestyle factor, could be a proxy of good health and general wellbeing. In contrast, persons experiencing cognitive decline or even with subclinical dementia, modify their dietary habits which includes reducing coffee consumption. This would therefore result in a protective association of high coffee consumption with incident dementia. This view is supported by our data, as we observed that the persons in >3 cups category were younger, more involved in work, more educated, and had a lower prevalence of hypertension and diabetes as compared to persons in the lower consumption categories at both rounds of coffee consumption assessment. Further evidence in support of this explanation comes from the observation in our study that persons who maintained their coffee consumption between the two assessments had a lower risk of dementia compared to those who reduced coffee consumption. Such healthy-worker effect leading to reverse causality may also explain similar short-term associations reported in previous studies as well as the lack of associations in studies with long follow-up.[3,6,10,11]

An alternate explanation for short-term association is that there is actually only a short-term beneficial association of coffee and incident dementia. Being a short-acting neuro-stimulant, caffeine may exert its beneficial effect on cognition in the beginning which subsequently wanes away with prolonged use. The beneficial effects of coffee on

hypertension might also explain this association.[26,27] Also, a short-term protective association might be a chance finding. This is however unlikely because a protective association was observed in 0-4 year periods after both coffee assessments: at baseline and at the follow-up round.

Interestingly, we found a harmful association between coffee consumption and incident dementia after excluding the first four years of follow-up. With these data, however, it is early to comment whether the increased risk after 4 years is driven by coffee consumption or by other confounding risk factors, or both. Coffee consumption especially of boiled coffee,[8] may increase the risk of dementia via hypercholesterolemia[1] with prolonged use. There is a possibility that high coffee consumption delays symptoms of cognitive decline and therefore diagnosis, which leads to more case detection later during follow-up. In the same vein, an increased risk of dementia after excluding first 4 years of follow-up could be a reactive phenomenon, which manifests because of the reduction in coffee consumption, removing its beneficial effects.

In conclusion, our data suggest that coffee consumption is not associated with incident dementia over a long follow-up. The observed short-term protective association likely represents reverse causality. Coffee consumption like other lifestyle factors is likely a proxy of good health and general well-being.

SUPPLEMENTARY INFORMATION

Supplementary Table 1. Hazard ratios (95% confidence intervals) for incident dementia by coffee consumption categories, stratifying follow-up time at cut-offs of 2, 3 and 5 years.

Coffee consumption		Risk of dementia			
		Strata of short follow-up		Strata beyond short follow-up	
	Cut-off point of follow-up	n/N	Hazard ratio (95% CI)	n/N	Hazard ratio (95% CI)
Coffee consumption at baseline exam (1989-1991)					
	2 years	51/5,408	0.93 (0.31, 2.81)	763/5,213	0.99 (0.76, 1.31)
	3 years	77/5,408	0.58 (0.28, 1.22)	737/5,093	1.06 (0.80, 1.41)
	5 years	167/5,408	0.86 (0.51, 1.43)	647/4,777	1.05 (0.77, 1.44)
Coffee consumption at repeated exam (1997-1999)					
	2 years	76/4,368	0.61 (0.31, 1.20)	502/4,055	1.10 (0.82, 1.47)
	3 years	138/4,368	0.73 (0.44, 1.24)	440/3,885	1.11 (0.81, 1.52)
	5 years	220/4,368	0.97 (0.63, 1.49)	358/3,505	1.03 (0.73, 1.44)

Hazard ratios for dementia are for >3cups of coffee/day versus 0-1 cup of coffee/day.
Hazard ratios are adjusted for age, sex, education level, body mass index, smoking, hypertension, diabetes mellitus 2, alcohol use, family history of dementia, and working status.

Supplementary Table 2. Hazard ratios (95% confidence intervals) for incident dementia stratified by age.

	≤ 75 years		> 75 years	
	0-4 years	> 4 years	0-4 years	> 4 years
n/N	37/4,360	486/4,119	75/1,048	216/816
Coffee consumption at baseline exam (1989-1991)				
0-1 cup/day	1 (ref)	1 (ref)	1 (ref)	1 (ref)
>1-3 cups/day	0.54 (0.17, 1.72)	1.11 (0.72, 1.71)	1.04 (0.50, 2.17)	0.72 (0.47, 1.10)
>3 cups/day	0.56 (0.18, 1.69)	1.26 (0.83, 1.93)	0.82 (0.38, 1.76)	0.93 (0.61, 1.42)
n/N	31/2,804	199/2,582	121/1,564	227/1,145
Coffee consumption at repeated exam (1997-1999)				
0-1 cup/day	1 (ref)	1 (ref)	1 (ref)	1 (ref)
>1-3 cups/day	0.38 (0.14, 1.06)	0.92 (0.53, 1.60)	1.04 (0.62, 1.74)	0.81 (0.55, 1.21)
>3 cups/day	0.38 (0.14, 1.01)	1.31 (0.78, 2.23)	0.81 (0.46, 1.46)	1.03 (0.69, 1.53)

Hazard ratios are adjusted for age, sex, education level, body mass index, smoking, hypertension, diabetes mellitus 2, alcohol use, family history of dementia, and working status.

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CHAPTER 3.2

Association of coffee consumption with MRI markers and cognitive function- A population-based study

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Submitted

ABSTRACT

Background: Coffee is one of the mostly consumed beverages worldwide, and has been of considerable interest in cognitive and dementia research due to its neuro-stimulatory effects. However, the associations of coffee consumption with MRI biomarkers of dementia, and pre-morbid cognitive performance have rarely been investigated.

Methods: In a community-dwelling sample of 2,914 older adults, we assessed coffee consumption habits. Moreover, these participants underwent brain MRI to quantify brain volume, hippocampal volume, and white matter lesion volume and to assess the presence of lacunar infarct. Finally, we examined cognitive function in these participants using a standardized neuropsychological test battery. We investigated associations of coffee consumption with MRI-markers and cognitive function using multiple linear and logistic regression models, and explored if MRI-markers affected the association between coffee consumption and cognition.

Results: We found that per cup increase in coffee consumption was associated with a lower prevalence of lacunar infarcts, Odds Ratio (OR) 0.88 (95% confidence interval (CI): 0.80, 0.97), and with smaller hippocampal volume, difference of the mean in hippocampal volume (mL): -0.01 (95% CI: -0.02, -0.00)]. Regarding cognitive function, we found that the consumption of >3 cups of coffee per day was associated with better performance on the Letter-Digit Substitution Task, difference of the mean: 1.20 (95% CI: 0.46, 1.94)], but worse performance on the Stroop color naming subtask, difference of the mean: 0.74 (95% CI: 0.22, 1.26), Stroop interference subtask, difference 1.81 (95% CI: 0.22, 3.40), and the 15-word verbal learning delayed recall, difference of the mean:-0.38 (95% CI:-0.74, -0.03)]. These associations were independent of the MRI findings.

Conclusions: Habitual coffee consumption was associated with a smaller hippocampal volume, and worse information processing speed and delayed memory. However, habitual coffee consumption seemed to be protective against lacunar infarcts, and was associated with better executive function.

INTRODUCTION

Coffee is one of the mostly consumed beverages worldwide and has therefore been of considerable interest regarding its potential effects on health.[1-4] Coffee is a complex mixture consisting of more than 1,000 different compounds with different physiological effects.[5] Compounds that are thought to be beneficial for health are caffeine, which is the major constituent of coffee and a strong neurostimulant, polyphenols, which have antioxidant properties.[5,6] Several studies have shown that the overall effect of coffee consumption is beneficial for various diseases, including diabetes,[2] and cardiovascular disease,[1,3] and all-cause and cerebrovascular, mainly stroke related mortality.[7-9] More recent evidence also links coffee consumption to a lower risk of dementia.[4,10-12]

Dementia is multifactorial disease with a long pre-clinical phase during which various pathologies steadily accumulate. Amyloid deposition and vascular brain disease are the two major pathologic processes involved in the development of dementia.[13,14] Clinically, these pathologies lead to subtle cognitive deficits that can be detected using cognitive testing, whilst structural magnetic resonance imaging (MRI) allows for in vivo visualization of markers of these pathologies, including brain atrophy, hippocampal atrophy, white matter lesions, and lacunar infarcts.[15] Elucidating the link between coffee consumption and these pre-clinical markers is essential to further disentangle the exact effect of coffee consumption on the brain. Preliminary evidence indeed links coffee consumption to cognition or brain MRI-markers, but these studies consisted of small samples, focused only one or two cognitive domains, or included persons not at risk of dementia (e.g. health young volunteers).[4,10,12,15,16] Therefore, it remains largely unknown how coffee consumption affects pre-clinical markers of dementia.

In a population-based setting of community-dwelling older adults, we investigated the association of habitual coffee consumption with several structural brain-MRI-markers and cognitive function.

METHODS

Setting

This study was embedded within the Rotterdam Study,[17] a population-based cohort study, aimed at investigating determinants of chronic diseases in the middle aged and elderly. The study started in 1990 with 7,893 persons, aged 55 years and older. In 2000, the cohort

was expanded with 3,011 participants, with the same inclusion criteria. In 2006, the cohort was again expanded with 3,932 participants aged 45 years or older. From 2005 onwards, all participants that came to the research center were invited for an MRI examination of the brain. For the current study, we included all participants that visited the research center from the first expansion between 2004 and 2006 and second expansion between 2006 and 2008, and who underwent MRI between 2005 and 2009. The Rotterdam Study has been approved by the medical ethics committee according to the Population Screening Act: Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. All participants provided written informed consent. A written informed consent was obtained from all participants.

Study population

A total of 3,871 persons underwent MRI between 2005 and 2009. Of these MRI examinations, 13 were excluded due to image artefacts. Moreover, we excluded all persons with prevalent dementia (n=16), cortical infarcts (n=99), and prevalent clinical stroke (n=55). Coffee consumption data was not available for 774 participants because not all participants received a food frequency questionnaire due to unavailability of trained personnel at times to administer the questionnaire. Therefore, information on coffee consumption was available for 2,914 persons, constituting the total sample available for analyses. Not all persons underwent all cognitive tests, resulting in slightly different totals for each separate cognitive test (**Figure 1**).

Assessment of coffee consumption

Coffee consumption was assessed as part of a validated semi-quantitative food frequency questionnaire indicating all foods and drinks consumed more than once a month during the preceding year.[18] The questionnaire comprised food items and all relevant beverages, including coffee, and was administered by a trained dietician. Participants reported their habitual coffee intake as number of cups per day, week, or month. The dietary coffee consumption was converted into miligrams (mg) of coffee per day, and then into cups of coffee per day.

Brain MRI and post-processing

Brain MRI-scanning was performed on a 1.5T-scanner with an eight-channel head coil (GE Healthcare, Milwaukee, Wisconsin, USA), and included a T1-weighted (T1w) sequence, a proton-density (PDw) weighted sequence, and a fluid-attenuated-inversion-recovery (FLAIR) sequence.[19] Automated brain tissue classification based on a k-nearest-neighbor-

classifier algorithm extended with white matter lesion segmentation was used to quantify brain volume, grey matter volume, white matter volume, white matter lesion volume, (in milliliters).[20,21] We also quantified left and right hippocampal volume (in millilitres) using a validated, custom atlas-based method.[22] Total hippocampal volume was defined as the sum of the left and right hippocampal volumes. Lacunar and cortical infarcts were rated on the FLAIR, PDw and T1w sequences.[23] Lacunar infarcts were dichotomized into present versus absent.

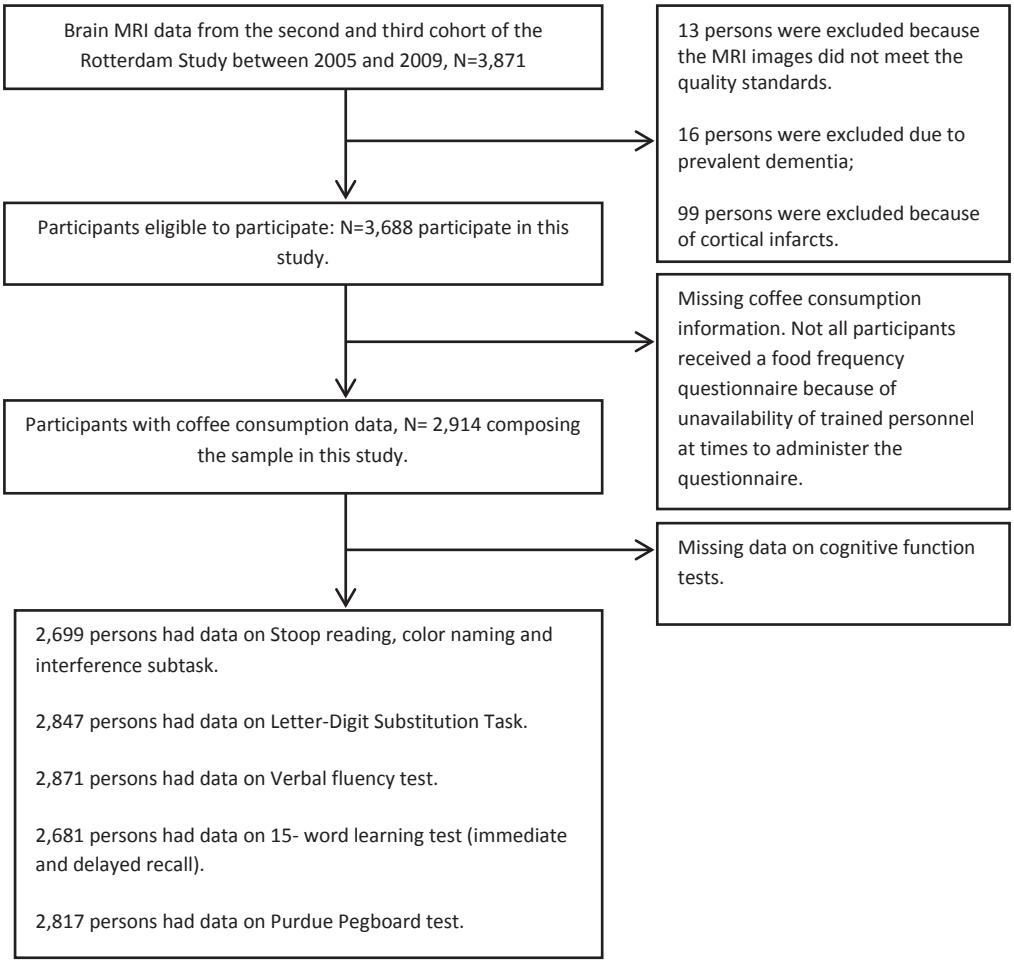

Figure 1. Flowchart demonstrating the selection of the study population.
The figure shows sequential exclusions of participants due to missing information on MRI markers, coffee consumption, or cognitive tests.

Assessment of cognitive function

All participant underwent a standardized neuropsychological test battery which included the following tests: Stroop tests[24] (which were used to evaluate speed of reading, color naming, and interference of automated processing and attention; Letter-Digit Substitution Task (LDST) to evaluate processing speed and executive function,[25] verbal fluency test to evaluate efficiency of long term memory,[26] 15-Word verbal immediate and delay recall tests to evaluate retrieval from verbal memory and recognition of verbal memory,[27] and Purdue Pegboard test to evaluate dexterity and fine motor skill.[28] Higher scores indicate a better performance on all cognitive tests, except for the Stroop tests in which a higher score indicates a worse performance as it measures time taken to complete the task. Accordingly, scores for the Stroop test were inverted for comparison to other tests.[29]

Other measurements

Information on educational level and cardiovascular risk factors was gathered by interview, physical examination and laboratory tests. We assessed the highest educational attainment of each participant (university degree, higher vocational education, general secondary education, intermediate vocational education, lower secondary education, lower vocational education, primary education). Body mass index was calculated as weight in kilograms/height in meters-squared (Kg/m^2). We measured systolic and diastolic blood pressures twice at the right arm and used the mean of the two measurements. Hypertension was defined as a blood pressure ≥140/90mmHg or use of blood pressure lowering medication prescribed for the indication of hypertension. Fasting blood samples were obtained and serum total cholesterol and high-density lipoprotein (HDL) cholesterol were measured using an automatic enzymatic procedure (Hitachi analyzer, Roche Diagnostics). Glucose was determined enzymatically by the Hexokinase method. Diabetes mellitus 2 was defined as a fasting serum glucose level ≥7.0 mmol/L, or use of anti-diabetic medication. A history of coronary heart disease was defined as myocardial revascularization and/or myocardial infarction. Alcohol consumption and smoking status were assessed by interview. For alcohol consumption, participants were categorized as users or nonusers, for smoking, participants were categorized as current, former, or never smokers.

Statistical analysis

We compared characteristics between the participants included and excluded from the analyses using Analysis Of Variance (ANOVA) for continuous variables, and chi-square tests for categorical variables adjusting for age and sex where applicable. We analyzed habitual

coffee consumption as cups per day. White matter lesion volume was natural log-transformed due to right-skewness of its distribution.

We used linear regression to investigate the relationship of coffee consumption with continuous MRI-markers (i.e. total brain volume, hippocampal volume and white matter lesion volume), and with cognitive tests. We used logistic regression to assess the association between coffee consumption and the presence of lacunar infarcts. Analyses were adjusted for age, sex and educational attainment in model 1. In a second model we additionally adjusted for body mass index, hypertension, diabetes mellitus 2, total cholesterol levels, HDL-cholesterol levels, previous coronary heart disease , alcohol consumption, and smoking (model 2). Analyses which included MRI-based brain volumes as outcome were additionally adjusted for intracranial volume (as measure of head size). For analyses with cognition as outcome, we constructed a third model, in which we included the MRI-markers that associated with coffee consumption to test whether the association between coffee consumption and cognitive performance was independent on these markers.

Finally, we repeated all analyses after categorizing coffee consumption into three groups: 0–1 cup/day, >1–3 cups/ day, and >3 cups/day.[30] Analyses were conducted using the Stata 12.0 (Stata Corporation, College Station, USA).

RESULTS

Characteristics of the study population are presented in Table 1. The mean age of the study population was 59.3 years, and 55% were women. Participants excluded due to missing data for coffee consumption were more likely to be younger, less educated, current smokers, non-users of alcohol, and had lower HDL-cholesterol levels and higher BMI than participants included in our analyses (**Table 1**).

Table 1. Characteristics of the included and excluded study population.

Characteristics	Included in analysis n=2,914	Excluded from analysis n=774
Women	1,608 (55.2)	415 (53.6)
Age, years	59.28 (7.2)	56.6 (7.2)[a]
Educational attainment		
University degree	166 (5.7)	40 (5.2)[a]
Higher Vocational Education	610 (20.9)	132 (17.1)
General Secondary Education	160 (5.5)	42 (5.4)
Intermediate Vocational Education	673 (23.1)	182 (23.5)
Lower Secondary Education	559 (19.2)	120 (15.5)
Lower Vocational Education	485 (16.6)	161 (20.8)
Primary Education	224 (7.7)	87 (11.2)
Missing	37 (1.3)	10 (1.3)
Body mass index (kg/m^2)	27.42 (4.1)	27.98 (4.4)[a]
Hypertension	1,559 (53.8)	351 (45.8)
Diabetes Mellitus type 2	62 (9.0)	76 (9.8)
Total cholesterol, (mmol/l)	5.63 (1.0)	5.60 (1.1)
HDL-cholestero,l (mmol/l)	1.45 (0.4)	1.37 (0.4)[a]
Previous coronary heart disease	181 (6.3)	36 (4.7)
Alcohol consumption	2,639 (90.7)	659 (86.9)[a]
Smoking		
Never	896 (30.8)	205 (27.0)[a]
Former	1,373 (47.2)	323 (42.6)
Current	643 (22.0)	231 (30.4)

Values are means (standard deviation) or counts (percentage).
[a] Significant difference ($p<0.05$) between included participants and excluded participants adjusting for age and gender where applicable.

Table 2 shows the associations between habitual coffee consumption as number of cups per day with structural brain MRI-markers. We found that coffee consumption was associated with a lower prevalence of lacunar infarcts, odds ratio (OR) per cup increase in coffee consumption 0.88 (95% confidence interval (CI): 0.80, 0.97). This association remained significant after additional adjustment for cardiovascular risk factors, OR 0.88 (95% CI: 0.78, 0.97). We also found coffee consumption to be associated with lower hippocampal volume but only in model 2, difference of the mean in hippocampal volume per cup increase in coffee consumption: -0.01 (95% CI:-0.02, -0.00).

Table 2. Association of coffee consumption and structural MRI-markers (45 to 89 years of age).

Coffee consumption (per cup increase per day)	Model 1[d]		Model 2[e]	
	Difference (95%CI)	p-value	Difference (95%CI)	p-value
Total Brain Volume[a]	-0.16 (-0.93, 0.61)	0.677	-0.39 (-1.14, 0.37)	0.318
White Matter Volume[a]	0.08 (-0.68, 0.83)	0.845	0.13 (-0.65, 0.91)	0.747
Grey Matter Volume[a]	-0.50 (-1.19, 0.18)	0.159	-0.51 (-1.21, 0.18)	0.150
Total Hippocampal Volume[a]	-0.01 (-0.02, 0.00)	0.108	-0.01 (-0.02, -0.00)	0.033
White Matter Lesions[a,b]	-0.00 (-0.02, 0.01)	0.531	-0.00 (-0.01, 0.00)	0.423
	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value
Lacunar Infarcts[c]	0.88 (0.80, 0.97)	0.014	0.88 (0.78, 0.97)	0.012

[a] Volumes measures were also adjusted for intracranial volume.
[b] White Matter Lesions values were log transformed.
[c] Performed by logistic regression
[d] Model 1: Adjusted for age, sex, and educational attainment.
[e] Model 2: Additionally adjusted for alcohol consumption, smoking, cholesterol total, HDL-cholesterol, hypertension, diabetes mellitus 2, body mass index, and coronary heart disease.

Table 3 shows the associations between coffee consumption and cognitive tests. We found that coffee consumption was associated with better performance on the LDST, but also with worse performance on immediate and delayed recall tests. Interestingly, these associations remained statistically significant even after adjustment for total hippocampal volume and lacunar infarcts, difference of the mean in LDST: 0.12 (95% CI: 0.02, 0.22); immediate recall -0.04 (95% CI:-0.07, -0.00), and delayed recall -0.06 (95% CI:-0.11, -0.01). Coffee consumption (cups/per day) was not associated with performance on the verbal fluency test, the Purdue pegboard test or any of the Stroop subtasks (**Table 3**).

In analyses with categories of coffee consumption, there were 296 persons (10.2%) in the 0-1 cup/day category, 703 persons (24.1%) in the >1-3 cups/day category, and 1,915 (65.7%) persons in the >3 cups/day category. We observed a lower prevalence of lacunar infarcts, when comparing the >1-3 cups/day and >3 cups/day categories to 0-1 cup/day. In contrast, the associations with hippocampal volume, LDST, and delayed recall were only found when comparing the >3 cups/day category to 0-1 cup/day. Additionally, we found significant associations between coffee consumption and worse performance on Stroop color, difference in test score when comparing >3cups/day to 0-1 cup/day: 0.74 (95% CI: 0.22, 1.26) and interference subtask, difference in test score when comparing >3cups/day to 0-1 cup/day: 1.81 (95% CI: 0.22, 3.39) (**Figure 2**). No significant associations were present anymore with immediate recall and hippocampus volume (data not shown).

Table 3. Association of coffee consumption and cognitive function tests (45 to 89 years of age).

Coffee consumption (per cup increase per day)	Model 1[a]		Model 2[b]		Model 3[c]	
	Difference (95%CI)	p-value	Difference (95%CI)	p-value	Difference (95%CI)	p-value
Stroop reading subtask (seconds)	0.02 (-0.04, 0.07)	0.554	0.00 (-0.05, 0.06)	0.807	0.00 (-0.05, 0.06)	0.883
Stroop color naming subtask (seconds)	0.07 (-0.00, 0.14)	0.065	0.07 (-0.00, 0.14)	0.067	0.06 (-0.01, 0.13)	0.092
Stroop interference subtask (seconds)	-0.02 (-0.23, 0.19)	0.827	-0.02 (-0.23, 0.20)	0.862	-0.03(-0.25, 0.19)	0.790
LDST (number of correct digits)	0.09 (-0.01, 0.19)	0.065	0.12 (0.02, 0.22)	0.021	0.12 (0.02, 0.22)	0.021
Verbal fluency test (number of animals listed)	0.06 (-0.04, 0.15)	0.216	0.03 (-0.06, 0.13)	0.491	0.03(-0.07, 0.12)	0.580
15- WLT, immediate recall (number of correct answers)	-0.04 (-0.07, -0.00)	0.037	-0.03 (-0.07, -0.00)	0.049	-0.04 (-0.07, -0.00)	0.034
15-WLT, delayed recall (number of correct answers)	-0.05 (-0.10, -0.01)	0.025	-0.05 (-0.10, -0.01)	0.027	-0.06 (-0.11, -0.01)	0.018
Purdue Pegboard test (number of pins placed)	-0.02 (-0.05, 0.00)	0.128	-0.01 (-0.04, 0.02)	0.362	-0.02 (-0.05, 0.01)	0.221

Abbreviations: LDST Letter-Digit substitution task; WLT Word Learning Test

[a] Model 1: Adjusted for age, sex and educational attainment.

[b] Model 2: Additionally adjusted for alcohol consumption, smoking, cholesterol total, HDL-cholesterol, hypertension, diabetes mellitus 2, body mass index, and coronary heart disease status.

[c] Model 3: Additionally adjusted for total hippocampal volume and lacunar infarcts.

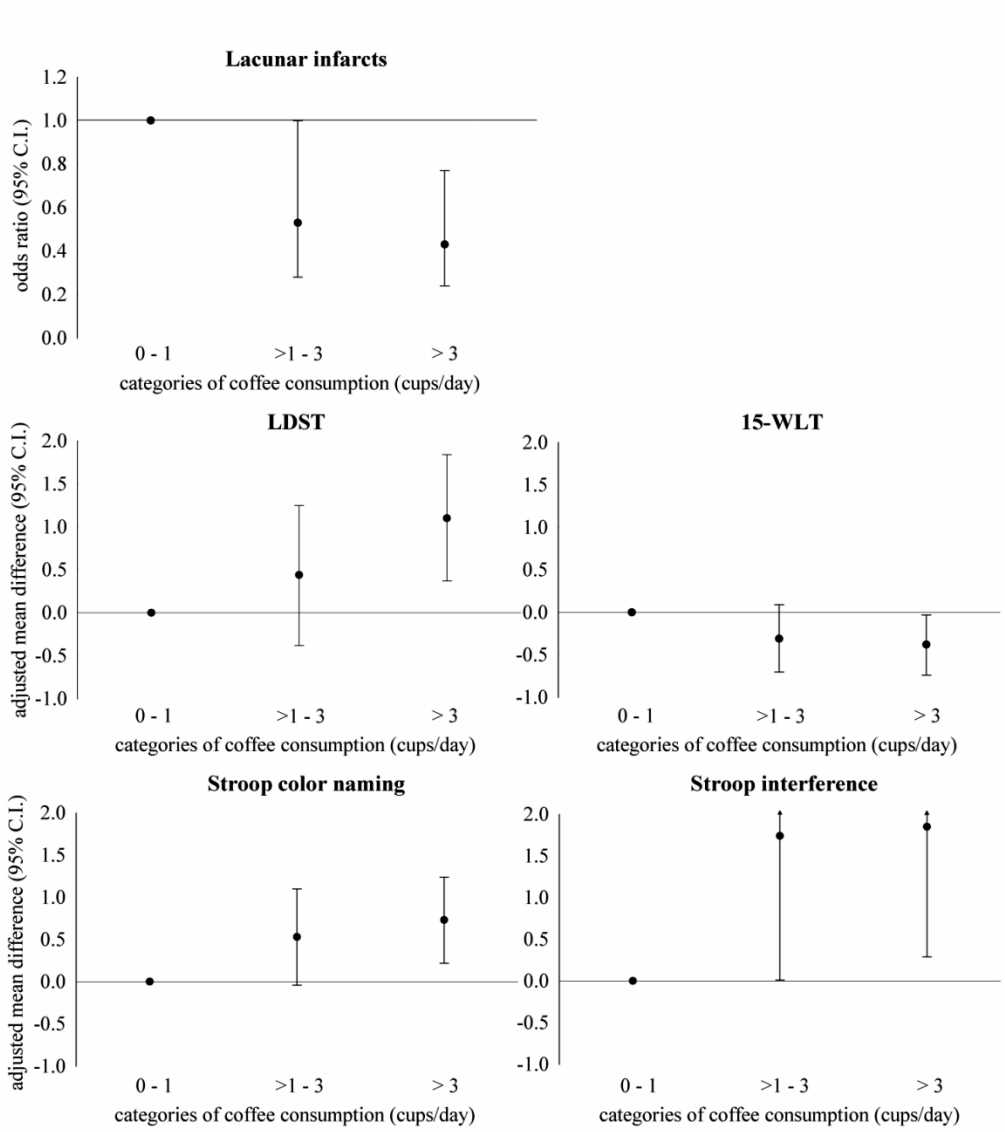

Figure 2. Association of coffee consumption categories with lacunar infarcts and cognitive function tests (45 to 89 years of age).

LDST= Letter-Digit Substitution Task; WLT= Word Learning Test

The upper figure shows odds ratios for the presence of lacunar infarcts for each category of coffee consumption as compared to the reference category (0-1 cups/day).

The lower four figures show differences in cognitive test scores for each category of coffee consumption as compared to the reference category (0-1 cups/day).

DISCUSSION

In this large sample of community-dwelling middle-age and older adults, we found that habitual coffee consumption was associated with a lower prevalence of lacunar infarcts, but also with a smaller hippocampal volume on structural MRI. Additionally, coffee consumption was related to better performance on LDST, but worse performance on the Stroop and memory tests, independent of MRI findings.

Coffee is a complex mixture of chemicals including caffeine, chlorogenic acid, polyphenols, melanoidins and diterpenes,[31,32] some of which have shown to have beneficial effects on neurodegeneration, as assessed in neuronal cell cultures,[6] or on cognition in aged rats.[14] Yet, diterpenes from the lipid fraction, such as cafestol, kahweol and especially 16-O-methylcafestol, if present in high amounts as in unfiltered or Turkish coffee, might impact circulating cholesterol levels.[32] However, at doses found in filtered coffee and espresso, these compounds largely have anti-inflammatory and anti-carcinogenic effects.[33,34]

We found that persons with higher consumption of coffee (>3 cups/day) had lower likelihood of having lacunar infarcts. Given the strong vascular origin of lacunar infarcts, it is likely that the beneficial effect of coffee on cardiovascular health might also underlie this association.[35,36] More specifically, studies have shown that coffee consumption is associated with a decreased risk of clinical stroke, probably due to its preventive effect on inflammation in the cascade of atherosclerosis.[3,12,35,36] Given the similarities between the pathophysiology of clinical stroke and lacunar infarcts, similar processes might underlie part of our observations.

Extending on our findings for lacunar infarcts, we also found coffee consumption to be associated with better performance on the LDST, which is a test of executive function. This is particularly noteworthy because of all cognitive domains executive function is thought to be primarily affected by vascular brain disease, including white matter lesions and lacunar infarcts.[15,37] Interestingly, the association of coffee with LDST was independent from lacunar infarcts, suggesting that other processes might also be involved. Future research should therefore focus on further unraveling the link between coffee, vascular health, lacunar infarcts, and executive function.

In contrast, we found coffee consumption to be associated with smaller hippocampal volume and poorer memory function. This finding is at odds with previous studies that suggest coffee to act as neurostimulant, improving memory function.[12,38] It is possible that the neurostimulatory effect of coffee might be short-acting, whereas in our study cognitive tests were administered as part of our study protocol and participants had not consumed

coffee for at least the preceding 60 minutes. Similarly, our MRI studies were not designed to find any short-acting effect of coffee, since hippocampus atrophy is a slow, protracted process. Alternatively, as indicated above, coffee contains numerous compounds, some of which might actually be detrimental at higher doses.[15,31] In our study, it was not possible to disentangle the potential consequences of different ways of coffee preparation (e.g. decaffeinate coffee, coffee with or without milk or sugar etc) to further unravel these associations.

From a practical point of view, the overall effect of coffee on brain needs further clarification. Indeed, the opposing effects we found for lacunar infarcts and hippocampus – and by extension LDST and delayed recall, are intriguing. In this regard, it is important to note that most studies investigating clinical neurological outcomes, have found protective effects of coffee consumption on clinical stroke, dementia, including Alzheimer's disease, and Parkinson's.[10,12,16,35,38-41] It seems thus that the net effect of coffee consumption is beneficial. Still, some caution is warranted, since most human studies have been observational by design, thereby thus being prone to methodological issues, such as recall bias and reverse causality.

Strengths of our study include the population-based design, large sample size, extensive phenotyping, and the focus on both cognitive function and brain MRI-biomarkers in the same population. There are also some limitations. Although we adjusted for known potential confounding factors in our study, there is a possibility of residual confounding due to other not measured factors, such as use of neuroleptics, anticonvulsants, anticholinesterase and antiparkinsonian drugs, or included confounders not being measured entirely accurately, such as alcohol or smoking. Also, there is the possibility of measurement error, which is especially true for questionnaire-based dietary exposures. We also did not take into account the method of brewing coffee such as filtering or boiling. Most importantly, our study was cross-sectional, which limits any causal inference. Nevertheless, the finding that associations with objective quantifiable structural MRI-markers correspond to those found for cognitive tests does add to the robustness of our findings.

In conclusion, habitual coffee consumption was associated with a lower prevalence of lacunar infarcts as well as better performance on executive function. At the same time, habitual coffee consumption was related to smaller hippocampal volumes and worse performance on speed tests and delayed memory. More studies are needed to further disentangle the subtle effects of coffee drinking on brain health, especially with respect to pre-clinical biomarkers.

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CHAPTER 3.3

Does cognitive reserve protect against dementia after a stroke or Tia? Results from the Rotterdam Study

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Submitted

Abstract

Background: Cognitive reserve may increase the tolerance to subclinical neurodegenerative pathology before it manifests clinically as dementia. However, it remains unclear if cognitive reserve also protects against dementia after clinical cerebrovascular events, such as stroke or transient ischemic attack (TIA).

Methods: Within the population-based Rotterdam Study, 12,561 participants (58.3% women, age-range 45-106 years) free of stroke, TIA, and dementia were followed for occurrence of stroke, TIA and dementia. Educational level was used as measure of cognitive reserve. We investigated associations of incident stroke or TIA with subsequent development of dementia across educational levels, using time-dependent Cox proportional hazard models. Additionally, we examined differences in cognitive decline across educational levels following stroke or TIA using linear regression.

Results: During 124,862 person-years, 1,463 persons suffered a stroke or TIA, 1,158 persons developed dementia, and 186 persons developed dementia after stroke or TIA. The risk of dementia in persons with stroke or TIA compared to persons without was highest in individuals with low education, HR 1.46 (95% CI: 1.18, 1.81), followed by those with intermediate education, HR 1.38 (95% CI: 1.04, 1.83). No significant association was observed in individuals with high education, HR 0.61 (95% CI: 0.25, 1.50). Additionally, persons with high education declined less in cognition following stroke or TIA compared to persons with low education.

Conclusions: Stroke or TIA increased the risk of subsequent development of dementia in persons with low and intermediate education, but not in persons with high education. Our results suggest a role of cognitive reserve in protection against dementia after stroke or TIA.

INTRODUCTION

The cognitive reserve hypothesis postulates that people with a higher reserve can tolerate more neurodegenerative pathology and maintain brain function for longer than people with low reserve, before the damage manifests clinically as dementia.[1-3] In the context of Alzheimer's disease, people with higher cognitive reserve can have more senile plaques and amyloid-β deposits before the disease manifests clinically.[4-7]

Although vascular disease is also an important risk factor for dementia including Alzheimer's disease,[8] it is less known if people with higher education, and thus more cognitive reserve, can also tolerate more cerebrovascular damage before dementia occurs. Evidence does suggest, however, that subclinical vascular lesions such as white matter lesions[9] or silent brain infarcts[10] result in less cognitive decline in people with higher education. Since clinical cerebrovascular events such as stroke and transient ischemic attack (TIA) are related to more tissue damage than silent lesions,[11] it remains to be established whether cognitive reserve also protects against dementia after clinical events. More importantly, stroke and TIA have a high clinical impact and may lead to considerable morbidity, which is further aggravated by sequelae like dementia.[12,13] Hence, identification of (modifiable) factors that act as protectors against dementia following a stroke or TIA is of major public health and clinical importance.

Therefore, in this population-based study we investigated whether cognitive reserve, operationalized using education, protects against dementia after a stroke or TIA. We also examined differences in cognitive decline after a stroke or TIA across levels of education.

METHODS

Setting and study population

This study was part of the population-based Rotterdam Study.[14] In 1990, 7,983 persons aged 55 years and older were recruited. In 2000, the cohort was expanded by 3,011 persons aged 55 and older, and in 2006 in a second expansion wave, an additional 3,932 persons aged 45 and older were added. Of these total 14,926 participants, 527 with prevalent dementia, and 902 with insufficient baseline information for dementia or no consent for the follow-up of stroke, TIA or dementia were excluded. We also excluded 354 persons with prevalent stroke, 324 with prevalent TIA, and 258 with missing education information, resulting in 12,561 participants eligible for the analyses of incident dementia.

The Rotterdam Study is approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands , implementing the "Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)". Written informed consent was obtained from all participants.

For the analyses of cognition, only participants who suffered a stroke or TIA between the two follow-up rounds in 2004-2008 and 2009-2013 were eligible, as these rounds comprised the most comprehensive cognitive test battery. In these examination rounds, 7,039 persons participated, of which 34 participants with no consent for collection of follow-up data, 435 participants with prevalent stroke or TIA at the first test date, 83 participants with prevalent dementia or insufficient information for dementia at the first test date, and 83 with missing data on education were excluded. Of the remaining 6,404 participants, 261 actually suffered a stroke or TIA between the two follow-up rounds. 205 of these participants had at least one cognitive test and were therefore eligible for analyses.

Educational level as a measure of cognitive reserve

We used educational level as a measure of cognitive reserve which was inquired during the home interview and participants were classified as having low (primary, unfinished secondary, and lower vocational), intermediate (secondary or intermediate vocational), or high education (higher vocational or university).

Assessment of stroke and TIA

At baseline, history of stroke and TIA was assessed using home interviews and verified using medical records. From baseline onwards, participants were continuously followed-up for occurrence of stroke and TIA through a computerized linkage between the study database and medical records of general practitioners (GPs). This data linkage system is highly efficient in the Dutch situation where the GPs receive all medical information about their patients if they contact any medical caregiver or professional, including specialists. Additionally, nursing home physicians' files and files from GPs of participants that moved out of the study area were checked on a regular basis. Information from GPs and hospital records was collected from participants with a potential stroke or TIA. Research physicians reviewed the information and an experienced vascular neurologist verified the strokes according to World Health Organization criteria.[15,16] We defined TIAs as temporary attacks with presence of focal symptoms, which are attributable to dysfunction of one arterial territory of the brain.[17] Follow-up for stroke and TIA was complete until 2013 for 98.5% of potential person-years.[18]

Assessment of dementia

Participants were screened for dementia at baseline and follow-up examinations using a three-step protocol.[19] First, screening was done using the Mini-Mental State Examination (MMSE)[20] and the Geriatric Mental Schedule (GMS) organic level. Second, screen-positives (MMSE < 26 or GMS organic level > 0) subsequently underwent an examination and informant interview with the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX). Participants who were suspected of having dementia, underwent if necessary, further neuropsychological testing. Additionally, the total cohort was continuously monitored for dementia through computerized linkage between the study database and digitized medical records from GPs and the Regional Institute for Outpatient Mental Health Care. Third, a consensus panel led by a neurologist decided on the final diagnosis in accordance with standard criteria using the DSM-III-R criteria for dementia, the NINCDS-ADRDA for Alzheimer disease,[21] and the NINCDS-AIREN criteria for vascular dementia.[22] Follow-up for dementia was complete until 2013 for 98.4% of potential person-years.

Cognitive tests

From 2004-2008 and 2009-2013, participants underwent extensive cognitive testing. Executive function was assessed by the Stroop test (interference task) which tests attention and concentration, Letter-Digit Substitution Task which tests processing speed, and Verbal Fluency Test which assesses verbal fluency. Memory was assessed by the 15-Word verbal Learning test including both immediate and delayed recall. Fine motor skills and coordination was assessed by the Purdue pegboard test for both hands.[23] A higher score indicates a better cognitive performance for all tests, except the Stroop test in which a higher score indicates a worse performance as it measures time to complete the task.

Covariates

Smoking habits and medication use were assessed during the home interview. Participants were categorized into current, former and never smokers. Body mass index was calculated as weight in kilograms/height in meters squared. Total cholesterol and high-density lipoprotein cholesterol were measured in serum in mmol/L. Blood pressure was measured twice at the right arm in sitting position at the research center and average of two blood pressure readings was used. Diabetes mellitus type 2 was diagnosed as fasting blood glucose ≥ 7.00 mmol/L, or use of anti-diabetic medication evaluated by interview and pharmacy records.[24] Cognitive score was assessed using the Mini-Mental State Examination.[20]

Statistical analyses

We examined the risk of dementia in people with stroke or TIA as compared to people without stroke or TIA using Cox proportional hazard models. Adherence to the proportional hazards assumption was tested by plotting smoothed Schoenfeld residuals against time; no violations of the assumption were identified. Stroke or TIA was used as time varying exposure, which took into account the incident cases of stroke or TIA as they occurred during follow-up. Participants were censored at date of dementia, date of death, or last date of follow-up, whichever came first. Subsequently, we examined this association across levels of education by stratifying on educational level as well as by including an interaction term. In secondary analyses, we examined this association in men and women separately since levels of education differ between older men and women, as well as their risk factors for stroke.[25]

Subsequently, we tested whether education is associated with change in cognitive test scores after a stroke or TIA using linear regression models. This was conducted in a subgroup of participants with stroke or TIA, for which cognitive test scores were available both before and after the stroke or TIA. Using low education level as the reference, we first examined the association of education with cognitive test scores before stroke or TIA. Second, we examined the association of education with cognitive test scores after stroke or TIA. Finally, the association of education with the change in cognitive tests scores after stroke or TIA was examined. This was tested by performing linear regression of education with cognitive test scores after stroke or TIA, adjusting for the test score before stroke or TIA.

For all analyses, two models were fitted. Model 1 was adjusted for age and sex only (where applicable). Model 2 was additionally adjusted for education level (where applicable), body mass index, smoking, total and high-density lipoprotein cholesterol, systolic and diastolic blood pressure, lipid- and blood pressure-lowering medication, diabetes mellitus type 2, and MMSE. The analyses of change in cognition were additionally adjusted for time between the two cognitive examinations. The dementia analyses were adjusted for covariates using their baseline values, while cognition analyses were adjusted using the values of covariates from the first visit of cognition assessment. Missing values on covariates (< 6%) were handled by multiple imputations.

Data were analyzed using the Stata Software Version 13 (StataCorp, College Station, TX, USA) and IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY).

RESULTS

This study included 12,561 dementia-, TIA-, and stroke-free participants at baseline. **Table 1** summarizes baseline characteristics of the study population. People with higher education were younger and more frequently men. During a mean follow-up of 9.9 ± 5.2 years, 1,463 persons suffered a stroke or TIA and 1,158 persons were diagnosed with dementia; 186 persons developed dementia after a stroke or TIA.

Table 1. Baseline characteristics of the study population, N=12,561.

Characteristics	Levels of education		
	Low	Intermediate	High
	N=5,299	N=5,342	N=1,920
Age, years	67.8 (9.9)	63.3 (8.8)	60.0 (8.2)
Women	3754 (70.8)	2838 (53.1)	735 (38.3)
Body mass index, kg/m^2	27.2 (4.2)	26.8 (4.1)	26.5 (3.9)
Smoking			
Never	1,993 (37.9)	1,558 (29.3)	539 (28.1)
Former	2,018 (38.4)	2,459 (46.2)	950 (49.6)
Current	1,247 (23.7)	1,301 (24.5)	428 (22.3)
Total cholesterol, mmol/L	6.4 (1.3)	6.1 (1.2)	5.8 (1.2)
HDL cholesterol, mmol/L	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)
Lipid lowering medication	409 (7.7)	556 (10.4)	222 (11.6)
Systolic blood pressure, mmHg	139.8 (22.2)	137.3 (20.9)	135.3 (21.1)
Diastolic blood pressure, mmHg	76.0 (12.0)	78.0 (11.6)	79.8 (11.7)
Blood pressure lowering medication	1,313 (24.8)	1,131 (21.2)	350 (18.3)
Mini Mental State Examination, points	27.2 (2.1)	28.2 (1.5)	28.6 (1.4)
Diabetes mellitus type 2	417 (8.6)	390 (7.7)	107 (5.8)

Abbreviations: HDL high density lipoprotein; N number of persons included in study.
Values are means (standard deviation) or counts (percentage).

People with a stroke or TIA had an increased risk of dementia compared to participants without stroke or TIA (multivariable adjusted hazard ratio (HR) 1.42 (95% CI: 1.20, 1.67)). In analyses stratified for education, this risk was highest in persons with low education, HR 1.46 (95% CI: 1.18, 1.81), followed by those with intermediate education, HR 1.38 (95% CI: 1.04, 1.83).In the high education group, people with a stroke or TIA did not have an increased risk of dementia compared to people without a stroke or TIA, HR 0.61 (95% CI: 0.25, 1.50) (**Table 2**).

Stratification by gender showed a similar pattern of associations, which was more pronounced in men than in women (**Table 3**). Interaction testing of educational level with stroke or TIA on the risk of dementia yielded p-value 0.71 in the overall population, 0.05 in men, and 0.79 in women.

Table 2. Risk of dementia after stroke or TIA by levels of education.

	Risk of dementia, hazard ratios (95% confidence intervals)			
	n/N[a]	n/N[b]	Model 1[c]	Model 2[d]
Total population	1,158/12,561	186/1,463	1.40 (1.19, 1.65)	1.42 (1.20, 1.67)
Strata of education				
Low education	732/5,299	112/749	1.47 (1.19, 1.81)	1.46 (1.18, 1.81)
Intermediate education	364/5,342	68/576	1.38 (1.04, 1.82)	1.38 (1.04, 1.83)
High education	62/1,920	6/138	0.74 (0.31, 1.77)	0.61 (0.25, 1.50)

Abbreviations: TIA transient ischemic attack
[a] n/N: number of dementia cases/total number of participants at risk for dementia.
[b] n/N: number of dementia cases after stroke or TIA/total number of stroke or TIA.
[c] Model 1: adjusted for age and sex.
[d] Model 2: additionally adjusted for age, sex, education (where applicable), Mini-Mental state Examination score, body mass index, smoking, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication use, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication use, and diabetes mellitus type 2.

People with high education scored better on 15-Word verbal learning test (both immediate and delayed recall), Verbal fluency test, and Letter Digit Substitution task both before and after stroke or TIA (**Table 4**). When we studied the change in cognitive test scores from before to after stroke or TIA, we found that that people with high education declined less in delayed recall compared to people with low education, β 1.42 (95% CI: 0.34, 2.50). Effect sizes of the Stroop interference task and Verbal fluency test also suggested a lower decline in the higher education group, although only borderline significant.

Table 3. Risk of dementia after a stroke or TIA by levels of education in men and women.

	Risk of dementia, hazard ratios (95% confidence intervals)							
	Men				Women			
	n/N[a]	n/N[b]	Model 1[c]	Model 2[d]	n/N[a]	n/N[b]	Model 1[c]	Model 2[d]
Total population	360/5,234	70/618	1.56 (1.18; 2.06)	1.67 (1.26; 2.21)	798/7327	116/845	1.33 (1.08; 1.63)	1.32 (1.07; 1.62)
Strata of education								
Low education	147/1,545	29/202	2.10 (1.36, 3.23)	2.20 (1.42, 3.42)	585/3,754	83/547	1.33 (1.04, 1.70)	1.32 (1.03, 1.68)
Intermediate education	172/2,504	36/320	1.36 (0.91, 2.03)	1.47 (0.98, 2.20)	192/2838	32/256	1.41 (0.95, 2.09)	1.40 (0.94, 2.08)
High education	41/1,185	5/96	0.87 (0.33, 2.30)	0.68 (0.24, 1.95)	21/735	1/42	0.33 (0.04, 2.61)	0.21 (0.02, 1.95)

Abbreviations: TIA transient ischemic attack.

[a]n/N: number of dementia cases/total number of participants at risk for dementia.

[b]n/N: number of dementia cases after stroke or TIA/total number of stroke or TIA.

Model 1: adjusted for age.

Model 2: adjusted for age, education (where applicable), Mini-Mental state Examination score, body mass index, smoking, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication use, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication use, and diabetes mellitus type 2.

Table 4. Change in cognitive test scores after stroke or TIA by levels of education.

	N	Stroop interference task (seconds)	LDST (correct answers)	VFT (animal names listed)	15-WLT-Immediate recall (correct answers)	15-WLT-Delayed recall (correct answers)	Purdue Pegboard (number of pins placed)
		Difference (95% confidence intervals)					
Before stroke or TIA							
Low-education	73	Ref	Ref	Ref	Ref	Ref	Ref
Int-education	89	-0.46 (-6.83; 5.92)	4.04 (1.79; 6.29)	1.32 (-0.39; 3.03)	2.55 (0.45; 4.65)	1.32 (0.31; 2.33)	0.56 (0.03; 1.08)
High-education	43	-4.29 (-12.15; 3.57)	6.01 (3.24; 8.78)	5.11 (3.03; 7.20)	4.77 (2.25; 7.30)	2.09 (0.87; 3.31)	0.36 (-0.28; 1.00)
After stroke or TIA							
Low-education	73	Ref	Ref	Ref	Ref	Ref	Ref
Int-education	89	-3.24 (-11.32; 4.85)	3.35 (1.16; 5.53)	1.64 (-0.16; 3.44)	3.41 (1.29; 5.52)	1.32 (0.33; 2.31)	0.20 (-0.39; 0.79)
High-education	43	-11.31 (-21.29; -1.33)	4.86 (2.17; 7.56)	4.66 (2.46; 6.85)	4.86(2.31; 7.40)	2.44 (1.25; 3.63)	-0.06 (-0.78; 0.65)
Decline after stroke or TIA		Difference in change in cognition (95% confidence intervals)					
Low-education	73	Ref	Ref	Ref	Ref	Ref	Ref
Int-education	89	-2.87 (-9.58; 3.85)	0.60 (-1.04; 2.25)	0.91 (-0.65; 2.47)	2.09 (0.24; 3.93)	0.68 (-0.20; 1.56)	0.02 (-0.55; 0.60)
High-education	43	-8.44 (-16.77; -0.11)	0.80 (-1.25; 2.84)	1.87 (-0.13; 3.88)	2.21 (-0.07; 4.49)	1.42 (0.34; 2.50)	-0.18 (-0.88; 0.51)

Abbreviations: Int. Intermediate; LDST Letter-Digit Substitution Task; VFT Verbal Fluency Test; 15-WLT 15-Word Learning Test; N number of persons with at least one cognitive test

Estimates represent differences in test score, and differences in change in cognitive test scores as compared to the low education category, with 95% confidence intervals. A higher score indicates a better cognitive performance for all tests (scores), except the Stroop test (time taken to finish the task, in seconds) in which a higher score indicates a worse performance.Change in cognition is defined as cognition after stroke or TIA, adjusted for cognition before stroke or TIA.

Estimates are adjusted for age, sex, body mass index, smoking, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication use, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication use, and diabetes mellitus type 2. Estimates for change in cognition are additionally adjusted for time between the two examination dates.

DISCUSSION

This population-based study showed that stroke or TIA increased the risk of subsequent dementia in persons with low and intermediate education, but not in persons with high education. Additionally, as compared to people with low education, those with high education not only scored better on cognitive tests both before and after stroke or TIA, but also declined less on cognitive test scores after a stroke or TIA.

It is known that people with stroke or TIA have an increased risk of dementia compared to those without stroke or TIA,[17,26] but we showed that this effect was dependent upon the level of education. This differing effect across levels of education, points towards a protective role for cognitive reserve. Cognitive reserve is an established concept and has been shown to protect against Alzheimer pathology. A major novelty of our study is that we demonstrated the protective role of cognitive reserve against clinical cerebrovascular pathology.

Here we point out that clinical studies have previously identified low education as a risk factor for dementia in patients with stroke, but because of the clinical setting, a comparison to risk of dementia in persons without stroke was lacking.[13,27-29] It is specifically this comparison with persons without stroke that provides evidence for cognitive reserve as a protective factor against post-stroke dementia. Therefore, the second major novelty of our study is that we were able to make this comparison and importantly showed that in persons with high education, stroke or TIA did not increase the risk of dementia. This suggests that people with higher education, and hence higher cognitive reserve can bear more cerebrovascular damage before it becomes clinically apparent as dementia.

The question remains what the underlying neural substrate is of cognitive reserve. One explanation is that people with higher cognitive reserve might be more resilient to the damage caused by a stroke or TIA, either due to better efficiency, capacity, or flexibility of brain networks already present before the damage occurred (neural reserve), or because of better compensation for the damage (neural compensation). Neural compensation pertains to the ability of persons with higher cognitive reserve, to form collateral networks in the brain, when the usual neuronal networks are compromised by the vascular damage.[2,30,31] Studies have suggested that cognitively stimulating activities, which are mostly experienced during education, not only promote neurogenesis, but also upregulate Brain Derived Neurotrophic Factor (BDNF) which in turn promotes plasticity.[1,31] However, an alternative explanation is that people with higher education have a more favorable

environment including a healthier lifestyle and better access to healthcare. This might lead to less severe strokes, perhaps better detection of less severe strokes, and more importantly, early hospitalization and thus fewer complications after stroke.

In analyses stratified on gender, we found that the pattern of associations was more pronounced in men than in women. This suggests a stronger protective effect of cognitive reserve against dementia following stroke in men. One explanation could be that only few women had dementia after a stroke or TIA in the high education group, which could have affected our power. For low and intermediate education groups, for which we had larger numbers, results were similar for men and women. Another explanation could be that perhaps in women from older generation birth cohorts, education is less representative of their cognitive reserve than in men, particularly for West-European populations. In our study, many women were born in a period when girls were not equally encouraged for education as boys, and often only completed limited years of education, which was not reflective of their potential. Instead, they quit school to work at home. Therefore, in this group, educational level might not be the best proxy for cognitive reserve and thus obscured any associations in women. The time spent on leisure activities including social, physical and recreational activities might have been a better proxy for cognitive reserve, but we did not have that information in our study.

The finding in our study of less cognitive decline in the high education category in people with stroke or TIA further supports the role of cognitive reserve. Unlike previous studies which only had information on cognitive decline after stroke or TIA,[28,29] we had cognition assessments both before and after the stroke or TIA. This allowed to demonstrate that the impact of a stroke or TIA on cognition was smaller in people with high education, suggesting that persons with higher education not only have a better cognition in the first place, they can also adapt better to the cerebrovascular damage, indicating cognitive reserve.

Strengths of this study include a large population-based sample representative of different levels of education, a long and robust follow-up of incident TIA, stroke, and dementia, and the availability of cognitive tests before and after stroke or TIA. However there are certain limitations. Only education as a measure of cognitive reserve was available. Activities in later life, such as occupation or leisure activities including recreation, physical and social engagements could not be taken into account, which might be important particularly in older adults. However, education is the most used measure of cognitive reserve in existing literature.[30,32] Furthermore, we could not adjust for brain reserve in our study, since brain volumes were not available in this population. This might have led to an overestimation of

results, as the observed associations could partly be explained by brain reserve. Another limitation is that we did not have information about the severity of stroke. It is possible that people with higher education had less severe strokes leading to less brain damage and therefore a smaller risk of dementia. Moreover, we did not have enough cases of dementia in the high education category for women and therefore the effect estimates might be underpowered. Finally, complete cognitive testing was available in a subgroup of our study population only, therefore the results might be influenced by selection.

In conclusion, our study shows that cognitive reserve protects against dementia after cerebrovascular events. Future studies should explore the exact mechanism of this protective effect as well as investigate whether improvement of cognitive reserve later in life, for instance using cognitively stimulating activities, might delay or prevent dementia in stroke patients.

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CHAPTER 4

BIOMARKERS AND NEUROPSYCHIATRIC OUTCOMES

CHAPTER 4.1

The Amino-terminal pro B-type natriuretic peptide and risk of dementia and cognitive decline: A 10-year follow-up study in the general population

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ABSTRACT

Background: The Amino-terminal pro B-type natriuretic peptide (NT-proBNP) has a well-documented prognostic value for cardiovascular disease (CVD) and higher levels have been associated with cognitive dysfunction in CVD patients. However, how NT-proBNP relates to incident dementia and cognitive-decline in community-dwelling persons is unknown.

Methods: Between 1997-2001, serum NT-proBNP was measured in 6,040 participants (mean age 69 years, 57% women) free of heart-failure and dementia from the population-based Rotterdam Study. Persons were continuously followed-up for incident dementia till 2012, for 56,616 person-years. Cognition was assessed at baseline and reassessed between 2002-2006 by Letter-Digit-Substitution-task, Stroop test, and Word-Fluency test. Associations of NT-proBNP with dementia (555 cases), Alzheimer's disease (357 cases), and vascular dementia (32 cases) were assessed linearly and in quartiles using Cox regression. Associations of NT-proBNP with cognitive-decline were assessed using multiple linear regression. All analyses were repeated after excluding persons with any CVD.

Results: Higher NT-proBNP was associated with a higher risk of dementia, even after excluding persons with CVD and adjusting for cardiovascular risk factors, HR per SD 1.27 (95%CI: 1.13, 1.44). Associations were particularly strong for vascular dementia, HR per SD 2.04 (95%CI: 1.18, 3.55), but also for Alzheimer's disease when comparing the second and third quartile with the first. Higher NT-proBNP was cross-sectionally associated with poorer performance in multiple cognitive tests but longitudinally only in Letter-Digit-Substitution-task.

Conclusions: NT-proBNP reflecting subclinical CVD is associated with dementia, particularly vascular dementia. NT-proBNP can be a useful marker of imminent cognitive-decline and dementia in the absence of clinical CVD.

INTRODUCTION

Dementia is a major public health concern, with a prevalence of 35.6 million patients, and 7.7 million new cases every year worldwide.[1] Dementia, including Alzheimer's disease, has a multifactorial etiology with a substantial vascular component.[2-5] Amino-terminal pro B-type natriuretic peptide (NT-proBNP) is an emerging serum marker for cardiovascular diseases.[6,7] NT-proBNP is the inactive amino terminal of proBNP, which is synthesized and released from the ventricular myocardium in response to stretch of cardiomyocytes due to volume or pressure overload, and higher levels mark the presence of cardiovascular disease, particularly heart failure (HF).[8] It has been shown that after adjustment for the classical cardiovascular risk factors, higher levels of NT-proBNP were associated with up to two-fold increased risk of coronary heart disease and ischemic stroke,[9-11]and 3-fold increase in the risk of HF as compared to those with lower levels.[9] Importantly, it has been suggested that NT-proBNP indicates vascular disease even in the absence of overt clinical cardiovascular disease.[12] Moreover, in absence of clinical cardiovascular disease, arterial stiffness was found to be independently associated with higher levels of NT-proBNP.[13] Previously, associations of NT-proBNP and BNP with dementia and cognition have been reported in relatively small studies or in clinical settings, precluding generalizability of the findings.[14-18] Therefore, two important knowledge gaps remain. First, it remains largely unknown how NT-proBNP relates to dementia and Alzheimer's disease in community-dwelling persons, which is important to assess its public health impact. Second, the role of clinical cardiovascular disease in the association of NT-proBNP with dementia is unclear. It is conceivable that many persons with subclinical vascular disease, as reflected by increased NT-proBNP, would first experience clinical cardiovascular disease and subsequently develop dementia. This issue can be addressed in a longitudinal design that censors for onset of clinical cardiovascular disease.

This paper aims to assess the association of NT-proBNP with incident dementia, Alzheimer's disease, vascular dementia, and cognitive decline in a large population-based cohort study. Importantly, we also studied these associations in persons without clinical cardiovascular disease to elucidate the role of NT-proBNP as a marker of subclinical cardiovascular disease and its association with dementia.

METHODS

Setting

This study was part of the Rotterdam Study, a population-based cohort ongoing since 1990, which studies chronic diseases in the elderly. In 1990, 7,983 participants were enrolled. In 2000, the original cohort was extended by additionally recruiting 3,011 participants. Follow-up examinations including home interviews and physical exams at the research center take place every 3 to 4 years.[19] The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. A written informed consent was obtained from all participants.

Study population

NT-proBNP was assessed between 1997 and 2001 in 3,930 participants of the original cohort, and 2,580 participants recruited in the second cohort. Of these 6,510 participants, 12 with an unreliable NT-proBNP test measure, and 47 not consenting to undergo dementia screening were excluded. In addition, 112 persons with prevalent dementia, 243 with prevalent HF, 24 with incomplete HF follow-up, and 32 persons not consenting for HF follow-up were excluded. This yielded a total of 6,040 participants eligible for analyses. Cognition was assessed at baseline (1997-2001) in 5,693 participants, and at a follow-up examination (2002-2006) in 4,509 participants.

Participants were censored if died or lost to follow-up. If participants were lost to follow-up, they were censored on the date they were last seen/contacted.

Assessment of NT-pro-BNP

Blood samples for NT-proBNP measurement were collected in glass tubes containing clot activator and gel for serum separation. After collection, samples were allowed to stand for 30 minutes for clotting and then centrifuged for 20 minutes at 3000 rpm at 4ºC. Subsequently, serum was stored at -80ºC. NT-proBNP was measured using commercially available electrochemiluminescense immunoassay (Elecsys proBNP, F.Hoffman-La Roche Ltd. Basel, Switzerland) on an Elecsys 2010 analyser.[13] The precision, analytic sensitivity and stability features of the system have been described previously.[20] NT-proBNP levels are reported in pmol/L.

Assessment of dementia

Participants were screened for dementia at baseline and follow-up examinations using a three-step protocol.[21] Screening was done using the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level. Screen-positives (MMSE < 26 or GMS organic level > 0) subsequently underwent an examination and informant interview with the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX). Participants who were suspected of having dementia, underwent if necessary, further neuropsychological testing. Additionally, the total cohort was continuously monitored for dementia through computerized linkage between the study database and digitized medical records from general practitioners (GPs) and the Regional Institute for Outpatient Mental Health Care. Finally, a consensus panel led by a neurologist, decided on the final diagnosis in accordance with standard criteria using the DSM-III-R criteria for dementia, the NINCDS-ADRDA for Alzheimer disease,[22] and the NINCDS-AIREN criteria for vascular dementia.[23] If required for differential diagnosis, neuroimaging was used. Cases of Alzheimer's disease that also showed evidence of cerebrovascular disease, were classified as Alzheimer's disease+cerebrovascular disease, which in all analyses is included as Alzheimer's disease (5% of all cases). The data linkage system mentioned above is highly efficient in the Dutch situation and the possibility of underestimation is very low. In this setting, the GPs receive all medical information about their patients if they contact any medical care-giver or professional including specialists. Although, it is difficult to ascertain the exact number of cases that could have been missed by the system, we calculated the potential and observed person-years to calculate the completeness of dementia follow-up[24] which was complete (97.92 %) till January 2012.

Assessment of cognition

The cognitive test battery comprised the Letter-Digit-Substitution task (LDST) which tests processing speed, Stroop test (including reading, colour-naming, and interference subtasks) which tests attention and concentration, and the Word-Fluency test (WFT) which assesses verbal fluency and executive function.[25] Cognition was assessed at baseline in 1997-2001, and reassessed at the follow-up examination round in 2002-2006.

Other measurements

NT-proBNP reflects cardiovascular health; body mass index, serum lipid levels, hypertension, diabetes mellitus type 2, level of education of individuals, *APOE* genotype, life-style habits such as alcohol intake and smoking, inflammatory markers such as C-reactive protein (CRP), atherosclerotic markers such as carotid intima-media thickness

(IMT), and cardio- and cerebrovascular events such as myocardial infarction (MI) and stroke are associated with cardiovascular disease.[26-29] All these factors are also independent predictors of dementia,[30-33] and therefore were considered as potential confounders in addition to age and sex. Body mass index (BMI) was calculated as weight in kilograms/height in meters squared. Total cholesterol and high density lipoprotein (HDL) cholesterol were measured in serum in mmol/L. Blood pressure was measured twice at the right arm in sitting position at the research center and average of two blood pressure readings was used. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, or diastolic blood pressure ≥ 90 mmHg, or use of antihypertensive medication assessed by interview and pharmacy records.[34] Diabetes mellitus type 2 (DM) was diagnosed as fasting blood glucose ≥ 7.00 mmol/L, or use of anti-diabetic medication evaluated by interview and pharmacy records.[35] Educational level was assessed during the home interview and people were classified into two categories: low level of education (primary only or unfinished secondary), and intermediate to high (secondary, vocational, or university). For *APOE*-ε4 carrier status, persons were classified in two categories; non-carriers of *APOE*-ε4 allele, or carriers of one or two *APOE*-ε4 alleles. Alcohol intake was inquired during the home interview and participants were dichotomized as consumers versus non-consumers. Smoking habits were also assessed during the home interview and participants were categorized into current, former and never smokers. High-sensitivity CRP was measured in serum in nmol/L.[36] An average of 3 measures of carotid IMT by ultrasonography on both sides was used to calculate a mean measure for analyses.[37] All covariates were measured at baseline and were modelled as fixed at baseline. Cardiovascular disease was defined as prevalent or incident HF, stroke, MI, or any coronary revascularization procedure, as described previously[14] and participants were continuously monitored for all major cardiovascular events through automated linkage of study database with GP files.[38] HF was assessed by a validated score similar to the definition of heart failure of the European society of Cardiology.[39] This score was based on the presence of at least two signs of symptoms of heart failure (shortness of breath, ankle swelling and pulmonary crepitations) confirmed by chest X-ray and echocardiography or use of medication for the indication of HF in combination with objective evidence of cardiovascular diseases.[40] Previous stroke and MI were determined by reported events on interview and confirmed by medical records. Stroke was defined according to the WHO 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.[41] MI was diagnosed by a cardiologist or an internist and is defined as pathology findings of an acute MI within 28 days of death, or a rise/fall in cardiac biomarkers and/or objective indicative ECG changes, and preferably the presence of signs

and symptoms of MI. Revascularization procedures included coronary artery bypass grafting (CABG) and percutaneous coronary interventions (PCIs).[42]

Statistical Analyses

We log-transformed the NT-proBNP data to achieve a normal distribution, and then standardized the values by creating z-scores (log-transformed NT-proBNP minus mean of log-transformed NT-proBNP, divided by standard deviation of log-transformed NT-proBNP). In addition, we classified persons into quartiles on basis of NT-proBNP levels.

Risk of dementia, Alzheimer's disease, and vascular dementia was assessed using Cox proportional hazards models both linearly and in quartiles. Adherence to the proportional hazards assumption was tested by plotting smoothed schoenfeld residuals against time; no violations of the assumption were identified. In addition, analyses were repeated with tertiles and quintiles to test the dose effect. As sensitivity analyses, a competing risk analysis was also performed to account for death as a competing risk for the incidence of dementia, Alzheimer's and vascular dementia. Cross-sectional associations between NT-proBNP and cognitive function tested by LDST, Stroop1, Stroop2, Stroop3, and WFT were assessed by multiple linear regression models. Decline in the scores of all cognitive tests from the baseline assessment to the end of follow-up was studied using multiple linear regression models which were adjusted for baseline test scores.

All analyses were repeated after excluding persons with cardiovascular disease. For Cox regression analyses, this meant that persons with prevalent cardiovascular disease were excluded, whereas all incident cases of cardiovascular disease were censored.

Two models were fitted for all analyses. Model 1 was adjusted for age and sex only. Model 2 was additionally adjusted for *APOE*-ε4 carrier status, education level, alcohol intake, BMI, smoking, hypertension, total cholesterol, HDL, CRP, carotid IMT, and DM. BMI was also tested as a quadratic term, BMI^2, but was not included in the final model as there was no evidence of a U-shaped association between BMI and dementia.

The following variables had missing values which were dealt with, using multiple imputations using all covariates of interest as predictors: *APOE*-ε4 carrier status (3%), education level (1.3%), alcohol intake (0.8%), BMI (0.9%), smoking (0.2%), hypertension (0.1 %), total cholesterol (0.6%), HDL (1.4%), CRP (4.2%), and carotid IMT (8.2%). Data were analyzed using the Stata Software Version 13 (StataCorp, College Station, TX, USA).

RESULTS

Table 1 summarizes baseline characteristics of the 6,040 persons followed-up for dementia for a mean follow-up of 9.4 years.

Table 1. Baseline Characteristics of the study population, N=6,040.

Characteristics	Descriptives
Age, years	69.0 (8.2)
Women	3,502 (56.9)
APOE-ε4 carriers*	1,643 (27.6)
Education	
Primary or below	1,889 (31.1)
Intermediate to high	4,177 (68.9)
Body mass index, kg/m^2	26.8 (3.9)
Smoking	
Never	1,970 (32.1)
Former	2,960 (48.2)
Current	1,212 (19.7)
Alcohol drinking	5,043 (83.6)
Total cholesterol, mmol/L	5.8 (0.9)
High-density lipoprotein, mmol/L	1.4 (0.4)
NT-proBNP*, pmol/L	9.5 (5.1-18.3)
C-reactive protein, nmol/L	17.4 (6.7-36.2)
Carotid intima-media thickness, mm	1.0 (0.2)
Prevalent disease	
Hypertension	3,536 (57.5)
Myocardial infarction	391 (6.4)
Diabetes mellitus type 2	557 (9.0)
Stroke	286 (4.5)

Values are means (standard deviations) or counts (percentages).
Abbreviations: *APOE*-ε4: Apolipoprotein E-allele 4; NT-proBNP: N-terminal pro B-type natriuretic peptide and C-reactive protein are presented as medians (inter-quartile ranges) because of skewed distribution.

A total of 555 persons developed dementia during 56,616 person-years, of whom 357 developed Alzheimer's disease and 32 developed vascular dementia. 697 persons had prevalent cardiovascular disease at baseline, and 935 suffered incident cardiovascular disease during follow-up. Participants who died or were lost to follow-up (N=1,545+13) were more likely to be older, male, smokers, hypertensive, diabetic, and more likely to have suffered from an MI or stroke (**supplement table 1**).

Higher levels of NT-proBNP were associated with an increased risk of dementia, HR per SD 1.17 (95%CI: 1.05,1.30), which was particularly driven by vascular dementia, though statistically non-significant (**Table 2).**

The risk estimates for dementia showed a gradual increase over the quartiles (**Table 2 and Figure 1**).

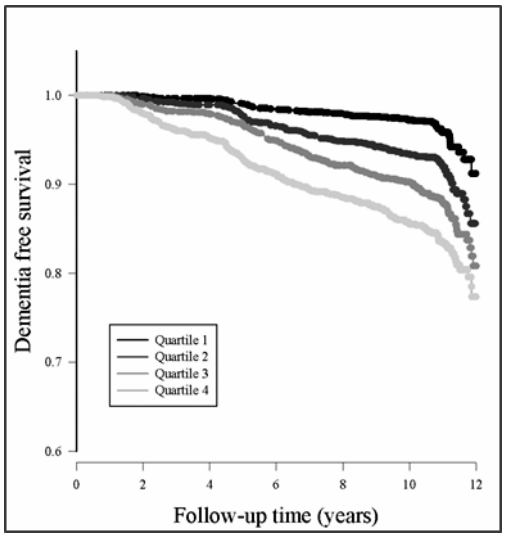

Figure 1. Kaplan-Meier survival curves for dementia across quartiles of NT-proBNP.
The curves show cumulative dementia-free survival across quartiles of NT-proBNP.

Persons in the second and third quartiles of NT-proBNP also showed a significantly increased risk of Alzheimer's disease compared to those in the lowest quartile. For vascular dementia, the number of cases was not sufficient to perform quartile analyses.

Adjusting for cardiovascular risk factors did not materially change these results. A significant p-trend was observed when assessing the dose effect for NT-proBNP (p-trend 0.02), and similar results were obtained in the analyses with tertiles and quintiles (data not shown).

In the competing risk analyses, we still found an increased risk of dementia per SD increase of NT-proBNP, HR per SD 1.05 (95% CI: 1.01, 1.09). In contrast, the risk for Alzheimer's disease in the continuous analyses inverted, HR per SD 0.92 (95% CI: 0.87, 0.97), possibly because the pattern across quartiles showed a very strongly increased risk

in quartile 2 and less strongly increased risks in quartiles 3 and 4, which were substantially increased nevertheless. Overall, results of these analyses did not reveal a substantial, but only a minor effect of competing risks on our main findings. (**Supplement table 2**)

After excluding persons with cardiovascular disease, NT-pro-BNP remained significantly associated linearly with dementia, HR per SD 1.27 (95%CI: 1.13,1.44), but now also with vascular dementia, HR per SD 2.04 (95%CI: 1.18,3.55) (**Table 3**).

Associations over the quartiles attenuated slightly, but remained statistically significant with dementia over all quartiles, and with Alzheimer's disease for the second quartile (**Table 3**). However, in a post-hoc power analyses, analyses for Alzheimer's disease lacked sufficient power both in analyses with and without excluding CVD. In the competing risk analysis after excluding persons with CVD, effect estimates slightly changed, but the pattern of results remained the same. (**Supplement table 2**)

Higher levels of NT-proBNP were associated with a poorer performance on LDST, and Stroop test cross-sectionally (**Table 4**). After excluding persons with cardiovascular disease, the association of NT-proBNP with LDST persisted (**Table 4**).

Longitudinally, higher NT-proBNP was only associated with a significant decline in performance on LDST, which remained significant after excluding persons with cardiovascular disease (difference in decline per SD -0.21, 95%CI: -0.37,-0.04) (**Table 5**).

Table 2. Hazard ratios and 95 % confidence intervals for risk of incident dementia, per standard deviation increase, and across quartiles of NT-proBNP.

NT-proBNP	Dementia			Alzheimer's disease			Vascular dementia		
	n/N[a]	Model 1[b]	Model 2[c]	n/N[a]	Model 1[b]	Model 2[c]	n/N[a]	Model 1[b]	Model 2[c]
Per SD	555/6,040	1.19(1.07,1.32)	1.17(1.05,1.30)	357/6,040	1.03(0.91,1.18)	1.03(0.90,1.18)	32/6,040	1.34(0.90,2.01)	1.27(0.85,1.91)
Quartile 1	57/1,511	1.00 (ref)	1.00 (ref)	32/1,511	1.00 (ref)	1.00 (ref)	2/1,511		
Quartile 2	117/1,510	1.45(1.05,2.00)	1.39(1.01,1.92)	86/1,510	1.76(1.17,2.65)	1.68(1.11,2.53)	7/1,510	—	—
Quartile 3	176/1,509	1.53(1.12,2.09)	1.45(1.06,1.99)	121/1,509	1.62(1.08,2.43)	1.54(1.02,2.32)	8/1,509	—	—
Quartile 4	205/1,510	1.59(1.16,2.18)	1.52(1.10,2.09)	118/1,510	1.34(0.89,2.04)	1.30(0.85,1.99)	15/1,510	—	—

[a] Dementia cases/total number of participants.
[b] Model 1: Adjusted for age and sex.
[c] Model 2: Additionally adjusted for *APOE*-ε4 carrier status, education, alcohol intake, body mass index, smoking, hypertension, total cholesterol, high density lipoprotein, C-reactive protein, carotid intima-media thickness, and diabetes mellitus 2.
Hazard ratios for vascular dementia were not calculated across quartiles of NT-proBNP because of small number of cases per quartile.

Table 3. Hazard ratios and 95 % confidence intervals for risk of incident dementia, per standard deviation increase, and across quartiles of NT-proBNP, in persons without cardiovascular disease.

NT-proBNP	Dementia			Alzheimer's disease			Vascular dementia		
	n/N[a]	Model 1[b]	Model 2[c]	n/N[a]	Model 1[b]	Model 2[c]	n/N[a]	Model 1[b]	Model 2[c]
Per SD	461/5,343	1.29(1.14,1.46)	1.27(1.13,1.44)	308/5,343	1.14(0.98,1.33)	1.13(0.96,1.31)	19/5,343	2.09(1.22,3.59)	2.04(1.18,3.55)
Quartile 1	46/1,336	1.00 (ref)	1.00 (ref)	32/1,336	1.00 (ref)	1.00 (ref)	1/1,336		
Quartile 2	94/1,337	1.38(0.97,1.97)	1.34(0.93,1.92)	71/1,337	1.63(1.04,2.55)	1.58(1.00,2.47)	2/1,337	—	—
Quartile 3	135/1,336	1.49(1.05,2.12)	1.42(1.00,2.01)	97/1,336	1.58(1.01,2.46)	1.49(0.95,2.33)	5/1,336	—	—
Quartile 4	191/1,334	1.68(1.18,2.39)	1.56(1.09,2.24)	115/1,334	1.38(0.87,2.18)	1.28(0.80,2.04)	11/1,334	—	—

[a] dementia cases/total number of participants.
[b] Adjusted for age and sex.
[c] Additionally adjusted for *APOE*-ε4 carrier status, education, alcohol intake, body mass index, smoking, hypertension, total cholesterol, high density lipoprotein, C-reactive protein, carotid intima-media thickness, and diabetes mellitus 2.
Hazard ratios for vascular dementia were not calculated across quartiles of NT-proBNP because of small number of cases per quartile.

Table 4. Cross-sectional association between per SD increase in NT-proBNP and cognition in non-demented persons.

Cognitive Tests	All participants		Participants without CVD	
	Difference (95% CI)		Difference (95% CI)	
	Model 1[a]	Model 2[b]	Model 1[a]	Model 2[b]
LDST, number of correct answers	-0.43 (-0.63, -0.23)	-0.35(-0.55,-0.16)	-0.40 (-0.61, -0.18)	-0.31(-0.53,-0.10)
Stroop 1, seconds	0.20 (0.07, 0.31)	0.19(0.07,0.31)	0.12 (-0.01, 0.25)	0.13(-0.01,0.26)
Stroop 2, seconds	0.27 (0.11, 0.44)	0.25(0.08,0.42)	0.19 (0.01, 0.36)	0.17(-0.02,0.35)
Stroop 3, seconds	0.72 (0.16, 1.29)	0.57(-0.01,1.14)	0.48 (-0.14, 1.10)	0.36(-0.25,0.98)
WFT*, number of animals listed	-0.12 (-0.28, 0.03)	-0.07(-0.23,0.10)	-0.07 (-0.24, 0.11)	-0.07(-0.25,0.11)

Abbreviations: CVD cardiovascular disease; LDST Letter-Digit Substitution task; WFT Word Fluency test
[a] Adjusted for age and sex
[b] Additionally adjusted for *APOE*-ε4 carrier status, education, alcohol intake, body mass index, smoking, hypertension, total cholesterol, high density lipoprotein, C-reactive protein, carotid intima-media thickness, and diabetes mellitus 2.

Table 5. Longitudinal association between per SD increase in NT-proBNP and cognitive decline in non-demented persons.

Cognitive Tests	All participants		Participants without CVD	
	Difference in decline (95 % CI)		Difference in decline (95 % CI)	
	Model 1[a]	Model 2[b]	Model 1[a]	Model 2[b]
LDST, number of correct answers	-0.22 (-0.36, -0.08)	-0.22 (-0.36, -0.08)	-0.19 (-0.35, -0.02)	-0.21 (-0.37, -0.04)
Stroop 1, seconds	0.08 (-0.05, 0.22)	0.07 (-0.07, 0.21)	-0.02 (-0.17, 0.12)	-0.02 (-0.17, 0.13)
Stroop 2, seconds	0.06 (-0.08, 0.21)	0.05 (-0.10, 0.21)	0.11 (-0.05, 0.28)	0.11 (-0.06, 0.28)
Stroop 3, seconds	-0.63 (-1.30, 0.02)	-0.72 (-1.40, -0.04)	-0.91 (-1.69, -0.14)	-0.84 (-1.62,-0.06)
WFT*, number of animals listed	-0.05 (-0.20, 0.10)	-0.03 (-0.18, 0.12)	0.01 (-0,18, 0.18)	0.01 (-0.18, 0.19)

Abbreviations: CVD cardiovascular disease; LDST Letter-Digit Substitution task WFT: Word Fluency test.
[a] Adjusted for age and sex.
[b] Additionally adjusted for *APOE*-ε4 carrier status, education, alcohol intake, body mass index, smoking, hypertension, total cholesterol, high density lipoprotein, C-reactive protein, carotid intima-media thickness, and diabetes mellitus 2.

DISCUSSION

In this prospective population-based study, higher levels of NT-proBNP were associated with a higher risk of dementia, including vascular dementia, and to a lesser extent with Alzheimer's disease. Moreover, higher levels of NT-proBNP were associated with poorer performance on multiple cognitive domains cross-sectionally, but only with poorer information processing speed longitudinally. All associations persisted after excluding persons with cardiovascular disease and after adjustment for sociodemographic and cardiovascular risk factors.

Strengths of this study are accounting for lifestyle and cardiovascular confounders, censoring for incident cardiovascular disease, and a long and robust follow-up for dementia. However, there are certain limitations. We did not have a specific test for memory at baseline, and therefore, the association of NT-proBNP and decline in memory could not be tested. Some selection based on NT-proBNP levels cannot be ruled out, as persons with severe cardiovascular disease, thus highest NT-pro-BNP levels, were less likely to attend the examination rounds. In addition, risk estimates for vascular dementia across quartiles of NT-proBNP could not be calculated due to small number of cases per quartile.

We found that higher levels of NT-proBNP were associated with increased risk of dementia, particularly vascular dementia. There are no longitudinal studies to show an association of NT-proBNP with risk of dementia, but previously, one study showed the association of BNP with incident dementia and decline on MMSE scores in 464 persons over a follow-up of 5 years.[17] The link between NT-proBNP and dementia can be explained in several ways. First, it is possible that persons with high NT-proBNP first suffer from a clinical cardiovascular event which subsequently leads to development of dementia. However, this is unlikely because we observed an association even after censoring persons with clinical cardiovascular disease during follow-up. Second, shared risk factors such as body mass index, smoking, or blood lipid levels could also explain the association between NT-proBNP and dementia. However, we observed that after adjusting for many potential confounders in this respect, associations attenuated only slightly, although some residual confounding cannot be fully ruled out. The third and most likely explanation is that NT-proBNP reflects the presence of subclinical cardiovascular disease. Even though in clinical practice NT-proBNP is used as a marker of HF, studies have increasingly shown a role of NT-proBNP to reflect subclinical disease, especially poor cardiac function and volume overload in relation with hypertension.[12] There is indeed evidence suggesting a role for subclinical cardiovascular disease in dementia, which is further supported by our data. The

association of NT-proBNP with dementia in a cardiovascular disease free population controlled for cardiovascular risk factors indicates that neurodegenerative changes commence very early in the course of cardiovascular disease even when it is below the diagnostic threshold. Given the strong association of NT-proBNP with HF, information on cardiac function such as ejection fraction could be further informative to identify HF which has not been diagnosed. Interestingly, we found associations not only with vascular dementia, but with Alzheimer's disease as well in the second and third quartiles of NT-proBNP, which further advocates the role of vascular pathology in development of Alzheimer's disease. Given the insidious onset of dementia through a long pre-clinical phase of cognitive decline, we also showed an important link of NT-proBNP with cognition cross-sectionally and to a lesser extent longitudinally. This suggests that subclinical cardiovascular disease already leads to subtle cognitive deficits without overt dementia and therefore pinpoints a potential window of opportunity for developing preventive strategies. Higher NT-proBNP was not associated with a significant decline in multiple domains because these analyses might be underpowered and changes would become detectable with increasing follow-up. In addition, there could be a selective attrition of persons with major cognitive deficits during follow-up. Further, it is possible that NT-proBNP associates more with changes in memory rather than these domains, but we did not have memory tests to assess this association in this study.

We did not observe statistical significance for the highest quartile of NT-proBNP in relation with Alzheimer's disease in contrast to dementia. However, since we lacked sufficient power for Alzheimer's disease analyses, definitive conclusions cannot be drawn from these estimates. Moreover, since dementia might represent multiple brain pathologies especially cerebrovascular, these findings possibly indicate that the higher risk for dementia was driven by the vascular component of dementia and NT-proBNP may be a marker of cerebrovascular pathology and associated cognitive deficits independent of Alzheimer's disease pathology Additionally, results of the competing risk analyses did not reveal a substantial, but only a minor effect of competing risks on our main findings.

The exact mechanism underlying the association between subclinical cardiovascular disease and brain damage remains unknown, but it is possible that a compromised systemic perfusion and vascular deterioration may affect cerebral homeostasis. It is known that autoregulation of cerebral vasculature augments the blood flow to the brain, during periods of reduced cardiac function. However, studies have suggested that these autoregulatory mechanisms are less effective when systemic flow reductions are subclinical or chronic.[43,44] Another possibility is that impairment in contractility of atria or ventricles leads to formation of thrombi. [45,46] Finally, it is also possible that a separate

mechanism, such as inflammation or oxidative stress, links cardiovascular disease and neurodegenerative changes.

Our data suggest that subclinical cardiovascular disease as reflected by serum NT-proBNP levels is associated with dementia. Since NT-proBNP marks the presence of subclinical cardiovascular disease in absence of overt cardiovascular disease, it can indicate persons at a higher risk of dementia. This provides a window of opportunity for timely preventive and treatment strategies for the subtle neurodegenerative changes caused by subclinical cardiovascular disease, leading to a delay in dementia onset or even prevention. However, certain prerequisites such as testing for the predictive accuracy of the marker and cost-effectiveness should be assessed. In addition, the risk factors which require intervention should be identified. In conclusion, NT-proBNP is a potential marker of subclinical cardiovascular disease that may cause neurodegenerative changes.

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CHAPTER 4.2

Cardiovascular, metabolic and renal biomarkers and their association with depression: a longitudinal population-based study of older adults

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ABSTRACT

Background: Several cardiovascular, metabolic, and renal biomarkers, are associated with depression. However, it is not known if these biomarkers also predict depression.

Methods: In the Rotterdam Study, 5,813 participants with a mean age of 69±8 years were followed from 1997 to 2011 for 'incident major depressive disorder (MDD)' and 'clinically relevant depressive symptoms' (mean follow-up:9±4 years). The following biomarkers were tested: NT-proBNP, CRP, glucose, total cholesterol, HDL, cystatin C, uric acid, creatinine, and homocysteine. To assess the joint effects of biomarkers, Principal Component Analysis was conducted, and four groups of renal, metabolic, cardiovascular, and lipid biomarkers were extracted. Data on incident MDD and incident depressive symptoms were obtained through repeated screening by a validated questionnaire, with subsequent semi-structured psychiatric interview, as well as continuous monitoring of general practitioners' records. Cox proportional hazards models adjusted for socio-demographics, cardiovascular risk factors, health markers, and prevalent chronic diseases were used to analyze the association of biomarkers with incident depression.

Results: Higher levels of NT-proBNP and CRP were each related to a higher relative risk of incident depressive symptoms, HR per SD 1.08 (95% CI: 1.00, 1.16), and 1.09 (95% CI: 1.02, 1.17) respectively. Higher levels of NT-proBNP were also associated with a higher risk of incident MDD, HR per SD 1.27 (95% CI: 1.05, 1.54). In joint analysis, cardiovascular (HR 1.14 (95% CI: 1.05, 1.23)), and metabolic biomarkers (HR 1.08 (95% CI: 1.00, 1.18)) were associated with incident depressive symptoms.

Conclusions: Cardiovascular, inflammatory, and metabolic pathways have a possible role in the etiology of depressive disorders and depressive symptoms in older adults.

INTRODUCTION

Major depressive disorder (MDD) and clinically relevant depressive symptoms are common in older age, and are associated with a poor quality of life and excess mortality.[1-3] The etiology of depression in older adults is poorly understood, and may differ from their younger counterparts. In older adults, depression is less related to family history of depression, presents with more cognitive symptoms, has a worse response to treatment than in younger adults,[3] and is highly prevalent in many chronic disorders, for instance diabetes mellitus or heart failure.

Many clinical studies tested biomarkers of cardiovascular, metabolic, or renal diseases, in relation to prevalent depression, and found positive associations.[4-7] Extending the findings from clinical studies, many population based studies also reported associations between biomarkers of cardiovascular, metabolic, and renal diseases, with prevalent depression in older adults. [8-11] However, these studies were largely cross-sectional. Given these associations in both clinical and population-based studies, it is possible that such biomarkers or the biological pathways they represent, are also involved in the etiology of depression in the general population. However, there are no prospective studies to investigate the associations of such biomarkers with incident depression in population-based studies. Although there are studies which suggest that depression is associated with vascular impairment, and shares risk factors with cardiovascular diseases such as smoking behavior and diabetes mellitus,[12,13] it remains largely unknown if biomarkers of these diseases are also longitudinally associated with incident depression.

Identifying biomarkers of cardiovascular, metabolic, and renal pathways that predict depression may help elucidate its pathophysiology in older adults. However, it should be noted that these pathways are interrelated. For instance, vascular impairment or diabetes mellitus can give rise to impairment renal function, and impaired renal function can lead to cardiovascular dysfunction. Therefore, biomarkers of these biological systems are also dependent on each other to some extent. However, the correlations between these biomarkers in relation to depression have not been taken into account by previous studies. Findings from studies, which have investigated individual biomarkers in relation to depression, are also inconsistent.[8-11,14] Understanding these correlations between biomarkers might be a beginning step to understand the interrelated pathways leading to depression in older adults. Further, testing biomarkers jointly may help tease out the true independent associations and thus improve precision.

Therefore, we aimed to investigate the associations of novel and traditional biomarkers, namely amino-terminal pro–B-type natriuretic peptide (NT-proBNP), C-reactive protein (CRP), glucose, total cholesterol, high-density lipoprotein cholesterol (HDL), cystatin C, uric acid, creatinine, and homocysteine, individually and jointly, with incident MDD and clinically relevant depressive symptoms in a population-based setting.

METHODS

Setting

This study was embedded in the Rotterdam Study, an ongoing population-based cohort addressing the incidence and determinants of chronic diseases in late life.[15] Every 3 to 4 years, all participants undergo an extensive home interview and physical examination at the research center. In addition, the study database is linked to medical files from general practitioners, ensuring continuous surveillance for all major events. Depressive disorders and symptoms were assessed for the first time in the examination round in 1997-2001 (response rate: 79%), which formed the baseline for this study.

Study population

In total, 7,808 participants attended the baseline assessment. Of these, 148 individuals who had prevalent dementia, 354 with no assessment for dementia or depression at baseline, and 5 persons with bipolar disorder, were excluded. We further excluded 975 participants who did not have data for any of the following biomarkers: cystatin C, uric acid, creatinine, homocysteine, glucose, total cholesterol, HDL-C, CRP and NT-proBNP. Of the 6,326 remaining participants, 513 had clinically relevant depressive symptoms at baseline, and were excluded. Thus, 5,813 participants were eligible for analyses.

Assessment of biomarkers

At baseline, fasting blood samples were collected to determine serum or plasma levels of biomarkers. NT-proBNP was measured using a commercially available electrochemiluminescence immunoassay (Elecsys proBNP; F Hoffman-La Roche Ltd., Basel, Switzerland).[16,17] High-sensitivity CRP measurement was performed using rate near-infrared particle immunoassay (Immage Immunochemistry System; Beckman Coulter, San Diego, CA).[18] Glucose was enzymatically determined using the Hexokinase method (Boehringer Mannheim, Mannheim, Germany).[19] Total and HDL cholesterol were determined using an automatic enzymatic procedure (Boehringer Mannheim).[19] Cystatin C

levels were attained using a BNII nephelometer (Dade Behring Inc., Deerfield, IL).[20] Uric acid was measured with Kone Diagnostica reagent kit and Kone autoanalyzer.[21] Creatinine level was assessed by a nonkinetic alkaline picrate (Jaffé) method (Kone Autoanalyzer; Kone Corp, Espoo, Finland, and Elan; Merck, Darmstadt, Germany).[22] Total homocysteine levels were measured using the Architect i2000 RS analyzer (VUmc), HPLC-method (WUR) and LC–MS/MS (EMC).[23]

Biomarker measurements were not available for all participants due to insufficient quantity of blood for some participants. This was not likely to introduce bias as the missings were completely at random.

Assessment of depression

Depressive disorders and depressive symptoms and were each determined from two sources of information as described previously.[24] The first source had two steps. In step 1, all participants were screened for depressive symptoms during the examination rounds with the Dutch version of the Centre for Epidemiologic Studies Depression (CES-D) scale[25] which consists of 20 items, each with a possible score of 0-3 scale. Maximum score is 60, and participants with a score of 16 or above are considered to have clinically relevant depressive symptoms (categorically defined). In the next step, all screen positives were interviewed by a clinician using the Dutch version of the Schedules for Clinical Assessment in Neuropsychiatry (SCAN).[26] MDD was diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, 4th revised edition (DSM-IV). The second source was the medical records of general practitioners, which were continuously monitored for occurrence of episodes of depressive disorders and depressive symptoms from baseline onward. All available information from these two sources was used to define the incident MDD episodes and incident depressive symptoms episodes that occurred first chronologically in any of the two data sources.

Assessment of covariates

Age, sex, education, civil status, home status, alcohol intake, smoking behavior, body mass index (BMI), hypertension and prevalent type 2 diabetes mellitus, myocardial infarction (MI) and stroke were considered possible confounders as these are known risk factors for depression in the elderly.[13,27,28] In addition, depressive symptoms score at baseline was also used as a covariate for the analyses of MDD.

Education was assessed during interview and people were classified into two categories, low level of education (primary only or unfinished secondary) and intermediate to high

(secondary, vocational or university). Civil status was defined as married (or cohabiting with a partner) or not married (single). Home status was defined as living independently or not living independently (including nursing homes). Alcohol intake was measured in grams of ethanol intake per week.[29] Participants were categorized into current, former and never smokers. BMI was assessed as weight in kilograms/height in meters squared. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, or diastolic blood pressure ≥ 90 mmHg, or use of antihypertensive medication assessed by interview and pharmacy records.[30] Diabetes Mellitus type 2 was diagnosed as fasting blood glucose ≥ 7.00 mmol/l or use of anti-diabetic medication evaluated by interview and pharmacy records.[31] Previous MI and stroke were determined by reported events in interview and confirmed by medical records. In addition, participants were continuously monitored for all major events through automated linkage of study database with general practitioners files.

Statistical analysis

We tested the associations of NT-proBNP, CRP, glucose, total cholesterol, HDL, cystatin C, uric acid, creatinine, and homocysteine, with incident MDD and clinically relevant depressive symptoms using Cox proportional hazards models. First, each biomarker was analyzed individually for MDD and depressive symptoms. Later, the biomarkers were tested jointly to improve precision and explore their interrelated effects, using the components derived from the PCA. All biomarkers were log-transformed to achieve a normal distribution, and standardized. In addition, we performed multiple imputations to handle missing values in the covariates, and winsorized the outliers at three standard deviations. As mentioned above, all analyses were conducted in a population (N=5,813), free of clinically relevant depressive symptoms at baseline (CES-D ≥ 16).

Principal Component Analyses (PCA): This technique allowed the highly correlated biomarkers to be interpreted as one domain. Additionally, PCA reduced the problem of multiple testing, as the number of tests was reduced from 9 to 4. Therefore, the analysis could be reasonably exempted from the requirement of adjustment for multiple testing. PCA were conducted using oblique rotation (direct oblimin), and the analyses were adequate (Kaiser-Meyer-Olkin score for the overall analysis: 0.725, all individual marker scores: >0.5). Correlations between the variables were sufficiently large to perform PCA (Bartlett`s test of sphericity, p-value <0.001). Given the large sample size and the average communality > 0.6, Kaiser's criterion was used to retain components with eigenvalues over 1, and the scree plot was in concordance with this criteria.[32] Therefore four components were retained, explaining, in total, 69% of the variation in biomarker levels.

Biomarker loadings of an absolute value > 0.4 on a factor were considered to have a strong association. Factors were described and labelled as follows, according to the principal biomarker loadings: 'Cardiovascular' (NT-proBNP and cystatin C), 'metabolic' (glucose, HDL and CRP), 'renal' (cystatin C, uric acid, creatinine, and homocysteine), and 'lipid' (total cholesterol). Each participant received an individual score of the four biomarker components based on levels of biomarkers and factor loadings.

For all analysis, two models were fitted: Model 1 was adjusted for age and sex only. Model 2 was additionally controlled for education, civil status, home status, BMI, hypertension, smoking status, alcohol intake, type 2 diabetes mellitus, previous MI and stroke. Data were analyzed using the Stata Software Version 13 (StataCorp, College Station, TX, USA).

RESULTS

The present study included 5,813 persons. Mean age at baseline was 69 years and 55% of the participants were women. During 52,536 person-years, 1,058 (18%) persons developed clinically relevant depressive symptoms, including 152 (3%) persons that developed MDD. Baseline characteristics of the study population are described in **Table 1**.

In the analyses of individual biomarkers, higher levels of NT-proBNP were associated with a higher relative risk of MDD, and remained associated with MDD after adjusting for potential confounders, HR per SD 1.27 (95% CI: 1.05, 1.54) (**Table 2**). None of the other biomarkers was related to incident MDD. For depressive symptoms, higher levels of NT-proBNP, CRP, and glucose were associated with a higher risk of incident depressive symptoms when adjusted for age and sex. After additional adjustment for all possible confounders, NT-proBNP and CRP remained associated to a higher risk of incident depressive symptoms, HR per SD 1.08 (95% CI: 1.00, 1.16) and 1.09 (95% CI: 1.02, 1.17) respectively (**Table 2**).

Table 1. Baseline characteristics of study population, N=5,813.

Characteristics	Descriptives
Social demographics	
Age, years	69.0 (8.1)
Women	3,198 (55.0)
Education – Intermediary or high	4,588 (79.7)
Civil Status – married or cohabiting	4,119 (70.9)
Home Status – Dependent	761(13.1)
Alcohol Intake, g/week	82.3 (108.1)
Smoking behavior	
Never	1,793 (30.8)
Past	2,885 (49.6)
Current	1,135 (19.5)
Health markers and diseases	
Body Mass Index, kg/m²	27.0 (4.0)
Hypertension	4,039 (70.3)
Prevalent type 2 diabetes mellitus	494 (8.5)
Prevalent myocardial infarction	368 (6.4)
Prevalent stroke	474 (8.1)
Blood measurements	
NT-proBNP, pmol/l	23.6 (75.8)
CRP, mmol/ml	32.3 (57.3)
Glucose, mmol/l	6.5 (7.1)
Total cholesterol, mmol/l	5.8 (1.0)
HDL cholesterol, mmol/l	1.4 (0.4)
Cystatin C, nmol/l	77.9 (20.0)
Uric Acid, moml/l	0.3 (0.1)
Creatinine, umol/l	79 (24.0)
Homocysteine, umol/l	14.7 (5.6)

Values are means (standard deviation)s or counts (percentage).

Table 2. Association of individual biomarkers with incident major depression and clinically relevant depressive symptoms.

Biomarkers	Incident Major depressive disorder, hazard ratios (95% confidence intervals)				
	n/N [a]	Model 1 [b]	P-value	Model 2 [c]	P-value
NT-proBNP	149/5,682	1.28 (1.06,1.55)	0.009	1.27 (1.05,1.54)	0.01
CRP	146/5,583	1.07 (0.91,1.27)	0.41	1.00 (0.83,1.20)	0.97
Glucose	151/5,777	1.11 (0.93,1.33)	0.24	1.10 (0.87,1.38)	0.44
Total cholesterol	151/5,777	1.00 (0.83,1.18)	0.93	1.01 (0.85,1.20)	0.90
HDL-C	149/5,713	0.85 (0.75,1.01)	0.07	0.90 (0.75,1.08)	0.26
Cystatin C	148/5,613	1.17 (0.96,1.42)	0.12	1.11 (0.90,1.35)	0.33
Uric Acid	149/5,682	1.11 (0.94,1.33)	0.21	1.07 (0.88,1.29)	0.50
Creatinine	149/5,682	0.92 (0.74,1.14)	0.46	0.92 (0.74,1.14)	0.46
Homocysteine	130/4,929	1.01 (0.83,1.23)	0.63	0.97 (0.79,1.19)	0.79
Biomarkers	**Incident clinically relevant depressive symptoms, hazard ratios (95% confidence intervals)**				
	n/N [a]	Model 1 [b]	P-value	Model 2 [c]	P-value
NT-proBNP	1,035/5,682	1.09 (1.01,1.17)	0.02	1.08 (1.00,1.16)	0.04
CRP	1,019/5,583	1.11 (1.05,1.18)	0.001	1.09 (1.02,1.17)	0.009
Glucose	1,055/5,777	1.07 (1.02,1.17)	0.01	1.08 (0.99,1.18)	0.09
Total cholesterol	1,055/5,777	1.00 (0.93,1.06)	0.79	1.00 (0.94,1.07)	0.92
HDL-C	1,043/5,713	0.92 (0.86,0.98)	0.01	0.95 (0.88,1.02)	0.14
Cystatin C	1,027/5,613	1.06 (0.98,1.15)	0.12	1.03 (0.95,1.12)	0.44
Uric Acid	1,035/5,682	0.97 (0.91,1.04)	0.45	0.95 (0.88,1.02)	0.13
Creatinine	1,035/5,682	0.95 (0.88,1.03)	0.25	0.95 (0.88,1.04)	0.26
Homocysteine	909/4,929	1.01 (0.93,1.08)	0.93	0.99 (0.92,1.07)	0.85

[a] Number of cases/population at risk.
[b] Model 1: Adjusted for age and sex.
[c] Model 2: Additionally adjusted for education, civil status, home status, alcohol intake, smoking behavior, BMI, hypertension and prevalent type 2 diabetes.

In joint analyses of biomarkers, we tested the components derived from PCA in relation to incident MDD. None of the cardiovascular, metabolic, renal, or lipid components was associated to incident MDD (**Table 3**). However, higher scores in the cardiovascular and metabolic factors were associated with an increased risk of incident depressive symptoms. These associations remained after adjusting for all potential confounders, cardiovascular: HR 1.14 (95% CI: 1.05, 1.23), and metabolic: 1.08 (95% CI: 1.00, 1.18). No associations were observed for the renal and lipid components with incident depressive symptoms (**Table 3**). Pattern matrix, eigenvalue and percentage of variance explained by each extracted PCA component are shown in **Table 4**.

Table 3. Association of PCA derived components of biomarkers with incident major depression and clinically relevant depressive symptoms.

Components	Incident Major depressive disorder, hazard ratios (95% CI)				
	n/N [a]	Model 1 [b]	P-value	Model 2 [c]	P-value
	124/4,668				
Renal		0.94 (0.78, 1.13)	0.51	0.95 (0.78, 1.15)	0.58
Metabolic		1.21 (0.98, 1.49)	0.08	1.16 (0.94, 1.44)	0.16
Cardiovascular		1.12 (0.95, 1.34)	0.18	1.08 (0.87, 1.34)	0.46
Lipid		1.10 (0.90, 1.35)	0.37	1.06 (0.85, 1.31)	0.61

Components	Incident clinically relevant depressive symptoms, hazard ratios (95% CI)				
	n/N [a]	Model 1 [b]	P-value	Model 2 [c]	P-value
	867/4,668				
Renal		0.96 (0.90, 1.03)	0.24	0.97 (0.90, 1.04)	0.39
Metabolic		1.09 (1.01, 1.18)	0.03	1.08 (1.00, 1.18)	0.05
Cardiovascular		1.14 (1.05, 1.22)	<0.001	1.14 (1.05, 1.23)	0.002
Lipid		0.99 (0.91, 1.07)	0.77	0.98 (0.92, 1.05)	0.64

Abbreviations: CI confidence intervals.
[a] Number of cases/population at risk.
[b] Adjusted for age and sex.
[c] Additionally adjusted for education, civil status, home status, alcohol intake, smoking behavior, BMI, hypertension and prevalent type 2 diabetes.

Table 4. Pattern Matrix (Principal Component Analysis).

Biomarkers	Components			
	Cardiovascular	Metabolic [a]	Renal	Lipid
NT-proBNP	0.85	0.08	0.07	-0.14
CRP	0.32	0.77	0.02	0.21
Glucose	-0.09	0.70	-0.10	-0.14
Cholesterol	-0.02	0.02	0.04	0.94
HDL-C	0.31	-0.44	-0.35	0.33
Cystatin C	0.46	0.05	0.66	-0.03
Uric Acid	-0.23	0.24	0.74	0.10
Creatinine	-0.06	-0.14	0.87	-0.08
Homocysteine	0.23	-0.12	0.70	0.02
Eigenvalues	1.07	1.36	2.76	1.02
% of variance	11.87	15.14	30.67	11.29

Loadings over 0.4 are in bold letters.
[a] Metabolic component was multiplied by (-1) to represent a risk factor.

DISCUSSION

In this prospective population-based study of older adults, we found that higher levels of NT-proBNP and CRP were each related to a higher risk of incident clinically relevant depressive symptoms. Higher levels of NT-proBNP were also associated with incident MDD. When biomarkers were tested jointly, cardiovascular and metabolic biomarkers were associated with an increased risk of incident depressive symptoms.

We found that NT-proBNP was associated with both incident MDD and depressive symptoms. This finding extends the results from cross-sectional studies, which demonstrated higher levels of NT-proBNP in depressed patients.[5,6,33] NT-proBNP, which is a well-documented prognostic marker of cardiovascular disease, is also an emerging marker for the presence of subclinical cardiovascular disease in apparently healthy individuals.[16] We also found that CRP, an inflammatory marker, predicted clinically relevant depressive symptoms. Previous studies have also suggested that immune dysregulation might result in depression in older adults.

When testing biomarkers jointly, the cardiovascular and metabolic components were associated with incident depressive symptoms. The cardiovascular component comprised NT-pro-BNP and cystatin C, of which, NT-proBNP was the principal loading, showing consistency with the analyses of individual biomarkers. These observed associations of NT-proBNP and cardiovascular component with incident depression, even after adjusting for cardiovascular events and risk factors, suggest a role of subclinical cardiovascular disease in the development of depression. Studies have suggested that ischemic lesions in the cerebral white matter may disrupt neural connections in regions regulating mood and cognition, such as the hippocampus. Such disruptions might lead to development of depression. Moreover, it is also possible that a reduction in cerebral blood flow can impair regional brain function, contributing to affective and cognitive symptoms.[34] In joint analyses, the metabolic component was also associated with incident depressive symptoms. This factor comprised CRP, glucose, and HDL. Therefore, these findings were consistent with those of the individual biomarker analyses, and suggest the interplay of inflammatory and metabolic pathways in the etiology of depression. There are studies that tested CRP individually in relation to risk of depression, and found that CRP was associated with depressive symptoms both cross-sectionally and longitudinally.[8,9] There could be a few possible explanations for these findings. It is possible that immune dysregulation promotes the development of depressive disorders in elderly by affecting neurotransmitters levels such as monoamines.[34] Moreover, it is also possible that metabolic imbalance may lead to subclinical vascular damage, which in turn may produce

depressive symptoms. It is therefore important to note that in most cases, depression is perhaps a result of interaction of multiple pathways such as vascular and inflammatory, which is also reflected in our findings. In our study, we could show that CRP is part of a group of related factors that leads to depressive symptoms. These findings are in line with the previous literature.[35] In a recent review, metabolic risk factors, such as central obesity and hypertriglyceridemia were associated with a higher risk of depression.[35] However, in the analyses of individual metabolic biomarkers in our study, glucose and HDL were not associated with depressive symptoms after adjusting for all potential confounders. Other studies that found an association of glucose and HDL with depression,[35,36] did not adjust for the confounding effects of social factors such as marital status, home status and education, which might explain the differences in results.

We did not find any associations of renal biomarkers with MDD or depressive symptoms. Several cross-sectional and few prospective studies investigated the association of these biomarkers with depressive disorders with conflicting results.[4,7,10,14] It is possible that these associations are only observed in patients with severe kidney disease. Therefore, the null association observed in our study might be a result of possible selection on health, as persons with more severe kidney disease are less likely to attend the examination rounds.

Strengths of the study include the population-based design, large sample size, long follow-up and a large number of incident depressive symptoms and MDD cases. The novelty of this study is that we analyzed several relevant biomarkers individually and jointly, in relation to incident MDD and clinically relevant depressive symptoms. The joint analysis allowed to assess the correlation patterns of biomarkers, and reduced multiple testing problems. Additionally, we also tested the confounding effects of social factors such as marital status, home status and education, all of which are associated with both physical and psychiatric health in the elderly.[27] There are certain limitations as well. First, we did not have information about prior depression in this population. Second, there is a possibility of selection bias, as persons with more severe kidney or cardiovascular disease are less likely to attend the examinations. Third, as biomarkers were assessed only at baseline, their levels might have changed over time, resulting in regression dilution.[37]

In conclusion, cardiovascular, inflammatory, and metabolic pathways, or their interactions have a role in the etiology of depression in older adults.

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CHAPTER 5

GENERAL DISCUSSION

This thesis is based on longitudinal studies assessing the associations of psychiatric determinants, life-style factors, and new biomarkers with neurological outcomes and mortality. All studies included in this thesis are embedded in the Rotterdam Study, an ongoing population-based cohort of older adults in Ommoord, a district of Rotterdam.[1] In 1990, 7,983 participants aged 55 and older were enrolled in the Rotterdam Study referred to as the Rotterdam Study I. In 2000, an expansion of the cohort was made by enrolling 3,011 participants, the Rotterdam Study II, who fulfilled the inclusion criteria. Finally, in 2002, the cohort was again expanded by 3,486 participants, the Rotterdam Study III, now including those aged 45 years or older. In addition to detailed interviews and examinations every 3-4 years, all participants are continuously monitored for all major life events via a computerized linkage of the study database with the files from general practitioners.

The strengths and limitations of individual studies have been described previously per chapter. In the general discussion, I will discuss the key findings of this thesis, and elaborate general methodological considerations. Lastly, I will provide clinical implications of this research and a broad viewpoint on directions for future research.

KEY FINDINGS

Depression and dementia

One of the key findings of this thesis is that depressive symptoms in older age represent a *prodrome* of dementia. A *prodrome* can be defined as a predementia syndrome in which underlying dementing process which is yet subclinical, manifests as neuropsychiatric symptoms.[2] Thus, appearance of depressive symptoms in late-life might mark the onset of dementia in near future.

Although the psychiatric symptoms of neurodegenerative pathology are not limited to depression,[3] I focus on depression associated with dementia because it is the most common psychiatric comorbidity of dementia.[4,5] Furthermore, comorbid depression in dementia is associated with accelerated cognitive decline, distress, poor quality of life, increased caregiver burden, and worsening of functional impairment.[3] The other common psychiatric manifestations of underlying neurodegenerative changes include anxiety, apathy, and sleep disturbance.[4,6] To assess the association between depression and dementia in this thesis, I used depressive symptoms as assessed by the Center for Epidemiological Studies Depression Scale (CES-D). CES-D assesses symptoms of depression

which do not reach the threshold to be diagnosed as major depression but are still of clinical relevance, and are associated with impairment similar to major depression.[7]

In **chapter 2.1**, I found that presence of clinically-relevant depressive symptoms in older adults predicted dementia and Alzheimer's disease, only in the short term, but not in the long-term. Associations were strongest for the short follow-up time, and attenuated with incrementally longer periods of follow-up time. The short-term associations became even stronger when excluding individuals who were also positive for depressive symptoms in a previous and a next round of depressive symptoms assessment, thereby eliminating the diluting effect of chronic symptoms. These findings of a short-term association suggested the role of *reverse causality*, suggesting that depressive symptoms did not increase the risk of incident dementia but rather manifested in response to the underlying cognitive impairment. It can be argued that the results of a null-finding in the long-term could be a case of *regression dilution*, which can frequently affect studies as the follow-up prolongs.[8] In this case, where depressive symptoms were measured at baseline, and were tested in relation to incident dementia over a long follow-up period, and in discrete follow-up periods, indeed results could have been diluted during 13 years. Therefore, the calculated results might be an underestimation of the true associations present. However, I observed a similar pattern of results when using the depressive symptoms from a previous assessment round, which reinforced that the pattern of results showing an increased risk of dementia in the short-term and a null association in the longer term was robust. Also, when I defined discrete time periods ensuring equal number of participants in each period, results were consistent. Nevertheless, use of one-time assessment of depression to investigate the associated risk of incident dementia (or any other long-term health outcome) was not only problematic because it might have led to *regression dilution*, but also because depression has a remitting and relapsing nature. By doing that, the course of depression over time was neglected, which was of crucial importance, as persons with more chronic high depressive symptoms might be at a higher risk of dementia than those who just had high symptoms acutely. This was not captured by a single assessment of depression. Therefore, as a further step, I used repeated measures of depressive symptoms to study the course of depression in relation to risk of dementia.

Therefore in **chapter 2.2**, using repeated measures of depression served two important purposes. First, the possibility of *regression dilution* was minimized. Second, and more important, repeated measures enabled to study the course of depression in relation to risk of dementia. Moreover, I exploited repeated measures of depressive symptoms to employ the latent-class trajectory modelling, which identified latent classes of depressive symptoms. The "term" latent signifies that there are existent classes of different courses

of the depressive symptoms in populations, but are unidentified or indistinct. This method served to sort individuals into groups that followed similar course of depressive symptoms over time. This was important as risk of dementia might differ significantly with the course of depressive symptoms over time. I hypothesized that depression affecting individuals at a point in time followed by full remission, might not contribute to any long-term health risks. Therefore, groups with transient depressive symptoms that perhaps are not the high risk groups for incident dementia should be reclassified, delineating those with sustained high or increasing symptoms, which are more likely the ones having a higher risk of dementia. In this study, I could demonstrate that the trajectory with increasing depressive symptoms was associated with a higher risk of dementia, reinforcing the prodromal hypothesis, and suggesting that indeed increasing depressive symptoms represent an early stage or prodrome of dementia. Further, as anticipated, the trajectories with decreasing or remitting depression were not associated with a higher risk of dementia. As a sensitivity analysis, I also performed survival analyses with incremental years of follow-up starting with a follow-up of 2 years and adding a year in every step, to test for *reverse causality*. The increasing trajectory was consistently associated with a higher risk of dementia in throughout the increments of follow-up. Interestingly, I observed that the remitting trajectory also showed a higher risk of dementia until the 5^{th} year of follow-up, which declined thereafter. This could mean that the escalation and peak of the remitting trajectory might also represented an early stage of dementia, which was reflected in higher risk of dementia in the early years of follow-up. In this case, the remitting trajectory might be following a similar course as the increasing one, with the only difference between the time of their peaks. To prove this proposition, it would have been useful if the course of depressive symptoms was known before the study period. This could be regarded as a limitation of the study, however, such limitations are inherent to adult cohort studies. Interestingly, the trajectory with sustained high depressive symptoms did not show a statistically significant higher risk, although I hypothesized that chronic high symptoms would be associated with a higher risk of dementia. However, it is very likely that I was unable to detect the risk difference because of insufficient power in this group. Nevertheless, for this group, it remains questionable that weather sustained high depression in older age poses a higher risk of dementia or not, as with younger ages.

An added advantage of using repeated measures was that in the increasing trajectory, I could demonstrate that depressive symptoms started to escalate several years before the diagnosis of dementia, indicating the long preclinical stage of dementia. This depicted that symptoms of depression appear as soon as individuals perceive a change in their cognitive abilities. It can be implied that depressive symptoms might only be apparent through a

phase in which participants are able to “recognize” this cognitive decline, or are “aware” of their deteriorating condition. Similarly, depressive symptoms might ameliorate once the neurodegenarative damage is severe enough to impact cognition and autonomy. This makes it imperative to timely recognize such behavioral or non-cognitive symptoms in the course of neurodegeneration, as earlier recognition might provide a wider window of opportunity for interventions. Therefore, as a further step to study the association of neurodegenerative damage and affective symptoms, I took a step back from clinical dementia to Mild Cognitive Impairment (MCI).

In **Chapter 2.6**, I studied MCI in association with incident depression and anxiety disorders, which further elucidated this complex association of neurodegenarative pathology with affective symptoms. Presence of MCI strongly predicted the incidence of both depressive and anxiety disorders. In this study, DSM depressive and anxiety disorders were studied instead of clinically relevant symptoms of depression and anxiety. Although I studied the association of MCI and depressive symptoms (Clinically relevant depressive symptoms=CES-D ≥ 16), I only reported results for disorders to maintain uniformity in the manuscript, since data on anxiety symptoms were not available. In the analyses depressive symptoms, I studied the associations of MCI with depressive symptoms as a continuous variable, both cross-sectionally and longitudinally using multiple linear regression models. In cross-sectional analysis, MCI was associated with depressive symptoms, difference 2.33 (95% confidence interval (CI): 1.77, 3.18). In longitudinal analysis also, MCI was associated with an increase in depressive symptoms over time, difference 0.92 (95% CI: 0.04, 1.81). The change in depressive symptoms was studied from the baseline round to the next examination round using multiple linear regression models which were adjusted for baseline depressive symptoms scores. However, I take caution in making any definitive comments about the associations of subtypes of MCI with depression or anxiety, because although these analyses were performed and reported, they included relatively smaller number of individuals, and thus results might not be very precise .

From these consistent findings, I inferred that depression or clinically relevant depressive symptoms constitute a neuropsychiatric stage on the continuum of cognitive decline, between preclinical dementia and clinical dementia. However, this stage of neuropsychiatric symptoms might only affect or more strongly affect those who are more vulnerable either genetically or environmentally, or both.

Coffee and dementia

As the most widely used beverage worldwide, coffee is considered more a part of a lifestyle, rather than just a dietary constituent. In **chapter 3.1**, I studied the longitudinal associations of coffee consumption and risk of dementia. The most robust finding of the several analyses carried out to test this association was that coffee consumption of 3 or more cups per day was protective of incident dementia in the short-term only, whereas no associations were found in the long-term. A recent systematic review concluded that evidence from population-based studies suggests a protective association of coffee intake with incident dementia and cognitive decline, particularly from studies with a shorter follow-up period.[9] However, a more recent meta-analysis of observational studies studying caffeine intake from tea or coffee, reported no association between caffeine intake by tea or coffee and risk of cognitive disorders, though a protective association was found for cross-sectional reports.[10]

The finding of a short-term protective association, and finding a similar pattern of associations using a repeated measure of coffee assessment in the same cohort strongly suggested *reverse causality;* a *"healthy coffee-drinker effect",* implying that those who were healthier, and socially and physically active, drank more coffee than those who were less healthy, and less active. In addition, perhaps the latter reduced their coffee intake due to the declining health or cognition, or switched to the generally perceived healthier options such as tea. However, it could not be completely ruled out if coffee actually has a beneficial influence on cognition, as there are several proposed biological pathways that may underlie this protective effect of coffee on neurodegeneration or its symptoms. The beneficial effects of coffee might be attributed to caffeine, antagonizing A1 adenosine receptors in the hippocampus and cortex[11-13] or reducing the permeability of blood brain barrier, with consequent reduction in the amount of amyloid passing into the brain, thus providing a neuroprotective response.[14] Although these highly specific biological underpinnings might have an active role in the protective effects conferred by coffee, it is more likely that more generic mechanisms are involved. Regarding the effects of caffeine metabolites on adenosine receptors, the antagonism of A1 receptors by caffeine in the renal tubules and vasculature results in vasodilation and consequent diuresis, which lowers the blood pressure.[15] In addition, the chlorogenic acid in coffee is a potent antioxidant, which maintains the endothelial and vascular integrity by increasing the availability of nitric oxide.[16] Whether these systemic effects operate through the anti-inflammatory, anti-oxidant, or vasoprotective pathways, they all contribute to cardiovascular health of individuals. Therefore, the lower risk of dementia in habitual coffee-consumers might be due to their improved cardiovascular health. These

mechanisms do not hold for total dementia or dementia of vascular origin only, but also for Alzheimer's disease (AD), and suggest a role of vascular pathology in AD. However, this hypothesis of protective effect of coffee on CVD is relatively recent, as earlier studies investigating the effects of coffee consumption and risk of cardiovascular diseases suggested a harmful association, and a higher incidence of cardiovascular diseases and hypertension among coffee drinkers.[17-22] Recent studies however tend to report neutral or favorable effects of coffee on cardiovascular health.[23,24] As concluded in a recent review, "coffee is safe to drink by healthy subjects or those with preexisting cardiovascular diseases or hypertension."[16]

As a further step to investigate whether the beneficial effects of coffee consumption only represent a *healthy-volunteer effect*, or there is an actual beneficial effect also, I investigated the association of coffee consumption with MRI markers and cognitive decline in **chapter 3.2**. I observed that moderate coffee consumption (1-3 cups/day) was associated with a lower prevalence of lacunar infarcts, and a better processing speed and executive function. This is plausible as studies show that executive function is a cognitive domain that is primarily affected by vascular brain disease, including white matter lesions and lacunar infarcts.[25,26] However, the association of coffee and better executive function was independent of lacunar infarcts suggesting the involvement of other pathways or presence of changes that were not detectable on MRI. Nonetheless, these findings reinforced the hypothesis that coffee intake might be protective against dementia or cognitive decline by improving vascular health. Although the cross-sectional design of this study precluded any conclusions about causality, it does provide evidence for cardiovascular health as a potential link between coffee intake and lower risk of dementia.

There were certain intriguing findings that I observed with the consumption of 3 or more cups of coffee per day with incident dementia longitudinally, and with cognition and brain volumes cross-sectionally. In **chapter 3.1**, after excluding the first few years of follow-up with a protective effect, a harmful effect of coffee consumption was observed in the later years, with 3 or more cups per day. This effect was not observed for moderate consumption of 1-3 cups per day. In addition, in the cross-sectional analyses of **chapter 3.2**, coffee consumption was associated with a smaller hippocampal volume and worse speed of color naming, interference of automated processing and attention, and retrieval from verbal memory. Where the null association over the overall follow-up period in **chapter 3.1** could be a case of *regression dilution*,[8] as coffee assessment was only assessed at baseline, the harmful effect observed after excluding the first few years is difficult to explain. Perhaps continued coffee consumption could mask the underlying cognitive deficits to a certain extent, and delayed the dementia diagnosis which resulted more

diagnoses in the later period. Alternatively, the symptoms might manifest only when cognitive decline reaches a certain threshold, and individuals reduce their coffee intake as part of a general change in their lifestyle as they perceive cognitive decline, hence alleviating the protective/masking effects of coffee. However, both these explanations are valid only if coffee actually has short-acting protective effects only, and therefore, maintaining regular consumption might be the key to maintain cognitive health. To test this, I also investigated change in coffee consumption and risk of dementia in **chapter 3.1**, which indeed showed that participants maintaining their coffee consumption over the years had a lower risk of dementia compared to those who reduced intake. However, another explanation could be that coffee consumption in older adults is beneficial in the short-term only, but becomes harmful after continued use. To disentangle whether this harmful association is a result of coffee or some other factors or a combination of both is a challenge. This is because habitual coffee consumption might be associated with certain other factors which are hazardous to health, such as smoking, or alcohol use. Moreover, certain ways of preparation like boiling are associated with an increase in total serum cholesterol.[27-31] However, we did adjust for smoking habits and alcohol consumption, and BMI as a general physical health indicator. In **chapter 3.2**, the association of 3 or more cups of coffee per day with a smaller hippocampal volume and poor memory was conflicting with the existing evidence. Previously, a U-shaped association between coffee and hippocampal volume has been reported, showing that low and high intakes of coffee are associated with larger hippocampal volumes, as compared to moderate intake.[32] Although the cross-sectional design precludes any conclusions about causality, higher coffee consumption was also associated with a poor memory in our study. Animal studies have shown that although short-acting effects of caffeine were neurostimulatory, long-term use of low-dose caffeine resulted in a slowed hippocampal-dependent learning and impaired long-term memory. In addition, caffeine consumption for 4-weeks significantly reduced hippocampal neurogenesis in rats.[33] For any dietary constituent, it is more likely that the effects are small and accumulate over time to produce an effect. However, even if coffee has neuroprotective effects , its consumption might be able to counteract or mask the effects of neurodegeneration to a certain threshold only.

Taken together, moderate intake of 1-3 cups of coffee, appeared beneficial and protected against dementia, cognitive decline, and vascular brain lesions, whereas, daily consumption of 3 or more cups of coffee were associated with an increased risk of incident dementia in later years, and a smaller hippocampal volume and poor memory cross-sectionally. These findings suggest that moderate consumption confers neuroprotection whereas higher consumption can prove to be harmful. Coffee is a

mixture of more than 1000 compounds,[16] and perhaps not all of them are advantageous to health. At higher intakes, the effects of such detrimental constituents of coffee might accumulate over time and result in unfavorable effects on cognition. Additionally, consumption of moderate amounts of coffee is certainly reflective of a healthy and active lifestyle in older adults.

Vascular pathology and Alzheimer's disease

The focus on the role of cardiovascular diseases and vascular impairment in the pathology of dementia including Alzheimer's disease is continuously growing. In **chapter 4.1**, I investigated the association of NT-proBNP with dementia, Alzheimer's disease, vascular dementia, and cognitive decline, both with and without including participants with overt cardiovascular disease. NT-proBNP is an emerging marker of cardiovascular diseases,[34,35] and is also suggested to be a marker of cardiovascular disease at a subclinical stage.[36] The association of NT-proBNP with cognitive dysfunction was shown in some clinical and smaller population-based studies,[37-41] but its association with incident dementia was not known even in clinical settings. Interestingly, NT-proBNP, which is an inactive amino terminal of proBNP, predicted dementia even after adjustment for several cardiovascular risk factors, and excluding participants with diagnosed cardiovascular diseases and events. Although the estimates were more strong for vascular dementia which is plausible, they were also significant for Alzheimer's disease. Thus providing further support to the involvement of vascular pathology in the etiology of Alzheimer's disease. I also observed that higher NT-proBNP was associated with a decline in processing speed and executive function. This finding suggested that neurodegenerative changes start very early in the course of vascular impairment, which may provide a wider window of opportunity for intervention. Interestingly, this finding was also consistent with the findings in **chapter 3.2**, and suggest that of all cognitive domains, the executive function is affected primarily by vascular dysfunction, and is perhaps affected earlier than other domains of cognition.

Cognitive reserve and dementia

In **chapter 3.3**, I investigated the role of cognitive reserve in the protection against dementia after clinical cerebrovascular events. I used level of education as a measure of cognitive reserve which is most widely used measure,[42] and found that risk of dementia was higher after a stroke or TIA in persons with low or intermediate education only. Those with higher education were protected against dementia after a stroke or TIA. However, this effect was present only in men, where I observed a dose-response association, showing a decreasing risk of dementia after a stroke or TIA with the increasing level of

education. A possible explanation for this discrepancy in results for men and women was the lack of power in analyses of women, particularly for the high education category where there was only one case of dementia that occurred after a cerebrovascular event. However, there could be other reasons as well, but before I discuss further, I would elaborate on the concept of reserve.

The concept of reserve comprises two distinct but *not* mutually exclusive components, the brain reserve, and the cognitive reserve.[43] The brain reserve refers to the amount of "available neural substrate", i.e. brain volume and neuronal and synaptic counts, and is thus referred to as the passive model of reserve. In contrast, the cognitive reserve is an active model which is the "ability" to maintain function to a certain extent despite brain damage.[43,44] Scientists believe that it's cognitive reserve which explains why some people can endure more brain damage than others and still maintain function, or how people with similar brain damage have different cognitive abilities.[43-46] Cognitive reserve is built and maintained throughout life by cognitively engaging activities such as educational and occupational attainment and also leisure activities.[47,48] Cognitive reserve in turn comprises two sub-concepts, the neural reserve and the neural compensation. As the name implies, neural reserve means functionally better and flexible cognitive networks which have the ability to better cope with damage. Instead, neural compensation pertains to the ability to compensate for the damage of standard brain networks by forming or utilizing alternative networks.[43] Although level of education, complexity of occupation, intelligence quotient, and participation in cognitively active activities throughout life are considered to build cognitive reserve, a comprehensive model of cognitive reserve is still lacking.[42,45] It is a challenging yet promising field. It is challenging because, first, a working model to operationalize cognitive reserve lacks,[42] second, it is difficult to understand completely how cognitive reserve works, third, it is difficult to discriminate cognitive reserve and brain reserve, and their overlap, and finally, it is hard to find ways not only to maintain, but also increase cognitive reserve in the older ages. At the same time, it is a promising domain to study because it is dependent on factors which are modifiable and thus there is room for intervention.

The two concepts of brain reserve and cognitive reserve are overlapping and a distinction is difficult. For instance, it can be argued that those with a higher brain volume and thus higher brain reserve are more likely to continue education or adopt more challenging and complex fields of education, and thus occupation also. This means that cognitive reserve is largely dependent on the brain reserve. On the other hand, a higher cognitive reserve may lead to a protective mechanism, resulting in less neuronal loss.[42]

To operationalize cognitive reserve is a challenge. Since cognitive reserve builds up by a range of activities and is dependent on several factors throughout life, assessment of no single factor precisely reflects cognitive reserve. An individual factor can thus serve only as a rough proxy of cognitive reserve. Also, a single factor such as education may depict cognitive reserve better in some groups than others. One such example is men and women as in our study where we faced the challenge that education seemed an acceptable measure of cognitive reserve for men but not for women. In an older population like the Rotterdam Study, women did not have equal opportunities for acquiring education as men. Thus, educational level of women was not reflective of their true potential. Therefore, assessment of other measures of cognitive reserve or a combination of many factors, which are considered to build cognitive reserve, might have served as a better indicator. However, we did not have information on any of the other factors in our study which could contribute towards cognitive reserve such as leisure or social engagements, which might have served better for women. We did have information about occupation, but similar to education, women in the study were mostly working at home and men were the main bread-winners. Therefore, occupation was also not a good measure of cognitive reserve for women. To investigate this further, I ran a sensitivity analysis in couples only (n=1,731). I assigned the educational level of men (head of the household) to their spouses to explore if that serves as a better indicator for cognitive reserve in married/partnered women. However, these analyses were underpowered, and this construct also did not provide any useful information. In this small group of married women, I first ran the analyses using the their own educational level. In these analyses, risk of dementia after a stroke or TIA tended to be lower in the low education group, hazard ratio (HR) (0.83, 95% CI: 0.46, 1.51), but high in the intermediate education group, HR 2.84 (95% CI: 1.38, 5.83). Risk could not be calculated in the high education group because there were no cases in this group. When I assigned the educational level of men to women in this group, results were similar. Women with low education tended to have a lower risk of dementia after a stroke or TIA, HR 0.91 (95% CI: 0.43, 1.93), whereas those with intermediate or high education tended to have a higher risk, HR 1.34 (95% CI: 0.74, 2.42), and HR 1.46 (95% CI: 0.30, 7.25) respectively. However, these estimates were not precise due to the lack of power, and thus inconclusive. Also, in our study, we could not adjust for any measure of brain reserve, such as total brain volume, as data on brain volumes were not available. We did have brain volumes in a small subset of individuals but were assessed several years after the baseline.

METHODOLOGICAL CONSIDERATIONS

Definition of depression

In the Rotterdam Study, depression is assessed by a two-step approach. The first step screens individuals for clinically-relevant depressive symptoms, and in the next step, screen positives are subjected to a semi-structured psychiatric interview to diagnose depressive disorders according to the American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders (DSM).[49]

In step 1, screening is performed by the Dutch version of the Center for Epidemiologic Studies-Depression Scale (CES-D) which is a self-report questionnaire comprising 20 items. CES-D is a frequently used valid and reliable instrument to assess symptoms of depression in older adults, and a cut-off score of 16 is established to indicate presence of clinically relevant depressive symptoms.[7] This scale, which includes items to assess symptoms of negative affect, absence of positive affect, behavior, sleep, and appetite, requires participants to indicate the frequency of experiencing these feelings during past week. In addition, it includes 4 items pertaining to positive emotions. Therefore, CES-D not only measures clinically relevant depressive symptoms reliably, which indicate sub-threshold depression, but it assesses a general emotional state of individuals. This is particularly valuable in older adults. The general emotional state of these older individuals might reflect many relevant underlying conditions other than major depression. Such conditions could be social, such as loss of a partner, which is a devastating life event especially in older age groups, cardiovascular events such as stroke or myocardial infarction, diagnoses of distressing illnesses like cancer, or neurologic, such as cognitive decline.

In the next step, screen positives undergo a semi-structured psychiatric interview, the Schedules for Clinical Assessment in Neuropsychiatry (SCAN).[50] In contrast to the fully structured interview, SCAN retains features of a clinical examination. This construct adds the advantage to identify subjects, who do not have a depressive disorder, but suffer from anxiety or another psychiatric disorder, and thus can be excluded from depression analyses.

The two-step approach used for depression assessment in the Rotterdam Study enabled to identify those who do not fulfill the criteria of DSM-depressive disorders, but have clinically-relevant depressive symptoms, and thus could be analyzed.

This measure of sub-threshold depression was meaningful in addition to depressive disorders, as in older adults, clinically relevant depressive symptoms are not only much

more common than depressive disorders, but they cause impairment similar to that of major depressive disorders.[51] As compared to the point prevalence of depressive disorders of 2% to 3% in the older adults, clinically relevant depressive symptoms, on average, affect approximately 10%-15% community dwelling older adults.[52,53] These clinically-relevant symptoms in older age-groups may stem from two broad categories of risk factors or their interaction, biological and psychosocial.[54] The common biological risk factors include 1) genetic predisposition, 2) vascular diseases[55] and events, such as myocardial infarction or stroke, 3) dementia,[56] 4) chronic diseases such as diabetes and cancer etc.[57] Common psychosocial factors include 1) personality attributes,[58] 2) life events,[59] 3) social-stressors,[60] and 4) bereavement.[61]

Therefore, in this thesis, to study the association of depression and risk of incident dementia, I have used data on these clinically-relevant depressive symptoms, which was also advantageous as symptoms could be used as a continuous score, which facilitated to use them repeatedly to follow the course of depression over time.

Selection

"Selection biases are distortions that occur from procedures used to select subjects and from factors that influence study participation." [62]

Selection bias is a broad term which encompasses several types of biases, but here I will only enumerate those which are relevant for cohort studies, such as bias resulting from *differential loss to follow-up, non-response bias, volunteer bias,* and *healthy-volunteer bias*. However, the common consequence of any type of *selection bias* is that the association between the exposure and outcome is different in participants that were selected, than among those who were eligible, but not selected.[63] For studies in this thesis, the frequently encountered *selection biases* were, bias due to *differential loss to follow-up*, and *healthy-volunteer bias*, which are common in cohort studies requiring active participation. Invariably, participants with more severe form of a disease under study (exposure), were more likely to be lost to follow-up, or, participants who are healthier were more likely to participate than those who were unhealthy. This phenomenon, which is referred to as the "*healthy volunteer effect*" might have resulted in an underestimation of the true associations between risk factors and diseases present in our population. For a cohort of older adults like the Rotterdam Study, this is particularly true as participation can be highly affected by progressing age and frailty. Studies from the Rotterdam Study showed that reasons for non-participation in order of frequency were, loss of interest, physical limitations, or frailty related to age.[64] In this thesis, selection

effect might have affected studies which required a visit to the examination center than those which utilized data from home interviews. For instance, **chapter 4.1** which studied the association of NT-proBNP in relation to incidence dementia, might be influenced by selection, as persons with severe cardiovascular disease were less likely to attend the visit to the examination center. However, when we investigated cognitive decline in this study, participants with substantial cognitive impairment were more likely to drop out from the cognitive reassessment resulting in possible underestimation of associations.

In a more complex instance, possible *selection bias* occurred when using repeated measures of depressive symptoms in **chapters 2.2** and **chapter 2.3.** If trajectories of depressive symptoms are defined by using repeated measures of depressive symptoms, the likelihood to be in a trajectory with remitting depression is higher for those who had at least two assessments. Therefore, when calculating risk of dementia or mortality across trajectories, results could have been influenced by *selection bias*, particularly for the remitting trajectory, as it might be conditioned on health. This was a situation with a very limited options of solutions. In the process of trajectories identification, those with mores assessments (and perhaps healthier) would invariably be more likely to fall in a remitting trajectory (if they had a remitting course), than the ones who only had one assessment. Although the research question, whether risk of dementia and mortality differ with the course of depression was a highly interesting and relevant, it was very challenging to design a valid study. I attempted to solve this concern of *selection bias* by only including participants with at least two assessments to identify trajectories. Although it could not eliminate the *selection bias*, it did condition all trajectories on somewhat similar criteria. With this approach, I obtained trajectories very similar to the ones which included all participants, with slight reclassification. Also, the results were consistent and showed that sustained high and increasing depression was associated with a higher risk of dementia and mortality, but not the trajectories with a remitting course.

Reverse causation

Another bias encountered when we studied psychiatric symptoms and lifestyle factors in relation to risk of dementia was *"reverse causality"*. For instance, when investigating the association of anxiety and risk mortality in **chapter 2.5**, I found very strong associations of anxiety with mortality in the first 3-4 years of follow-up, whereas no associations were found in the long-term (~20 years). Similarly, in **chapter 3.1**, I observed a short-term protective association between higher coffee intake (>3 cups/day) and incident dementia, which disappeared when I tested the association over a long follow-up of 20 years. These findings where strong associations between exposure and outcome were observed in

short-term only, and disappeared in long-term follow-up, are classical examples of *reverse causation*. This meant that what appeared to be a strong causal association, was actually a phenomenon where preclinical symptoms of disease (for example dementia) led to exposure (for example anxiety). To confirm that these results were influenced by *reverse causality*, I repeated the analyses after excluding the first few years of follow-up, which is the most common strategy to address *reverse causality*. Further, I tested several cut-offs for follow up time for these studies, and also tested incrementally increasing years of follow-up to get a thorough understanding of our data.

Regression dilution

Studies included in this thesis have an average follow-up time of 10 years. For some studies with follow-up longer than 10 years in this thesis, for instance **chapters 2.1, 2.4, 3.1**, I did not find an association in the long-term, which could be an effect of *regression dilution*.[8] Therefore, the estimates presented in these studies might be underestimation of the true associations.

Generally, in prospective studies, the risk ratios are estimated using the values of the determinant measured at baseline only. However, such an approach tends to underestimate the real associations. As exposures could change over time, the real associations would have been stronger, if the actual values of exposures were used to estimate the associations under study. The phenomenon of *regression dilution* is a general concern in long follow-up studies, and has been demonstrated with examples of measurements of blood pressure and serum cholesterol.[8] One potential solution to this problem is the use of *regression dilution* ratios for correction of the derived estimates for *regression dilution*. These ratios describe the steepness of the uncorrected (obtained) estimates, to the steepness of the real association. These ratios can be calculated by, reassessing the level of exposure in a small but representative subsample of the study population after an interval which is approximately equivalent to the mid-point of the follow-up period under study. For most studies included in this thesis, I could not calculate *regression dilution* ratios to correct the calculated risk estimates, and therefore, it is limitation of studies with long follow-up. However, **chapters 2.2 and 2.3** in this thesis utilize repeated assessments of the exposure of interest and thus overcome the influence of dilution by time.

Competing risks

In cohort studies, where the outcome of interest is not inevitable, participants may suffer from a *competing risk*, and thus get eliminated from the follow-up.[62] For example, in a

study in which the outcome of interest is dementia, some subjects may die and thus removed from follow-up without getting demented. However, some participants may be lost to follow-up due to other reasons as well. In survival analyses, the usual practice is to treat both the losses to follow-up due to various reasons (true losses), and losses to *competing risk* (e.g. death) as forms of censoring. However, since loss to follow-up, and loss due to *competing risks* can have different relations to the study variables (both exposure and outcome), both forms of censoring can affect the results differently. For studies included in this thesis, dementia was largely the outcome of interest. For such studies, death was the main *competing risk*.

In **chapter 4.1**, in which I studied NT-proBNP and the risk of dementia, a small number of participants were lost to follow-up, which could be because of several reasons which may or may not be related to both NT-proBNP and dementia. However, during follow-up, people were also lost to follow-up because they died (death as a *competing risk* for dementia).

Similarly, in **chapter 2.2**, in which I studied the risk of dementia across different trajectories of depressive symptoms, death was an important *competing risk*. I identified different trajectories of depression in this study, namely, trajectory with no or few depressive symptoms, decreasing depressive symptoms, remitting symptoms, increasing symptoms, and a trajectory with persons who had sustained high depressive symptoms throughout the study period. It is well-documented that chronic depression is associated with a higher risk of mortality.[65,66] Therefore, it was expected that death as a *competing risk* of dementia should have affected the estimates of dementia risk in the high symptoms trajectory most, than any other trajectory of depressive symptoms. In this case, not only *competing risk* was an issue but it was even complex as it could have differentially affected the risk estimates for dementia across trajectories.

To address this, I conducted *competing risk analyses* in these studies, which were presented in the respective chapters. The competing risk analysis, models cumulative incidence of the outcome of interest (e.g. dementia) in the presence of competing risks (e.g. death), i.e. competing events are not censored. In this example, the probability of dementia is not only a function of the hazard of dementia, but a function of mortality as well, because death impedes dementia to occur. This analyses thus provides sub hazard ratios (SHRs) for dementia, which measures the effect of covariates on the cumulative incidence of dementia.

Repeated measures and Latent-class trajectory modelling

This thesis largely included longitudinal studies with a considerably long follow-up. In such studies, the use of repeated measures (where available) was valuable because it could handle the issue of *regression dilution* which arises when we study disease risks in the long-term, but the exposure assessment is only available from the baseline.[8] More importantly, repeated measures are of importance when studying psychiatric of psychological traits and disorders, because such exposures are very likely to fluctuate over time. Therefore, an assessment of such a trait, for example depression, at a single time point is not sufficient to derive conclusions about the risk of a long-term health outcome, or might even result in invalid results.

In this thesis, I investigated the associations of psychiatric disorders with dementia and mortality. Given the remitting and relapsing nature of depression, it was possible that the true long-term associations of depression with dementia and mortality were not captured by a single assessment of depression. Therefore, as a further step, I used repeated measures of depressive symptoms to define the course of depression in **chapters 2.2 and 2.3**. Not only did I define the course of depression, but also identified different trajectories of depressive symptoms in the study population, and investigated the risks of dementia and mortality across these trajectories.

For identification of different trajectories of depression, the Latent-Class trajectory modelling was employed, which is a specialized form of finite mixture modelling.[67] This technique identifies latent classes of individuals following similar progression of an outcome over time or with age. In a simplistic manner, for each subject, a probability to belong to each class (trajectory) was calculated, and the trajectory with the highest probability was assigned to the subject. These trajectory classifications were then used as an indicator variables to calculate hazard ratios for However, I faced methodological issues when calculating trajectories and estimating risks, which I discussed in detail in the respective chapters.

IMPLICATIONS AND DIRECTIONS FOR FUTURE RESEARCH

Although the ultimate goals of medical research are to cure or even prevent the disease, a better understanding of the course of the disease, identification of disease markers, and risk factors that cause or modify the course of disease are the beginning steps to this aim. Therefore, there might not be direct clinical implications of many of the studies in this

thesis, but there are certainly important findings that fill many knowledge gaps in the respective literature and can guide future research.

The first point of importance is that the appearance of depressive symptoms, or, more inclusively, affective symptoms in older age might be a sign of dementia and should be carefully considered. Any sign of the disease in its preclinical period may serve as a window for meaningful interventions. Since CES-D not only gauges depressive symptoms but a general emotional state of individuals, all behavioral change including symptoms of negative affect or lack of positive affect, life style habits, sleep or appetite might indicate underlying cognitive impairment. Although not much can be done to stop the pathologic process at this stage, steps can be taken to slow progression to delay the onset of dementia, or at least to improve the quality of life of patients, whether it is through pharmacological, or non-pharmacological interventions. Future studies should also explore the risks associated with chronic high depression of late-life, as in this thesis, there was an evidence of a high risk of dementia for chronic late-life depression, but the statistical power lacked to derive definitive conclusions. Moreover, etiological research should aim to unravel common etiological pathways between cognitive impairment and depression. In this regard, investigating depression in relation to subclinical vascular lesions such as microbleeds or white matter lesions may provide informative underpinnings for in depth etiologic research .

I did not find any association between anxiety symptoms or anxiety disorders with dementia. It is possible that a single anxiety assessment was not sufficient to find associations, however, it is more likely that there are no health effects independently associated with anxiety. Finally, it would be highly interesting to assess the influence of anti-depressant or anti-anxiety medication on these associations by mediation studies.

The second set of evidence which has more direct clinical implications is that cardiovascular health predicts dementia, and therefore improving and maintaining cardiovascular health should be a priority not only to prevent cardiovascular events such as myocardial infarction, stroke, or transient ischemic attacks, but also to prevent dementia. More importantly, cardiovascular disease is not only an important risk factor vascular dementia, but for Alzheimer's disease as well. I found that NT-proBNP reflects subclinical cardiovascular disease in persons who are free of any overt cardiovascular disease, and predicts dementia including Alzheimer's disease. The importance of this finding is that it shows that subclinical cardiovascular disease leads to neurodegenerative damage early in its course and it can be detected by NT-proBNP. This may provide a wider window of opportunity for prophylaxis and treatment. Early detection of subclinical

cardiovascular disease followed by vigorous intervention including treatment and controlling for modifiable risk factors might be very fruitful to reduce dementia burden by preventing it, or at least delay its onset. Moreover, these results should be taken further, and certain prerequisites such as predictive accuracy of NT-proBNP and cost-effectiveness should be assessed, as it may prove to be a non-invasive and cost-effective way to identify persons at a higher risk for dementia.

The third point of interest is that how lifestyle can affect the risk of dementia. In the realm of modifiable risk factors, education is a factor which can be modified to increase the cognitive reserve and consequently reduce the risk of dementia. The role of education is substantial in gaining cognitive reserve, however, it is only a part of many other factors that build up cognitive reserve. Similarly, cognitive reserve cannot be operationalized by educational level only, and a comprehensive construct to gauge cognitive reserve is lacking . Therefore, future studies should aim to build a model of cognitive reserve which is comprehensive and encompasses several determinants of cognitive reserve, such as education, occupation complexity, leisure and social activity, and other cognitive exercises. Since the concept of cognitive reserve deals with modifiable factors, and is thought to build up throughout life by cognitively active endeavors, it is highly promising for its preventive potential for dementia.

EPILOGUE

This thesis presents longitudinal studies investigating the associations of depression and anxiety with dementia and mortality. From very consistent finding across studies, I suggest the following chronological order of events in the process of cognitive decline: Subclinical neurodegeneration/cognitive impairment →neuropsychiatric symptoms →overt dementia. I also argue that cognitive symptoms are mental health symptoms, and that the interface of psychiatric and cognitive symptoms is not a well-defined one. These symptoms, which are the non-cognitive symptoms of neurodegenerative disease include changes in comportment or behavior, emotion, or personality. Examples of such symptoms include mood fluctuations, restlessness, agitation, apathy, loss of empathy, lack of motivation or initiative, inattention, reduced interest, social withdrawal, or even socially unacceptable behaviors. Neuropsychiatric symptoms appearing in advanced age not only might reflect underlying cognitive impairment, but also indicate that interventions are possible. Other studies in this thesis suggest an important role of cardiovascular disease and lifestyle factors in the etiology of dementia.

Since all these studies were based on considerably long follow-up, this thesis also highlights some common but frequently neglected methodological problems and biases in studies with a longer follow-up, and some potential solutions to these problems. For instance, if short follow-up studies are influenced by reverse causation, studies with long follow-up might be affected by regression dilution. Therefore, as discussed, common approaches to solve common methodological problems in epidemiology should be undertaken to prevent reporting of spurious results.

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CHAPTER 6

SUMMARY/ SAMENVATTING

SUMMARY

Dementia and neuropsychiatric disorders are mental disorders, which frequently co-occur, and have indistinct boundaries. Although they both are very important public health issues, their comorbidity is associated with an even greater public health impact. The association of depression and anxiety with dementia is complex and debated. One hypothesis suggests that psychiatric disorders are risk factors for dementia, while the other suggests that pathological changes in the preclinical phase of dementia, lead to neuropsychiatric symptoms. This might also be true for lifestyle factors which could be influenced when participants perceive a decline in their cognitive abilities, but are yet non-demented clinically. In addition, it is also suggested that both dementia and psychiatric symptoms result from a common pathological process, such as vascular impairment. Therefore, in **Chapter 2**, we studied psychiatric determinants of dementia, cognitive decline and mortality. In **Chapter 2.1**, we investigated the association of depressive symptoms with risk of dementia. Depressive symptoms only predicted dementia in the short-term but not in the long-term. This suggested that depressive symptoms are a response to the underlying cognitive impairment which accumulates over decades before manifesting as clinical dementia. Therefore, they are more likely to be a prodrome of dementia rather than a risk factor *(Analyses: Cox proportional hazards models).* This was further supported by our findings in **Chapter 2.2**. In this chapter we used repeated measures of depressive symptoms to study the course of depression. We related this course to the risk of dementia and found that the trajectory with increasing depressive symptoms was, in particular, associated with a higher risk of dementia. This also suggests that depressive symptoms escalate with the cognitive impairment and are a prodrome of dementia rather than a risk factor. High depression at one time point followed by remission was not associated with a higher risk of dementia *(Analyses: Latent class trajectory modelling, and Cox proportional hazards models with competing risk analyses).* In **Chapter 2.3**, we studied trajectories of depressive symptoms and risk of mortality associated with these trajectories. Chronic high depression was associated with a higher risk of mortality, whereas high symptoms at one time point followed by remission were not associated with a higher risk. The chronicity and course of depression predicted mortality better than depression assessed at one time point *(Analyses: Latent class trajectory modelling, and Cox proportional hazards models)*. In **Chapter 2.4**, we studied the association of anxiety with the risk of dementia. Neither participants with anxiety symptoms, nor those with anxiety disorders at baseline had a higher risk of dementia than those without anxiety. We concluded that anxiety is neither a risk factor, nor a part of prodrome of dementia *(Analyses: Cox proportional hazards models)*. In **Chapter 2.5**, we

studied anxiety symptoms and disorders in relation to risk of mortality. Anxiety symptoms were associated with a higher risk of mortality, but after adjusting for comorbid depression, this association disappeared. We concluded that anxiety is not associated with a higher risk of mortality. The associations observed with anxiety symptoms were largely explained by comorbid depression (*Analyses: Cox proportional hazards models).* As a further step, in **Chapter 2.6**, we studied associations of Mild cognitive impairment (MCI) with depressive and anxiety disorders. Persons with MCI were not only more likely to have prevalent depression and anxiety, but also had a higher relative risk of developing these disorders. We concluded that MCI is not only a forerunner of dementia but also a harbinger of affective disorders suggesting common etiological pathways between the two entities *(Analyses: Multiple logistic regression).*

In **Chapter 3**, we**,** studied lifestyle factors in relation to dementia and cognitive function. In **Chapter 3.1,** we studied the association of coffee consumption and risk of incident dementia over short and long follow-up periods. We found that drinking >3 cups/day was protective of dementia only in the short-term (~4 years), but not in the long-term. We found a similar result when we tested these associations, 4 years later in the same group of people. This phenomenon was a classic example of reverse causation, which means that those who were consuming more coffee were healthier, and had a healthier lifestyle. In older age, coffee consumption, like other lifestyle factors is perhaps a proxy of good health and general well-being *(Analyses: Flexible parametric models for survival analyses)*. To extend these findings, in **Chapter 3.2**, we assessed the associations of coffee consumption with MRI markers of brain volume and brain damage, namely, total brain volume, hippocampal volume, lacunar infarcts, and white matter lesions, and cognitive function. Habitual coffee consumption (≥1 cup/day) was associated with a lower prevalence of lacunar infarcts and a better information processing speed. However, coffee consumption of > 3 cups/day was also associated with a smaller hippocampal volume and worse information processing speed and memory. We concluded that the beneficial effects of coffee might be attributable to its beneficial effect on the cardiovascular system. However, the harmful associations require further investigation in longitudinal designs *(Analyses: Multiple linear, and logistic regression)*. In **Chapter 3.3**, we studied the role of cognitive reserve in protecting against dementia after a stroke or TIA. Education was used as a measure of cognitive reserve, and was stratified in three categories, low, intermediate, and high. Risk of dementia after a stroke or TIA was higher in persons with low and intermediate education groups but not in the high group. This effect was more pronounced in men. We concluded that cognitive reserve might protect against dementia

after clinical cerebrovascular events *(Analyses: Cox proportional hazards models with stroke or TIA as time-varying exposure).*

In **Chapter 4**, we assessed serum biomarkers in relation to incident dementia and depression. In **Chapter 4.1,** the association of NT-proBNP (a cardiovascular disease marker) with dementia and cognitive decline was assessed, both including and excluding persons with overt cardiovascular disease. Higher NT-proBNP was associated with a higher risk of dementia, including Alzheimer' disease, and was associated with a decline in processing speed and executive function, even in the absence of cardiovascular risk factors or disease. We concluded that NT-proBNP reflecting subclinical cardiovascular disease, predicts dementia, and can be a useful marker of imminent cognitive decline and dementia in absence of clinical cardiovascular disease *(Analyses: Cox proportional hazards models with competing risk analyses, and multiple linear regression).* In **Chapter 4.2** we examined the association of cardiovascular, renal, and metabolic biomarkers with incident depression. NT-proBNP was associated with major depression and depressive symptoms both, whereas CRP predicted incident depressive symptoms only. Additionally, we used Principle Component Analyses to combine correlating biomarkers in groups: cardiovascular, renal, and metabolic. We found that the cardiovascular component (NT-proBNP and cystatin C) and metabolic component (CRP, glucose, and HDL cholesterol) were associated with a higher risk of depressive symptoms. These findings suggested a role of cardiovascular, inflammatory, and metabolic factors in the etiology of depression in older adults *(Analyses: Principle Component Analyses, Cox proportional hazards models, and Generalized Estimation equations).*

SAMENVATTING

Dementie en neuropsychiatrische stoornissen zijn mentale stoornissen die vaak samen voorkomen, hun scheidslijn is onduidelijk. Beiden zijn het erg belangrijke problemen van de volksgezondheid en hun comorbiditeiten zijn met nog grotere volksgezondheidsproblemen geassocieerd. Hoe depressie en angst tot dementie gerelateerd zijn is onduidelijk en of er zo'n associatie is valt te betwisten. Er is een hypothese die suggereert dat psychiatrische stoornissen risicofactoren zijn voor dementie en een andere suggereert dat de pathologische veranderingen in de preklinische fase van dementie leiden tot neuropsychiatrische symptomen. Dit zou ook het geval kunnen zijn voor leefstijlfactoren die beïnvloed kunnen worden als deelnemers een afname van cognitieve functie merken, maar nog niet dement zijn. Daarnaast kan het zo zijn dat dementie en psychiatrische symptomen beiden komen door een gedeeld pathologisch proces, bijvoorbeeld vasculaire schade. Om hier meer inzicht in te krijgen, bestudeerden we in **Hoofdstuk 2** de psychiatrische determinanten van dementie, cognitieve achteruitgang en mortaliteit. In **Hoofdstuk 2.1** onderzochten we de relatie tussen depressieve symptomen en het risico op dementie. Depressieve symptomen voorspelden dementie alleen op de korte termijn, niet op de lange termijn. Dit suggereert dat depressieve symptomen een reactie zijn op een onderliggende cognitieve achteruitgang. Deze achteruitgang is een proces van jaren voordat het zichtbaar wordt als dementie. Depressieve symptomen zijn dan ook eerder een voorstadium dan een risicofactor voor dementie (*Analyses: Cox proportional hazards modellen*). Dit werd verder ondersteund door onze bevindingen in **Hoofdstuk 2.2.** In dit hoofdstuk gebruikten we herhaalde metingen van depressieve symptomen om het beloop van depressie te bestuderen. Dit beloop relateerden we tot het risico op dementie en we vonden dat voornamelijk een toename van depressieve symptomen gerelateerd was aan een hoger risico op dementie. Dit suggereert ook dat depressieve symptomen gelijk opgaan met de cognitieve achteruitgang en meer een voorstadium zijn van dementie dan een risicofactor. Veel depressieve symptomen op één tijdspunt gevolgd door een remissie was niet geassocieerd met een hoger risico op dementie (*Analyses: Latent class trajectory modelling en Cox proportional hazards modellen met competing risk analyses*). In **Hoofdstuk 2.3** bestudeerden we het beloop van depressieve symptomen en het risico op overlijden geassocieerd met dit beloop. Chronisch veel depressie was geassocieerd met een verhoogd risico op overlijden, terwijl het hebben van veel symptomen op één tijdspunt gevolgd door een remissie niet geassocieerd was met een verhoogd risico. De duur en het beloop van depressie voorspelden het risico op overlijden dus beter dan depressie gemeten op één tijdspunt (*Analyses: Latent class trajectory modelling en Cox proportional*

hazards modellen). In **Hoofdstuk 2.4** onderzochten we hoe angst gerelateerd is aan het risico op dementie. Zowel deelnemers met angstsymptomen als deelnemers met angststoornissen aan het begin van de studie hadden geen hoger risico op dementie vergeleken met deelnemers zonder angst. We concludeerden dat angst geen risicofactor en geen voorstadium van dementie is (*Analyses: Cox proportional hazards modellen*). In **Hoofdstuk 2.5** onderzochten we hoe angstsymptomen en angststoornissen gerelateerd zijn aan het risico op overlijden. Angstsymptomen waren geassocieerd met een hoger risico op overlijden, maar deze associatie verdween na het corrigeren voor depressie. We concludeerden dat angst niet gerelateerd is aan een hoger risico op overlijden. De associaties die we met angstsymptomen vonden werden grotendeels verklaard door het gemeenschappelijk optreden van depressie (*Analyses: Cox proportional hazards modellen*). Als volgende stap bestudeerden we in **Hoofdstuk 2.6** hoe een milde cognitieve achteruitgang (Mild cognitive impairment, MCI) geassocieerd is met depressieve stoornissen en angststoornissen. Deelnemers met MCI hadden niet alleen vaker prevalent depressie en angst, maar hadden ook een groter risico om deze stoornissen te ontwikkelen. We concludeerden dat MCI niet alleen een voorstadium is van dementie, maar ook van angst- en gemoedsstoornissen. Dit suggereert dat beide processen een gezamenlijke ontstaanswijze hebben (*Analyses: Multiple logistic regression*).

Hoofdstuk 3 richt zich op leefstijlfactoren in relatie tot dementie en cognitieve functie. In **Hoofdstuk 3.1** bestuurden we de associatie tussen koffieconsumptie en het risico op dementie gedurende korte en lange follow-up periodes. We vonden dat het drinken van >3 koppen koffie per dag beschermend was tegen dementie op de korte termijn (ongeveer 4 jaar), maar niet op de lange termijn. We vonden hetzelfde resultaat toen we deze associaties op een tijdspunt vier jaar later testten in dezelfde groep mensen. Dit fenomeen is een klassiek voorbeeld van een omgekeerde causaliteit (reversed causality). Dit betekent dat de mensen die meer koffie dronken gezonder waren en een gezondere levensstijl hadden. Op oudere leeftijd is koffieconsumptie, net als andere leefstijlfactoren, mogelijk een weerspiegeling van een goede fysieke en mentale gezondheid (*Analyses: Flexible parametric models voor survival analyses*). Om hierop door te gaan, onderzochten we in **Hoofdstuk 3.2** de associaties tussen koffieconsumptie en MRI markers van hersenvolume en hersenschade, namelijk totaal hersenvolume, hippocampusvolume, lacunaire infarcten en wittestofafwijkingen, en cognitieve functie. Het drinken van koffie (≥1 kop per dag) was geassocieerd met een lagere prevalentie van lacunaire infarcten en een betere informatieverwerkingssnelheid. Een consumptie van >3 koppen per dag was echter ook geassocieerd met een kleinere hippocampusvolume en een slechtere informatieverwerkingssnelheid en geheugen. We concludeerden dat de gunstige werking

van koffie zou kunnen komen door het gunstige effect op het cardiovasculaire systeem. Voor de schadelijke associaties is verder onderzoek nodig in de vorm van een longitudinale studieopzet (*Analyses: Multiple linear and logistic regression*). In **Hoofdstuk 3.3** bestudeerden we de rol van een cognitieve reserve in het beschermen tegen dementie na een beroerte of TIA. Onderwijsniveau was gebruikt als maat voor deze cognitieve reserve en was gestratificeerd in drie categorieën, namelijk laag, gemiddeld en hoog. Het risico op dementie na een beroerte of TIA was verhoogd in de mensen met een laag of gemiddeld opleidingsniveau, maar niet in de mensen met een hoog opleidingsniveau. Dit vonden we voornamelijk voor mannen. We concludeerden dat een cognitieve reserve mogelijk beschermt tegen dementie na een beroerte of TIA. (*Analyses: Cox proportional hazard modellen met beroerte of TIA als time-varying exposure*).

Hoofdstuk 4 beschrijft serum biomarkers in relatie tot het ontstaan van dementie en depressie. In **Hoofdstuk 4.1** onderzochten we de associatie tussen NT-proBNP (een marker voor cardiovasculaire ziekten) en dementie en cognitieve achteruitgang, zowel inclusief als exclusief de mensen met klinische cardiovasculaire ziekten. Een hoger NT-proBNP was geassocieerd met een verhoogd risico op dementie, inclusief de ziekte van Alzheimer, en was geassocieerd met een afname in verwerkingssnelheid en uitvoerende functie, zelfs in de afwezigheid van cardiovasculaire risicofactoren of cardiovasculaire ziekten. We concludeerden dat NT-proBNP, als marker voor subklinische cardiovasculaire ziekte, leidt tot een hoger risico op dementie. Het zou dan ook een waardevolle marker kunnen zijn voor een dreigende cognitieve achteruitgang en dementie, in de afwezigheid van klinische cardiovasculaire ziekten (*Analyses: Cox proportional hazard models met competing risk analyses, multiple linear regression*). In **Hoofdstuk 4.2** onderzochten we hoe verschillende cardiovasculaire, renale en metabole biomarkers samenhangen met het risico op depressie. Een hoger NT-proBNP was gerelateerd aan een hoger risico op zowel depressie als depressieve symptomen, terwijl een hoger CRP alleen gerelateerd was aan incidente depressieve symptomen. Daarnaast gebruikten we een 'Principle Component Analysis' om de markers in groepen in te delen, namelijk cardiovasculair, renaal en metabool. We vonden dat zowel het cardiovasculaire component (NT-proBNP en cystatine C) als het metabole component (CRP, glucose en HDL cholesterol) geassocieerd waren met een verhoogd risico op depressieve symptomen. Deze bevindingen suggereren een rol van cardiovasculaire en metabole factoren in het ontstaan van depressie in ouderen (*Analyses: Principle Component Analyses, Cox proportional hazards modellen, en Generalized Estimation equations*).

CHAPTER 7

Acknowledgements/Word of Thanks
PhD Portfolio
Complete List Of Publications
Curriculum Vitae

WORD OF THANKS

Alhamdulillah after almost 4 years of effort, this journey ends successfully into a brand new beginning, and this journey could not have been possible without the support of several inspirational colleagues and friends around, and especially the love and understanding of my family who despite being thousands of miles away, kept on supporting me with their love.

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Fabi Ayye Aalaa -e- Rabbi Kuma tukazziban.

Translation: So which of the favors of your Lord would you deny?

Saira, Rotterdam 2016.

PHD PORTFOLIO

Name	Saira Saeed Mirza
Erasmus Medical Center Department	Epidemiology
PhD period	2012-2016
Research School	NIHES
Promotor	Prof.dr. H. Tiemeier
Co-promotor	Dr. M. A. Ikram

Training	Year	ECTs
Courses and workshops		
Master of Science, Genetic Epidemiology, NIHES	2012-2014	70
SNPs and Human Diseases	2013	2.0
Linux for Scientists	2013	0.6
Course for the Quantitative researcher	2014	1.4
An Introduction to the Analysis of Next-generation Sequencing Data	2014	1.4
Physical Activity and Nutrition, Cambridge University, Cambridge, United Kingdom	2013	1.4
Integrity in Scientific Research, Erasmus MC	2014	2.0
Meetings and Conferences		
MELODEM research meeting, Paris, France	2013	0.5
CHARGE meeting, Rotterdam, the Netherlands	2013	0.5
Final Congress, NCHA, The Hague, the Netherlands	2013	0.5
Meeting, Institute for Scientific Information on Coffee, Paris, France	2013	0.5
6th Congress of The International Society for Vascular Behavioral and Cognitive disorders, Toronto, Canada (poster presentation)	2013	1.0
Alzheimer's Disease International Conference, Copenhagen, Denmark (two oral presentations)	2014	1.0
Alzheimer's Disease International Conference, Washington DC, United States (poster presentation)	2015	1.0

Teaching and Supervision		
Teaching assistant, The Practice of Epidemiological Analysis, NIHES	2013	1.0
Supervisor undergraduate medical students, Joanne Polak, Jolien van den Worm, Timothy Chin See Chong, Karlien Veldscholte. GTF2I and positive emotionality in preschool children. A candidate gene study. Department of Psychiatry, Erasmus MC	2013	2.0
Supervisor undergraduate medical students, Sjoerd Driessen, Tomas van der Velde, Redwan Azouagh. Does depression, anxiety and schizophrenia have a common genetic basis? Department of Psychiatry, Erasmus MC	2014	2.0
Lecturer and workshop facilitator undergraduate medical students, Research Methodology and Statistics. Department of Psychiatry, Erasmus MC	2015	1.0
Supervisor MSc student, Mayara Auler. Cardiovascular, metabolic and renal biomarkers and their association with depression: a longitudinal population-based study of older adults.	2014-2015	2.0
Other		
Peer review for scientific journals	2013-2015	2.0

COMPLETE LIST OF PUBLICATIONS

1. Otowa T, Hek K , Lee M, Byrne E, **Mirza SS**, Michel Nivard,. Bigdeli T, Aggen S, Adkins D, Wolen A, Fanous A, Keller M, Castelao E, Kutalik Z, Van der Auwera S, Homuth G, Nauck M, Teumer A , Hottenga JJ, Direk N, Hofman A , Uitterlinden A, MulderC , Henders A, Medland S , Gordon S, Heath A , Madden P, Pergadia M, van der Most P, Nolte I, van Oort F, Hartman C, Oldehinkel A Preisig M , Grabe HJ, Middeldorp C, Dr. Penninx BWJH, Boomsma D, Martin N, Montgomery G, Maher B, van den Oord E, Wray N, Tiemeier H, Hettema J. Meta-analysis of genome-wide association studies of anxiety disorders. Molecular Psychiatry 2015.
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MK, Kubo M, Kuusisto J, Lahti J, Launer LJ, Lea RA, Lehne B, Lehtimaki T, Liewald DC, Lind L, Loh M, Lokki ML, London SJ, Loomis SJ, Loukola A, Lu Y, Lumley T, Lundqvist A, Mannisto S, Marques-Vidal P, Masciullo C, Matchan A, Mathias RA, Matsuda K, Meigs JB, Meisinger C, Meitinger T, Menni C, Mentch FD, Mihailov E, Milani L, Montasser ME, Montgomery GW, Morrison A, Myers RH, Nadukuru R, Navarro P, Nelis M, Nieminen MS, Nolte IM, O'Connor GT, Ogunniyi A, Padmanabhan S, Palmas WR, Pankow JS, Patarcic I, Pavani F, Peyser PA, Pietilainen K, Poulter N, Prokopenko I, Ralhan S, Redmond P, Rich SS, Rissanen H, Robino A, Rose LM, Rose R, Sala C, Salako B, Salomaa V, Sarin AP, Saxena R, Schmidt H, Scott LJ, Scott WR, Sennblad B, Seshadri S, Sever P, Shrestha S, Smith BH, Smith JA, Soranzo N, Sotoodehnia N, Southam L, Stanton AV, Stathopoulou MG, Strauch K, Strawbridge RJ, Suderman MJ, Tandon N, Tang ST, Taylor KD, Tayo BO, Toglhofer AM, Tomaszewski M, Tsernikova N, Tuomilehto J, Uitterlinden AG, Vaidya D, van H, V, van SJ, Vasankari T, Vedantam S, Vlachopoulou E, Vozzi D, Vuoksimaa E, Waldenberger M, Ware EB, Wentworth-Shields W, Whitfield JB, Wild S, Willemsen G, Yajnik CS, Yao J, Zaza G, Zhu X. Directional dominance on stature and cognition in diverse human populations. Nature 2015;523:459-62.

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CURRICULUM VITAE

Saira Saeed Mirza was born on March 13th, 1980 in Karachi Pakistan. After matriculation from Seven Oaks High School, Karachi (1995), Saira completed her high school in pre-medical major from St. Joseph's Convent College, Karachi (1997). Saira started her medical school in 1998 at the Dow Medical College, University of Karachi, and graduated as a medical doctor in 2005. After completing her internships in Medicine and Surgery form Civil Hospital Karachi, Saira was invited to teach Physiology to undergraduate Medical course temporarily at the Dow University of Health Sciences in 2007. In the same year, she started a postgraduate residency training in Internal Medicine at the Jinnah Postgraduate Medical Center Karachi as a fellow of College of Physicians and Surgeons Pakistan. Due to several reasons, Saira chose teaching and research, and quit residency, and got enrolled in an MPhil programme in Physiology at the Dow University of Health Sciences, where she continued serving as a lecturer and a research associate. In 2011, she defended her MPhil thesis and continued working as a Physiology lecturer to undergraduate medical students. In April 2012, Saira started her PhD at the department of Epidemiology at the Erasmus MC, Rotterdam, under the supervision of Prof. Henning Tiemeier and Dr. Arfan Ikram. After her PhD, Saira plans to pursue her research career and continue with a post-doctoral training in the field of Epidemiology.

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