Unravelling the Link Between Cancer, Cognition, and Dementia

Causes, biomarkers, and methods

Kimberly Dieudonnee van der Willik

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Unravelling the Link Between Cancer, Cognition, and Dementia

Causes, biomarkers, and methods

Het ontrafelen van de link tussen kanker, cognitie en dementie

Oorzaken, biomarkers en methoden

Proefschrift

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Voor mijn pappa in de hemel
en mijn mamma hier op aarde

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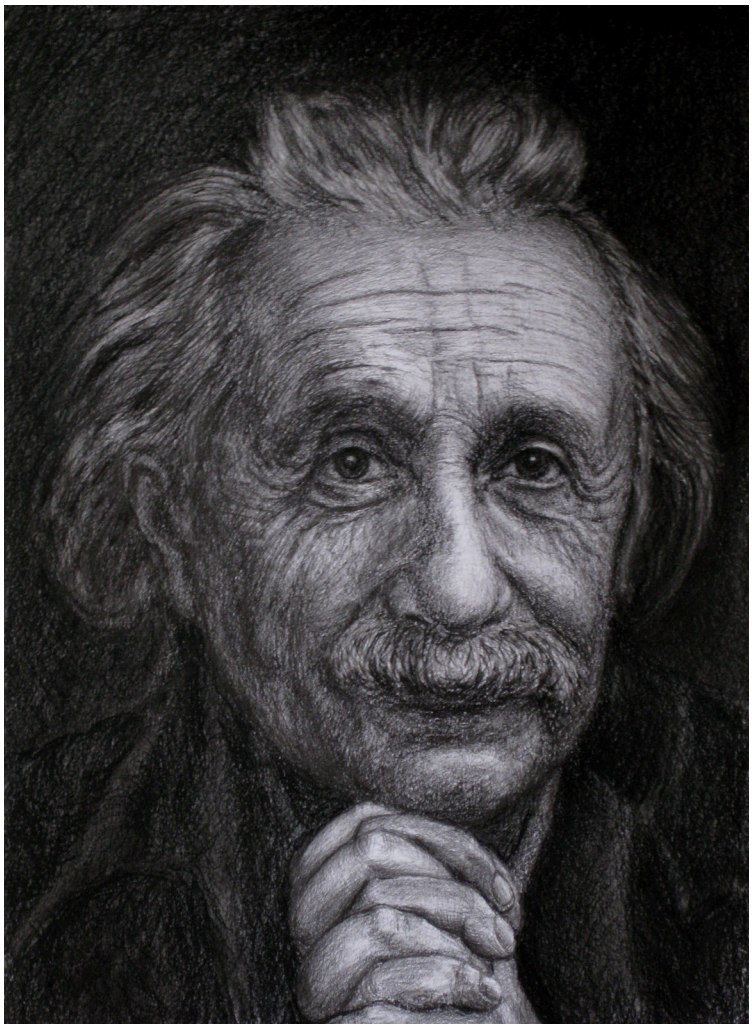
* Both authors contributed equally to this study

** Both last authors contributed equally to this study

Prologue



Ten years ago, I started my medical training with the intention of becoming a neurologist. I was intrigued by the mysterious function of the brain. Sometimes however, our actual life paths differ from our expectations. During the second year of my medical training, my dad was diagnosed with pancreatic cancer. Cancer. Metastatic. Incurable. Hearing that diagnosis changed my life path. I was determined to become a medical oncologist.

This thesis combines my interest in neurology, my passion for oncology, and my love for science. During my dad his illness, his cognition was influenced by the cancer, the chemotherapy, the pain killers, and all psychological factors that accompanied his cancer diagnosis and disease process. During this period, he painted the illustration that I used as the cover of this thesis. This painting is based on a photograph of me horse riding on the beach of Texel. The first horse rider is the leader of the group that is shown at the end of the original painting. I am the second horse rider, the one who is not completely painted yet. I am just a sketch. He did not have enough time left to finish the painting.

Throughout this thesis you will find more of his illustrations. Although the illustrations do not complement the text of this thesis, sometimes – just like in research – you have to work with what you have.

This thesis is the result of many people, including my dad.

Chapter 1

General introduction

Oncology – the study 'logos' of the tumour 'ónkos'

'A bulging tumour of the breast. Treatment: none.'

These words mark the first description of cancer that originates from Ancient Egypt 3000 years Before Christ. For many years, prominent historical physicians including Hippocrates – who introduced the term 'karkinos' (Latin: cancer) based on the shape of a crab – and Galenus – who proposed the term 'ónkos' (Latin: onco) – have been intrigued by tumours.[1] Also famous artists such as Rubens and Rembrandt were probably fascinated by this disease, because they spent many hours illustrating the appearance of tumours on their canvas.[2,3] Despite many efforts, it took almost five thousand years after this first known description before the first effective cancer-specific treatment – apart from mutilating surgery – was discovered: radiotherapy.[4] Since this discovery, the options of cancer treatment have further been expanded with chemotherapy,[5] hormonal therapy,[6,7] and more recently with immunotherapy.[8] Improvements in cancer treatments are necessary, because the number of cancer patients is growing considerably due to ageing populations worldwide.

In 2017, 16.8 million persons worldwide were diagnosed with cancer and 9.6 million persons died of cancer, making cancer the second leading cause of death.[9] Advances in screening methods and improvements in treatments have ensured longer survival of cancer patients, which in turn has led to higher rates of long-term and late side effects, both of cancer itself as well as of the aggressive treatments.[10] Such side effects include fatigue, infertility, secondary tumours, and cardiovascular diseases. In addition, cognitive problems are amongst the most frequently reported complaints by cancer patients and survivors that can negatively impact their quality of life and daily life functioning.[11-13]

Neurology – the study 'logos' of the nerve 'neûron'

Many persons have been intrigued by the complexity of the brain and its relation with behaviour, including Hippocrates, who referred to the brain as 'the organ of the intellect', the centre of all mental functions.[14] Diseases of the brain and spinal cord, i.e., the central nervous system (CNS), can manifest in different ways, such as epilepsy, multiple sclerosis, stroke, Parkinson's disease, and cognitive problems. Cognitive problems refer to disruptions in mental functions, including memory, learning, attention, concentration, executive functioning, and information processing speed.

Cognitive function declines gradually during brain ageing. Accelerated decline in cognitive function can result in cognitive impairment and may reflect the preclinical phase of neurodegenerative diseases, including dementia. The increase in life expectancy has not

only resulted in a higher number of cancer patients, but has also led to a growing number of patients with dementia. At present, around 50 million persons worldwide are living with a dementia diagnosis, and almost 10 million persons are diagnosed with dementia every year.[15]

Although many studies have shown that cancer patients often have impaired cognitive function, their trajectory of change in cognitive function and their risk of dementia remain poorly understood. It has been proposed that cognitive function declines shortly after diagnosis and treatment and then parallels the trajectory of cognitive function in persons without a history of cancer (phase shift hypothesis) or that decline in cognitive function is accelerated in comparison to cognitive function in persons without a history of cancer (accelerated ageing hypothesis). Longitudinal, population-based studies are needed to explore these hypotheses.[16]

Epidemiology – the study 'logos' of what is upon 'epi' the people 'demos'

Although modern epidemiology has been established from the 1980s onwards, Hippocrates already contributed to the foundation of epidemiology by studying the frequency of diseases and the causes of variation in this frequency. He focused however on the individual patient, rather than studying a group of patients. This touches upon an important epidemiological principle, i.e., group thinking.[17] Group thinking is a mode of conceptualising issues for a whole group of persons, i.e., the population. At a population-level, we can compare groups by contrasting what is observed in the group in presence of the exposure to what is occurred in the group that has not been exposed.

The aim of this thesis is to understand the origin and course of cognitive decline in cancer patients and survivors, their risk of dementia, and the mechanisms underlying these cognitive problems and dementia. The focus will be on comparing the cognitive function in cancer patients and survivors (exposed persons) to that in persons without a history of cancer (unexposed persons) at a population-level. In order to do so, the work presented in this thesis studies participants from the Rotterdam Study, a prospective population-based cohort study that was established in 1989 to study the occurrence and determinants of common diseases in the elderly. This thesis is divided into five Parts, which I will introduce in more detail.

Part I – Cancer registration

Before investigating cognitive function in cancer patients, it needs to be determined whether data on cancer events in the Rotterdam Study is complete and accurate. Lack of perfection in data collection may lead to an incorrect estimate of the true effect.[18] The Rotterdam Study collects data on cancer events using medical records of general practitioners and through linkage with the national hospital discharge registry and histology and cytopathology registries. To determine the completeness and accuracy of cancer registration in the Rotterdam Study,

Chapter 2 compares the registered cancer events in the Rotterdam Study to those in the Netherlands Cancer Registry. The Netherlands Cancer Registry is the oncological hospital registry in the Netherlands that collects data about all cancer patients. Cancer events in the Rotterdam Study were updated according to the results of this comparison in order to achieve accurate and complete cancer registration and to minimise measurement error. **Chapter 3** subsequently focuses on a specific group of cancers that are often missed by cancer registries: cancers that are not confirmed by pathology. Apart from pathological confirmation, patients with non-pathology-confirmed cancers have undergone the same extensive diagnostic work-up as patients with pathology-confirmed cancers. To estimate whether missing data on these cancer events may influence cancer statistics and may bias aetiological studies, this Chapter determines the characteristics and survival of patients with non-pathology-confirmed cancers.

Part II – Cancer and cognition

We have known for many years that patients with CNS cancer can develop cognitive problems.[19] These cognitive problems can be caused by local damage due to the tumour itself or by the harmful effects of cancer treatment on healthy brain tissue. In the early nineties, several neuropsychologists and oncologists noticed that also patients with cancer outside the brain – non-CNS cancer – experienced cognitive problems. Since then, the number of scientific publications on cognitive function in cancer patients has increased substantially (**Figure 1**). These studies have shown that about 20% to 30% of all non-CNS cancer patients have cognitive problems, with a subgroup of non-CNS cancer survivors having long-term cognitive problems that can last up to more than twenty years after cessation of treatment.[16,20]

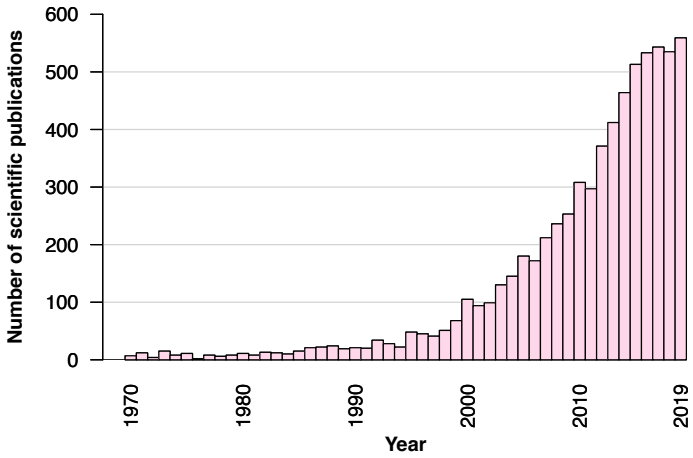


Figure 1 Number of scientific publications per year on cognition and cancer.
Numbers are obtained from the PubMed library using the search terms "cancer" and ("cognition" or "chemobrain") not ("brain tum" or "glioma" or "mening*" or "brain met*").*

Research has primarily been directed to chemotherapy as the driving force behind disturbances in the normal function of the brain, dubbed by some cancer survivors as 'chemobrain'. Different mechanisms for chemotherapy-induced cognitive problems have been suggested and revealed, including toxicity to neural progenitor cells, DNA damage in post-mitotic neurons and telomere shortening, deregulation of cytokines, and hormonal changes.[21,22] However, studies that have examined the consequences of chemotherapy on brain function were often cross-sectional and could therefore not provide information about the baseline cognitive function in cancer patients.[23]

More recent longitudinal studies have incorporated baseline assessments of cognitive function after surgery and before initiation of systemic adjuvant treatment. These studies have revealed that chemotherapy may not be the only cause of cognitive problems, because some patients had lower than expected cognitive function before they received chemotherapy.[24-27] In addition, imaging studies have shown that before patients received chemotherapy, some had altered brain structure and function, including lower white matter integrity and hyperactivation of different brain regions, in particular the frontal and parietal lobes.[28-32] Hyperactivation is often seen as a compensatory mechanism to maintain adequate levels of test performance during inadequate functioning of the brain.[33] Changes in brain functions were not fully explained by anxiety, depression, or fatigue. However, the time of study entry may not be appropriate, as the impact of anaesthesia and side effects of surgery could also induce changes in cognitive function.

Less is known about cognitive function in cancer patients prior to surgery. Thus far, seven studies have assessed cognitive function in newly diagnosed cancer patients.[34-40] Interestingly, these patients also showed worse neuropsychological test performance than cancer-free controls and had alterations on brain magnetic resonance imaging (MRI) scans. Preclinical support for this observation comes from studies that have shown that immunodeficient mice engrafted with patient tumour tissue had molecular changes in the brain similar to those seen in neurodegeneration and brain ageing.[41,42] This suggests that cancer itself may induce changes in the normal function of the brain, although clinical studies cannot fully exclude psychological factors that accompany a new cancer diagnosis.

Besides the role of cancer itself, cognitive problems in newly diagnosed cancer patients could also be explained by a shared pathology. For instance, genetic susceptibility, inflammation, and oxidative stress are processes related to cancer and cognitive decline.[43,44] Furthermore, shared risk factors such as ageing, smoking, lack of physical activity, and a poor diet, could also play a role in the development of both conditions. The potential different causes of cognitive problems in cancer patients are summarised in **Figure 2**.

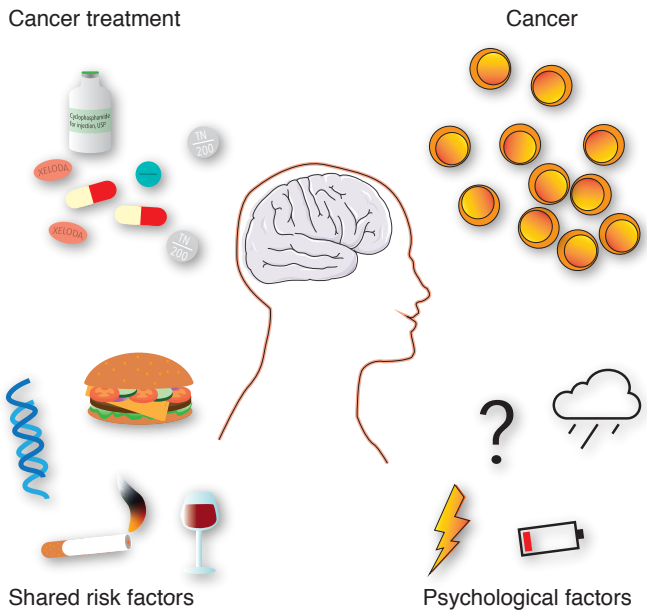


Figure 2 Overview of the potential causes of cognitive problems in cancer patients.

In this Part, **Chapter 4** first describes the change of cognitive function in the general population between the ages of 45 and 90 years. Understanding the natural course of cognitive function during ageing is necessary to identify persons who deviate from the mean trajectory of decline. This standard could therefore be used to contrast the trajectory of cognitive function in patients with cancer. **Chapter 5** subsequently delineates the change in cognitive function in cancer patients before they are diagnosed with cancer. In the Rotterdam Study, participants are invited to visit the research centre every three to six years to undergo several examinations including cognitive function assessments. Some of these participants will eventually be diagnosed with cancer. This enables us to investigate their cognitive function before the clinical manifestation of cancer, thereby excluding the psychological effects of a new cancer diagnosis. The underlying hypothesis is that if the cancer itself can affect cognitive function, cognitive function would already be altered before the diagnosis of cancer, because the time between the first cancer cell and clinical manifestation of the disease ranges between five and forty years for solid tumours (**Figure 3**).[45] Also based on this hypothesis, **Chapter 6** investigates the brain structure of patients before their cancer diagnosis using brain MRI. Lastly, **Chapter 7** investigates the change of cognitive function from before cancer diagnosis to late-life after cancer.

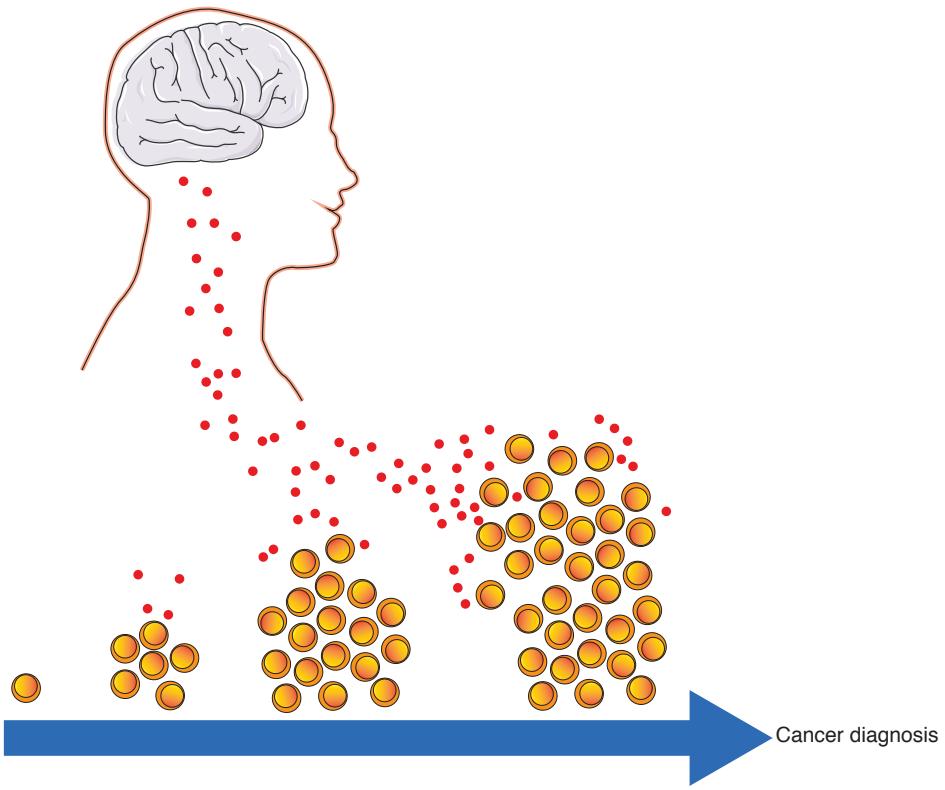


Figure 3 Preclinical phase of cancer.
Cancer cells can be present in the body years before the cancer is diagnosed (i.e., latency period). During this period, cancer cells can produce different factors that may affect the brain. Therefore, cognitive function and brain structure might already be affected before a person is diagnosed with cancer.

Part III – Cancer and dementia

Since a shared pathology between cognitive problems and cancer has been hypothesised and given that dementia is preceded by cognitive decline, a logical question emerges whether cancer and cancer treatment are also associated with a higher risk of dementia. Cancer and dementia share different biological processes, including inflammation, oxidative stress, DNA damage, and angiogenesis which may support a higher risk of dementia in cancer patients and survivors.[46] In contrast to these expectations, a substantial body of literature suggests an inverse link between cancer and dementia, i.e., in comparison with healthy persons, cancer patients have a lower risk of dementia, and patients with dementia have a lower risk of subsequently being diagnosed with cancer.[46-62]

The first study on the link between cancer and dementia originates from 1990, in which Yamada et al. investigated risk factors for dementia in atomic-bomb survivors.[60] They have

observed that the odds of having cancer prior to Alzheimer's disease (AD), the most common type of dementia, was 70% lower in patients with AD than in persons without AD. More than a decade later, longitudinal studies have confirmed that cancer patients had a lower risk of developing dementia than persons without a history of cancer. These studies have also shown that patients with dementia were less likely to be diagnosed with cancer than persons without dementia. These findings suggest an inverse association between cancer and dementia in both directions. This inverse association was observed for most cancer types, including non-melanoma skin cancer, and was consistent across different studies. An overview of the individual studies investigating this association is provided in **Table 1** at the end of this Chapter.

In addition to the role of cancer itself, few retrospective studies have evaluated the effect of chemotherapy on dementia in breast cancer survivors.[63-66] All these studies have used data from the linked Surveillance, Epidemiology, and End Results (SEER)-Medicare database. For this reason, the outcomes cannot be interpreted as independent. Nevertheless, these studies have demonstrated contrasting results with only one study showing a higher incidence of dementia in patients treated with chemotherapy than in patients without chemotherapy treatment.[65] Comparison of the risk of dementia in cancer survivors after chemotherapy with the dementia risk in cancer-free controls showed again an inverse association.[64]

Multiple biological mechanisms have been proposed supporting this inverse association between cancer and dementia in both directions. Promotion of genetic pathways involved in cell proliferation and survival could result in an increased cancer risk, while dementia is associated with increased cell death. For instance, the expression of the tumour suppressor protein p53 is often decreased in cancer, whilst elevated in AD brains.[67] Furthermore, the enzyme pin1 is involved in protein folding and cell cycle regulation, and is often overexpressed in tumours whereas it is depleted in AD. Other candidate processes are opposite disturbances of the epigenome and ultraviolet radiation exposure.[58,68]

Despite consistent results and suggested biological mechanisms, several methodological issues potentially driving this inverse association have not completely been ruled out. Therefore, careful interpretation and critical evaluation of the observed link is needed. Cancer and dementia are accompanied by multiple symptoms, which can mask symptoms of other, yet undiagnosed diseases. Additionally, physicians could be less willing to refer diseased patients, resulting in surveillance bias. Also, studying diseases in the older population may be subject to survival bias. These two types of biases are discussed in more detail in **Box 1** and **Box 2**.

Box 1 Surveillance bias.

Surveillance bias arises when patients with a certain disease undergo more or less intensive disease screening, resulting in a respectively higher or lower probability to be diagnosed with the studied outcome.[69] Patients with cancer or dementia may be less likely to be screened and diagnosed with other diseases. Several observations support this conception.

Firstly, patients with dementia are not always able to communicate symptoms such as pain.[70] This is supported by the finding that cancer in dementia patients is often diagnosed in a more advanced stage than in persons without dementia, since pain is an important symptom of a variety of cancers.[71] In turn, symptoms of comorbid diseases in cancer patients may be attributed to cancer, leaving the other underlying disease unrecognised.[72] Secondly, when a patient has a serious illness with a limited life expectancy, physicians may be less prepared to start a diagnostic work-up for new symptoms. In the case of dementia, patients undergo less often screening for cancer.[73,74] Also, it can be difficult for these patients to understand the risks and benefits of screening and the benefits may not outweigh the harms such as overdiagnosis and overtreatment.[75] A study under elderly care physicians in nursing homes has shown that end stage dementia was the primary reason not to refer patients with suspected breast cancer.[76] In cancer patients, cognitive problems remain often unrecognised, because cognitive assessment is not standard practice.[77] Thirdly, when a dementia patient is suspected to have cancer, pathological confirmation through biopsies is often omitted since it does not have therapeutic consequences.[78] Several studies have demonstrated that patients with dementia and cancer often do not receive cancer treatment.[71] Since many cancer registries only register pathology-confirmed tumours, these tumours will remain unnoticed.[79]

Box 2 Survival bias.

Survival bias is considered as a special case of selection bias and may occur when the studied exposure is associated with survival.[80] When the exposure negatively influences survival, those exposed persons who will survive are likely to have some other, protective characteristics helping them to survive. This results in a lower frequency of the exposure among the survivors, which can be observed as an inverse association between the exposure and outcome. **Figure 4** shows an illustration of non-medical survival bias.[81]

Both cancer and dementia are potentially fatal diseases and can affect survival. Survival rates for patients with cancer differ per cancer type and depend on the stage at diagnosis.[82] For dementia, the median overall survival depends on the age of the patient and ranges between 6.0 years for persons aged below 75 years, and 3.5 years for those aged over 85 years.[83] Importantly, patients who have developed both cancer and dementia have a higher overall mortality and disease-specific mortality than patients with only one of these conditions.[84] This suggests that survival bias could affect estimates of the association between cancer and dementia, resulting in lower exposure rates among the diseased (i.e., lower numbers of prevalent cancer diagnosis in patients with dementia, and less diagnoses of dementia before cancer development).

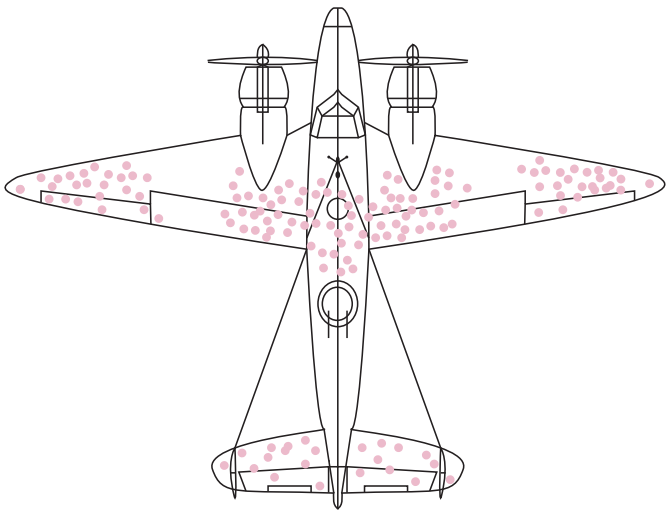


Figure 4 Illustration of survivor bias during World War II.
During World War II, researchers studied the damage done to army planes that had returned from missions. To reinforce the planes, they recommended to add additional armour on those places that showed most damage: the wings and tail (pink dots). The statistician Abraham Wald noticed that the researchers only investigated planes that had returned from their missions (survivors). The planes that had been lost during the missions were not observed (non-survivors). Therefore, the unscathed parts – the engines – instead of the damaged parts needed to be reinforced (image shows hypothetical data).

Different studies have tried to overcome surveillance and survival bias. For instance by restricting the analyses to persons who survived to at least the age of eighty years,[48,49] by studying the relation between cancer and negative control diseases such as automobile injuries and stroke,[49-51] and by focussing on different cancer types and stages.[46-48,50-53] Despite these strategies, the potential effects of surveillance and survival bias have not been satisfactory ruled out.

This thesis provides an alternative approach to elucidate the biological link between cancer and dementia. First, **Chapter 8** studies AD as a multistep process using multistage models. These models have frequently been used in cancer research to gain more insight in the number of steps (mutations) needed before manifestation of the cancer. If AD complies with the multistep process, this could support that AD and cancer follow a similar biological process. Next, the **Chapters 9**, **10**, and **11** focus on the preclinical stages of one disease – either cancer or dementia – and link it to the other disease. Preclinical stages of the disease share often the same biological underpinnings as the clinically manifested disease. If there is a biological link between cancer and dementia, this should extent across all preclinical stages of the diseases. Persons who have a preclinical stage of one of these diseases have often a longer life expectancy than those with clinically manifested disease. Therefore, studying the preclinical stage of one disease and linking it to the other disease can provide more insight in the biological relation between cancer and dementia by circumventing the effects surveillance and survival bias. **Chapter 9** describes the relation between mild cognitive impairment – a preclinical stage of dementia – and the risk of cancer. **Chapter 10** subsequently determines the relation between plasma amyloid-β – one of the earliest detectable changes in preclinical dementia – and the risk of cancer. Next, **Chapter 11** examines the relation between the tumour marker carcinoembryonic antigen as proxy for preclinical cancer and the subsequent risk of dementia. Lastly, **Chapter 12** presents alternative methods that can deal with selection bias to further examine the relation between cancer and dementia.

Part IV – Underlying mechanisms

To be able to develop prevention and intervention strategies for cognitive problems in cancer patients, it is necessary to understand the mechanisms by which cancer and cancer treatment can lead to disruptions in cognitive function. Proposed underlying mechanisms are the release of extracellular vesicles, inflammation, oxidative stress, vascular changes, changes in hormonal levels, and telomere shortening (**Figure 5**).[21,85] These mechanisms are partly based on the biological similarities between cancer and dementia.[86] As yet, it is unknown whether these mechanisms have already a role before clinical manifestation of cancer, and whether these mechanisms underlie late cognitive problems and dementia in cancer patients. This

thesis explores the role of the following two proposed mechanisms: inflammation and vascular factors.

Exact quantification of chronic systemic inflammation is often challenging. Well-known markers of inflammation have different limitations. For instance, C-reactive protein is not only elevated during chronic inflammation, but also during acute inflammatory processes, and the erythrocyte sedimentation rate is a non-specific measure of inflammation. Cytokines are more specific, but exact quantification is limited because of the wide variety of cytokine panels and the high costs. Recent evidence has suggested the use of easily obtainable measures of blood cells that can capture chronic systemic inflammation and have reliable prognostic and predictive value in cancer patients.[87-92] These blood cells are the neutrophils, lymphocytes, and platelets. In cancer, higher levels of neutrophils and platelets are associated with promotion of tumour growth and metastasis, whereas higher levels of lymphocytes are associated with tumour growth inhibition.[93,94] The measurements of these blood cells can be combined into ratios, i.e., the neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and systemic immune-inflammation index. **Chapter 13** studies the levels of these inflammatory ratios in relation to cognitive function in breast cancer survivors who were treated with surgery, radiotherapy, and chemotherapy on average twenty years ago. **Chapter 14** subsequently focuses on the same inflammatory markers in preclinical stage of dementia. In these studies, the granulocyte count is used as proxy for the neutrophil count.

Next to inflammation, cancer and cancer treatment can induce vascular changes such as a hypercoagulable state, atherosclerosis, and injury to cardiac myocytes.[95,96] Because of these vascular changes, cancer patients are often at a higher risk of developing thromboembolic complications and cardiovascular diseases than persons without a history of cancer.[97-99] Less is known about vascular changes before cancer diagnosis. **Chapter 15** contributes to this understanding by studying the presence of atherosclerotic calcification in the aortic arch – as proxy for systemic atherosclerosis – and the subsequent risk of cancer. Vascular changes might result in altered brain perfusion, which in turn can lead to long-term cognitive problems.[100,101] **Chapter 16** therefore evaluates atherosclerotic carotid disease and brain perfusion in breast cancer survivors on average twenty years after cancer treatment.

Part V – General discussion

In this last Part, I summarise the main findings of this thesis in the context of current knowledge on cognitive problems and dementia in non-CNS cancer patients. In addition, I discuss methodological considerations, define the implications of these findings, and provide suggestions and challenges for further research.

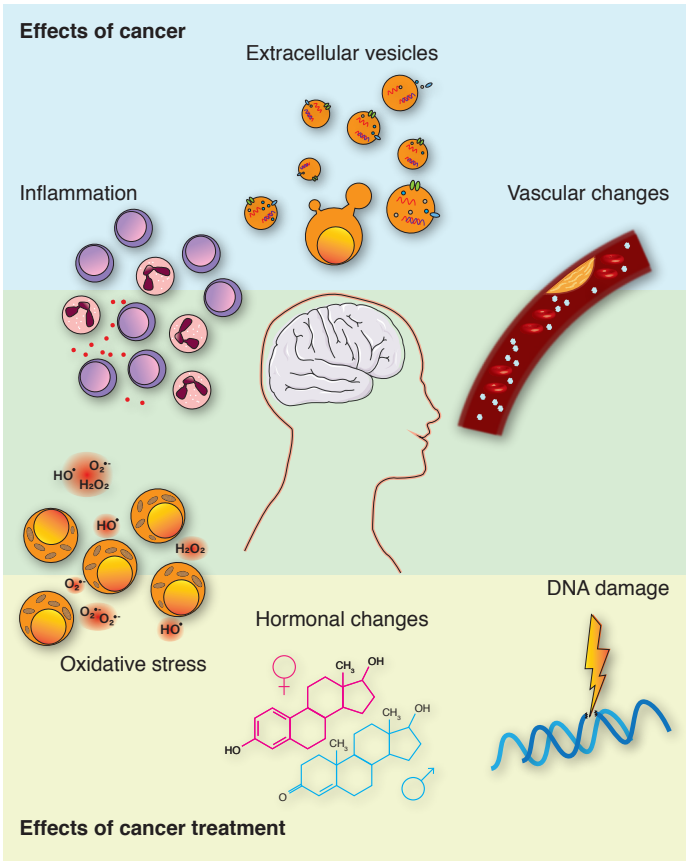


Figure 5 Overview of different mechanisms underlying cognitive problems in cancer patients.
Cancer might lead to differences in extracellular vesicles (blue background). Inflammation, oxidative stress, and vascular changes can be induced by both cancer and cancer treatment (green background). Lastly, cancer treatment can result in changes in hormonal levels and in DNA damage (yellow background).

Table 1 Overview of studies investigating the association between cancer and dementia.

Study	Study design	Study participants	Effect estimate (95% CI)	Controlling for bias	Conclusion
Studies investigating the risk of dementia in patients with cancer or cancer survivors					
Yamada (1999)	Prevalence study in atomic bomb survivor cohort	Total N=2222 (aged ≥60 years, 28.7% men). 230 participants had (a history of) cancer. 74 participants had AD. Unknown how many AD patients had a history of cancer.	OR 0.3 (0.05 to 0.98)	None	Decreased risk of AD in cancer patients/survivors
Realmuto (2012)	Case control study	Total N=378 (no age criterion, 28.6% men). 84 participants had (a history of) cancer. 126 participants had AD, of whom 23 with a history of cancer (18.3%).	OR 0.6 (0.4 to 1.1)	Different cancer types	Decreased risk of AD in cancer patients/survivors
White (2013)	Population-based cohort study	Total N=1102 (aged ≥70 years, 39.3% men). 141 participants had (a history of) NMSC. 100 participants developed AD, of whom 6 with prevalent NMSC (6.0%).	HR 0.47 (0.21 to 1.09)	None	Decreased risk of AD in NMSC patients/survivors
Nudelman (2014)	Case control study	Total N=1609 (aged ≥50 years, 51.3% men). 503 participants had (a history of) cancer. 446 participants had AD, of whom 83 with a history of cancer (18.6%).	OR 1.5 (1.3 to 1.8)*	Different cancer types	Decreased risk of AD in cancer patients/survivors, driven by NMSC
Frain (2017)	Retrospective cohort study of US veterans	Total N=3 499 378 (aged ≥65 years, 98.0% men). 771 285 participants had (a history of) cancer. 82 998 participants developed AD. Unknown how many AD patients had a history of cancer.	HR 1.00 (0.97 to 1.03)	- Risk over four time intervals following cancer diagnosis - Negative control diseases - Different cancer types	Decreased risk of AD in some cancer type patients/ survivors, but not for all cancer types together

** Cancer history positive is used as reference.*
AD = Alzheimer dementia, CI = confidence interval, HR = hazard ratio, NMSC = non-melanoma skin cancer, OR = odds ratio.

Table 1 Overview of studies investigating the association between cancer and dementia (continued).

Study	Study design	Study participants	Effect estimate (95% CI)	Controlling for bias	Conclusion
Bowles (2017)	Prospective population-based cohort study	Total N=4357 (aged ≥65 years, 41.3% men). 756 participants had prevalent cancer. 583 participants developed incident cancer. 877 participants developed AD, of whom 126 with prevalent cancer (14.4%) and 73 with (a history of) incident cancer (8.3%).	Prevalent cancer: HR 0.95 (0.77 to 1.17) Incident cancer: HR 0.73 (0.55 to 0.96)	- Risk of dementia per cancer stage - Analysis in participants who survived at least to age 80 - Different cancer types	Only a decreased risk of AD in incident cancer patients/survivors, not in prevalent cancer patients/ survivors
Studies investigating the risk of cancer in patients with dementia					
Attner (2010)	Case control study	Total N=167 080 (no age criterion, unknown % men). 2985 participants had a history of AD. 19 756 had cancer, of whom 253 with a history of AD (1.3%).	RR 0.60 (0.52 to 0.69)	Different cancer types	Decreased risk of cancer in dementia patients
Ou (2013)	Retrospective population-based cohort study	Total N=6960 (aged ≥40 years, 39.7% men). All 6960 participants had AD. 405 of these participants developed cancer (5.8%).	SIR 0.88 (0.80 to 0.97)	- Stratified analysis by duration of AD diagnosis - Different cancer types	Decreased risk of cancer in AD patients
Romero (2014)	Prospective population-based cohort study	Total N=4197 (aged ≥65 years, 42.0% men). 467 participants had AD. 441 participants died of cancer, of whom 16 had AD (3.6%).	HR 0.50 (0.27 to 0.93)	None	Decreased risk of cancer specific mortality in AD patients

AD = Alzheimer dementia, CI = confidence interval, HR = hazard ratio, MCI = mild cognitive impairment, RR = risk ratio, SIR = standardised incidence ratio.

Table 1 Overview of studies investigating the association between cancer and dementia (continued).

Study	Study design	Study participants	Effect estimate (95% CI)	Controlling for bias	Conclusion
Studies investigating both the risk of dementia in patients with cancer or cancer survivors and the risk of cancer in patients with dementia					
Roe (2005)	Prospective cohort study	Total N=594 (aged ≥47 years, 35.7% men). 50 participants had (a history of) cancer. It is unknown how many participants developed AD.	HR 0.34 (0.10 to 1.12)	None	Decreased risk of AD in cancer patients/survivors
	Prospective cohort study	Total N=249 (aged ≥47 years, 37.3% men). 395 participants had AD. 45 participants developed cancer. Unknown how many cancer patients had AD.	HR 0.34 (0.18 to 0.62)	None	Decreased risk of cancer in AD patients
Roe (2010)	Prospective cohort study	Total N=2151 (aged ≥65 years, unknown % men). 390 participants had (a history of) cancer. It is unknown how many participants developed AD.	HR 0.72 (0.52 to 1.00)	None	Decreased risk of AD in cancer patients/survivors
	Prospective cohort study	Total N=2225 (aged ≥65 years, unknown % men). 118 participants had AD. Unknown how many participants had cancer hospitalisations.	HR 0.41 (0.20 to 0.84)	None	Decreased risk of cancer in AD patients
Driver (2012)	Prospective population-based cohort study	Total N=1278 (aged ≥65 years, 38.8% men). 423 participants had (a history of) cancer. 256 participants developed AD. Unknown how many AD patients had a history of cancer.	HR 0.81 (0.59 to 1.11)	- Different cancer types - Analysis in participants who survived to age ≥ 80 - Negative control disease	Decreased risk of AD in cancer patients/survivors
	Nested case control study	Total N=1980 (aged ≥65 years, unknown % men). 376 participants had AD. 252 participants developed cancer. Unknown how many cancer patients had AD.	HR 0.38 (0.25 to 0.56)	Different cancer types	Decreased risk of cancer in AD patients

AD = Alzheimer dementia, CI = confidence interval, HR = hazard ratio.

Table 1 Overview of studies investigating the association between cancer and dementia (continued).

Study	Study design	Study participants	Effect estimate (95% CI)	Controlling for bias	Conclusion
Musicco (2013)	Prospective/ retrospective historical cohort study	Total N=21 451 (aged ≥60 years, 57.0% men). All of these participants had (a history of) cancer. 161 participants developed AD of whom 68 with (a history of) cancer (42.2%).	RR 0.64 (0.50 to 0.81)	- Retrospective and prospective follow-up - Separate analyses for persons surviving/ dying during follow-up - Different cancer types	Decreased risk of AD in cancer patients/survivors
	Prospective/ retrospective historical cohort study	Total N=2832 (aged ≥60 years, 33.4% men). All of these participants had AD. 161 participants developed cancer, of whom 93 with AD (57.8%).	RR 0.79 (0.64 to 0.97)	- Retrospective and prospective follow-up - Separate analyses for persons surviving/ dying during follow-up - Different cancer types	Decreased risk of cancer in AD patients
Freed-man (2016)	Prospective cohort study in Medicare population	Total N=1 163 327 (aged ≥66 years, 50.4% men). 742 809 participants had (a history of) cancer. 21 526 developed AD of whom 11 812 with (a history of) cancer (54.9%).	HR 0.87 (0.84 to 0.90)	- Negative control disease - Different cancer types	Decreased risk of AD in cancer patients/survivors

AD = Alzheimer dementia, CI = confidence interval, HR = hazard ratio, RR = risk ratio.

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Part I

Cancer registration

Chapter 2

Ascertainment of cancer in longitudinal research: the concordance between the Rotterdam Study and the Netherlands Cancer Registry

van der Willik KD, Ruiter R, van Rooij FJA, Verkroost-van Heemst J, Hogewoning SJ, Timmermans KCAA, Visser O, Schagen SB, Ikram MA, Stricker BHCh

ABSTRACT

Background Complete and accurate registration of cancer is needed to provide data on cancer incidence and to investigate aetiology. Such data can be retrieved from national registries, but also from large population-based cohort studies. Yet, the concordance and discordance between these data sources remain unknown.

Methods We evaluated completeness and accuracy of cancer registration by studying the concordance between the population-based Rotterdam Study and the Netherlands Cancer Registry between 1989 and 2012 using the independent case ascertainment method. We compared all incident cancers in participants of the Rotterdam Study (aged 45 years and older) to registered cancers in the Netherlands Cancer Registry in the same persons based on the date of diagnosis and the International Classification of Diseases (ICD) code.

Results In total, 2977 unique incident cancers among 2685 persons were registered. Two hundred eighty-eight cancers (9.7%) were coded by the Rotterdam Study that were not present in the Netherlands Cancer Registry. These were mostly non-pathology-confirmed lung and haematological cancers. In addition, 116 cancers were coded by the Netherlands Cancer Registry, but not by the Rotterdam Study (3.9%), of which 20.7% were breast cancers. Regarding pathology-confirmed cancer diagnoses, completeness was more than 95% in both registries. Eighty percent of the cancers registered in both registries was coded with the same date of diagnosis and ICD code. Of the remaining cancers, 344 (14.5%) were misclassified with regard to date of diagnosis and 72 (3.0%) with regard to ICD code.

Conclusions Our findings indicate that multiple sources on cancer are complementary and should be combined to ensure reliable data on cancer incidence.

INTRODUCTION

With an estimated number of 3.9 million new diagnoses and 1.9 million deaths from cancer in Europe in 2018, cancer poses a huge burden on societies.[1] Optimal cancer registration is not only crucial to provide reliable estimations of incidence and mortality,[2] but is also pivotal to better understand risk factors of cancer.[3] Extensive quality checks are performed before cancer registry data are accepted in Cancer Incidence in Five Continents, the reference source of data on international cancer incidence.[4] However, the number of validation studies of cancer registries is limited.

Methods to assess completeness and accuracy of cancer registries can be classified into two categories, i.e., qualitative and quantitative methods.[5] Qualitative methods include comparison of performance of a cancer registry with other registries, such as comparison with historical data or other populations. In contrast to qualitative methods, quantitative methods including independent case ascertainment, flow method, or capture-recapture methods provide a numerical evaluation of the extent to which all eligible events are registered and are therefore more appealing.

Several studies have compared cancer registries in Europe using quantitative methods.[6-17] In the Netherlands, the Netherlands Cancer Registry managed by the Netherlands Comprehensive Cancer Organisation (IKNL) registers cancers nationwide and provides information regarding cancer incidence, prevalence, risk, mortality, and survival of cancer.[18] Completeness of registration by the Netherlands Cancer Registry has been estimated at 98.7% in 1990 based on cancers registered by general practitioners.[6] A second evaluation in 1993 has shown completeness of 96.2%.[7] However, the potential added value of a large prospective population-based cohort study to the completeness and accuracy of cancer registration by the national cancer registry has not been evaluated.

Therefore, in this study, we investigated the concordance of cancer registration by the Netherlands Cancer Registry with a large population-based cohort study, the Rotterdam Study.

METHODS

Setting

This study is embedded within the Rotterdam Study, an ongoing population-based cohort study in Rotterdam, the Netherlands, designed to study the occurrence and determinants of diseases in the elderly population. Besides cancer, the Rotterdam Study focuses on the aetiology, prediction, and prognosis of cardiovascular, endocrine, hepatic, neurological, ophthalmologic, psychiatric, dermatological, otolaryngologic, locomotor, and respiratory diseases. The Rotterdam Study started in 1989 with 7983 participants (response of 78%) aged 55 years and older and residing in the district Ommoord, a suburb of Rotterdam. This first subcohort (RS-I) was extended with a second subcohort (RS-II) in 2000, consisting of 3011 participants (response of 67%) and with a third subcohort (RS-III) in 2006, composed of 3932 participants aged 45 years and older (response of 65%). The design of the Rotterdam Study has been described in detail.[19] In total, the Rotterdam Study comprises 14 926 participants aged 45 years and older at study entry.

The Rotterdam Study has been approved by the Medical Ethics Committee of Erasmus Medical Centre and by the board of The Netherlands Ministry of Health, Welfare, and Sports. A written informed consent was obtained from all participants.

Assessment of cancer

The Rotterdam Study

Diagnosis of incident cancer is based on medical records of general practitioners (including hospital discharge letters) and furthermore through linkage with the national hospital discharge registry (Landelijke Medische Registratie [LMR]) hosted by Dutch Hospital Data and histology and cytopathology registries in the region (part of the nationwide network PALGA). Cancer diagnosis is coded independently by two physicians and classified according to the International Classification of Diseases, 10th revision (ICD-10). In case of discrepancy between sources, consensus is sought through consultation with a physician specialised in internal medicine. Date of diagnosis is based on the pathology date, or – if unavailable – date of hospital admission or hospital discharge letter. Level of uncertainty of diagnosis is established as: certain (pathology-confirmed), probable (e.g., based on imaging features or elevated tumour markers without pathological confirmation), and possible (e.g., based on symptoms and physical examination, or suspicion based on imaging features or elevated tumour markers without pathological confirmation). Possible cancers were not included in the current study. Registration of cancer diagnoses is completed up to January 1st, 2013.

The Netherlands Cancer Registry

The Netherlands Cancer Registry is a population-based cancer registry with nationwide coverage since 1989. Cancer diagnoses are notified by the nationwide network and registry of histology and cytopathology (PALGA) and in addition through linkage with the LMR hosted by Dutch Hospital Data. Each cancer is coded by trained registration clerks (internal education of one year) according to the International Classification of Diseases for Oncology, 3rd edition (ICD-O-3) based on information gathered from medical files at the hospital. Date of diagnosis is coded according to international coding rules and mostly based on the date of first pathological confirmation, or – if unavailable – date of first hospital admission. In addition, information about tumour histology, tumour stage, and primary treatment was retrieved.

Linkage

All persons from the Rotterdam Study (N=14 926) were linked with patients in the Netherlands Cancer Registry based on the following characteristics: date of birth, sex, birth name, initials, zip code, and – if applicable – date of death. If a participant had multiple zip codes due to moving, historical zip codes were also included. All data were pseudonymised using a double-pass procedure beforehand. Data exchange took place between secured encrypted data servers. All cancers diagnosed between 1989 and 2012 were included. To make an equal comparison between the two cancer registries, we excluded the following cancers: cancers diagnosed before entry in the Rotterdam Study or after January 1st, 2013, cancers solely coded as cause of death, skin cancers (due to different registration methods), benign or borderline tumours, and carcinomas in situ other than ductal carcinoma in situ of the breast (**Figure 1**).

If a cancer was only coded by the Rotterdam Study or the Netherlands Cancer Registry (unmatched cancers), we performed a second linkage with previously excluded cancers (i.e., for instance, date of diagnosis prior to study entry or cancer solely registered as cause of death). In case of multiple cancers per patient, we included all different cancers.

We were interested in (i) the completeness and; (ii) the accuracy of both registries. Since we do not know the true number of cancers in the study population, we defined completeness as the proportion of cancers in one registry in relation to the total number of cancers coded by at least one of the registries. Completeness was determined for pathology-confirmed diagnoses of cancer and non-pathology-confirmed diagnoses separately, and for all cancers combined.

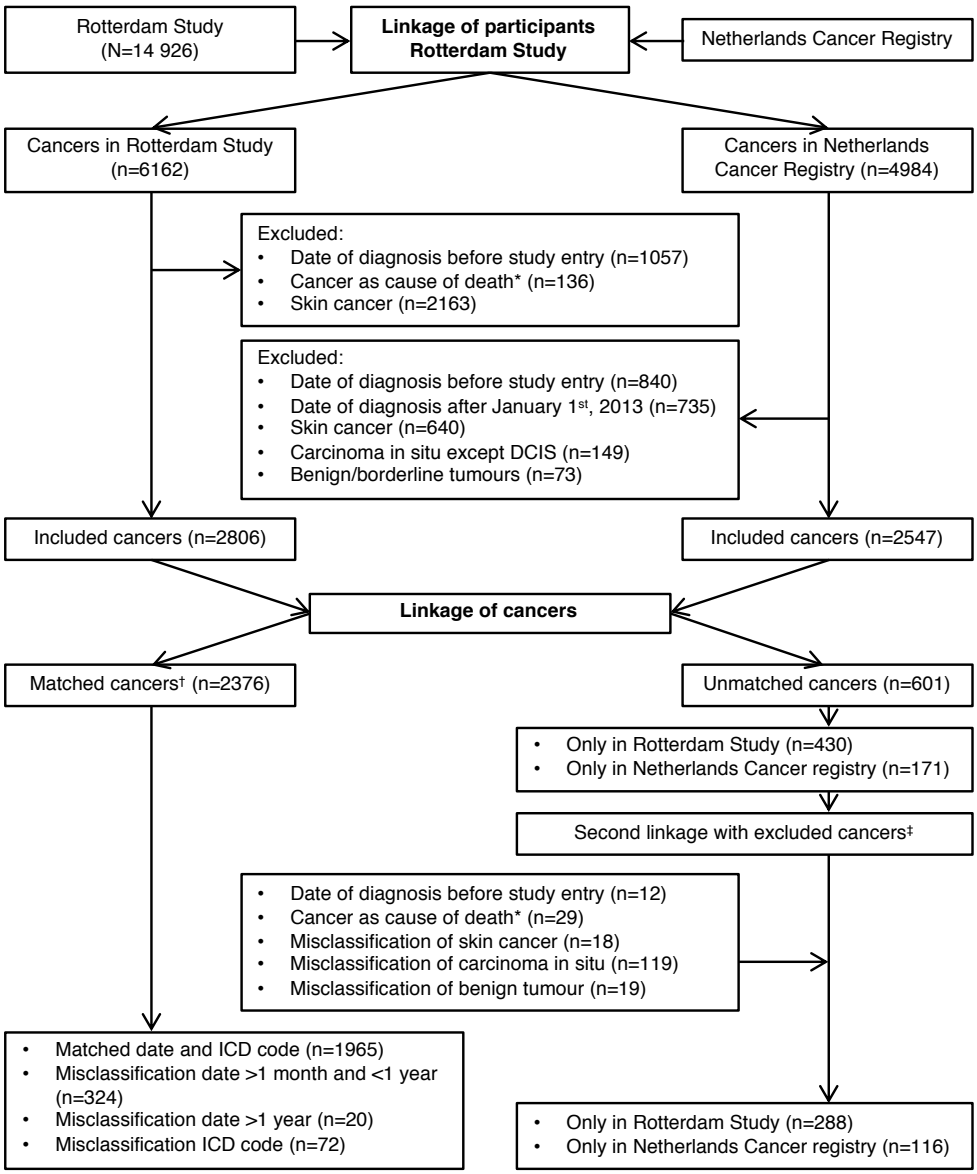

Figure 1 Flowchart of matched and unmatched cancers after linkage between Rotterdam Study and Netherlands Cancer Registry.

** Cancer as cause of death corresponds to cancer solely coded as cause of death, without a date of incident cancer diagnosis. † Five cancers were both misclassified with regard to date >1 month and <1 year and with regard to ICD code. Therefore, the number of matched cancers is lower than the total number of cancers in the different misclassification categories. ‡ A second linkage was performed to preclude whether unmatched cancers were present in both databases, but were excluded prior to the linkage of cancers based on the exclusion criteria.*

DCIS = ductal carcinoma in situ, ICD = International Classification of Diseases.

Accuracy of the date of cancer diagnosis and ICD code was investigated for cancers that were present in both registries (matched cancers). We digitally converted the ICD-O-3 codes into ICD-10 codes. These matched cancers were classified into the following categories: matched date of diagnosis (difference in date of diagnosis of one month or less) and ICD code, misclassification of date of diagnosis (two categories: (i) difference in date of diagnosis of more than one month but less than one year; and (ii) difference of more than one year), or misclassification of ICD code (different ICD code and different organ system). An overview of the different ICD-10 codes used for the categorisation into different organ systems is presented in **Supplementary Table 1.**

Unmatched cancers only coded by the Rotterdam Study and cancers misclassified with regard to date of diagnosis or ICD code were reassessed through evaluation of the patient's original medical files collected by the Rotterdam Study.

Statistical analyses

Differences in patient characteristics were evaluated using an independent samples *t* test (continuous variables) or a chi-square test (categorical variables). Two-sided $P<.05$ was considered statistically significant. Statistical analyses were performed using SPSS and the 'UpSetR' package from R software Version 3.3.2.[20]

RESULTS

In the same source population based on 14 926 participants of the Rotterdam Study, 2806 incident cancers among 2579 persons were coded by the Rotterdam Study and 2547 cancers among 2342 persons were coded by the Netherlands Cancer Registry (**Figure 1**). Linkage of the two registries resulted in a total of 2977 unique cancers among 2685 persons.

Completeness of registries

After the first linkage, 2376 cancers among 2227 persons were coded by both registries. The remaining 601 unmatched cancers were coded solely by one of the two registries, of which 197 cancers could eventually be matched after a second linkage with previously excluded cancers. This resulted in 288 cancers (9.7%) among 284 persons coded solely by the Rotterdam Study, of which 105 cancers (36.5%) were pathology-confirmed. Furthermore, 116 cancers (3.9%) among 115 persons were coded solely by the Netherlands Cancer Registry, of which 109 cancers (94.0%) were pathology-confirmed. Taking only cancers after the second

linkage into account, the Rotterdam Study had a completeness of 95.8% (2664 out of 2780 cancers) and the Netherlands Cancer Registry of 89.6% (2492 out of 2780 cancers) of all cancers. Regarding pathology-confirmed cancers (2475 cancers), completeness was 95.3% in the Rotterdam Study and 95.2% in the Netherlands Cancer Registry. Completeness of non-pathology-confirmed cancers (305 cancers) was 97.7% in the Rotterdam Study and 40.0% in the Netherlands Cancer Registry.

Persons with matched cancer diagnoses were significantly younger at baseline and at first cancer diagnosis than those coded solely by the Rotterdam Study ($P<.001$ and $P<.001$, respectively) or by the Netherlands Cancer Registry ($P<.001$ and $P<.001$, respectively, **Table 1**).

Cancer sites that were most frequently registered by both registries were gastric and oesophagus (93.4% of all these cancers were included in both registries), head and neck (91.0%), and male genital organs (90.0%, **Table 2**). Lung and mesothelioma was the most common cancer site among cancers coded solely by the Rotterdam Study (20.5% of all cancer cases solely coded by the Rotterdam Study).

Haematological cancer represented the second most frequent diagnosis that was coded solely by the Rotterdam Study (16.0%), of which chronic lymphocytic leukaemia was the most common diagnosis (39.1%). The distribution of different cancer sites among cancers coded solely by the Netherlands Cancer Registry was comparable to the distribution among the matched cancers, with breast as the most frequently diagnosed cancer site (20.7%). One-third of all cancers solely coded by the Netherlands Cancer Registry were second primary cancers of the same cancer site, with the highest numbers for breast (75.0%) and colon cancers (56.3%).

Accuracy of registries

One thousand nine hundred sixty-five cancers out of 2376 matched cancers (82.7%) were coded with the same date of diagnosis and ICD code by both registries. Cancer sites that were often correctly classified were colorectal (91.9% of all matched colorectal cancers), breast (88.3%), and oesophagus and gastric (87.9%). The remaining cancers were misclassified with regard to date of diagnosis (344 cancers [14.5%]) or ICD code (72 cancers [3.0%]).

Misclassification of date was further divided into a difference in date of diagnosis more than one month and less than one year (324 cancers), and more than one year (20 cancers, **Table 3**). Male genital cancer with prostate cancer as most frequent cancer was the most common cancer site among cancers with a difference in date of diagnosis of more than one month (24.4%) and the second among cancers misclassified for more than one year (25.0%),

Table 1 Characteristics of persons with matched and unmatched cancers in the Rotterdam Study and the Netherlands Cancer Registry.

Characteristic	Persons with matched cancers (n=2227)	Persons with unmatched cancers (n=397)	
		Rotterdam Study (n=284)	Netherlands Cancer Registry (n=115)
Age at study entry, years, median (IQR)	65.1 (60.3 to 71.8)	71.5 (64.8 to 77.7)	69.0 (63.6 to 75.3)
Women, No. (%)	1081 (48.5)	151 (53.2)	61 (53.0)
Educational level*, No. (%)			
Primary	395 (17.7)	69 (24.3)	22 (19.1)
Lower	897 (40.3)	104 (36.6)	43 (37.4)
Intermediate	647 (29.1)	89 (31.3)	33 (28.7)
Higher	261 (11.7)	19 (6.9)	15 (13.0)
Age at first cancer diagnosis, years, mean (SD)	74.0 (8.5)	80.5 (8.6)	78.2 (9.1)
45-65 years	355 (15.9)	16 (5.6)	10 (8.7)
65-75 years	856 (38.4)	50 (17.6)	27 (23.5)
75-85 years	794 (35.7)	126 (44.4)	51 (44.3)
>85 years	222 (10.0)	92 (32.4)	27 (23.4)

Persons in Rotterdam Study or Netherlands Cancer Registry do not sum up to total number of persons with unmatched cancers since some persons with unmatched cancers overlap. Missing values of educational level are not imputed and therefore numbers do not always sum up to 100%.
** Educational levels were assessed during home interviews according to the following categories: primary: primary education, lower: lower or intermediate general education, or lower vocational education, intermediate: intermediate vocational education or higher general education, or higher: higher vocational education or university.*
IQR = interquartile range, SD = standard deviation.

after haematological malignancies (40.0%). Date of diagnosis was more often accurately registered by the Netherlands Cancer Registry than by the Rotterdam Study based on evaluation of the original medical files (**Supplementary Table 2**).

Misclassification regarding ICD code was less common, with 72 cancers (3.0%) classified as misclassification of ICD code and organ system (**Supplementary Figure 1**). Most differences in ICD code were found for lung cancers or cancers coded as tumour of unknown primary origin (**Supplementary Figure 1**).

Table 2 Cancer sites according to matched and unmatched cancers.

Cancer site	Matched cancers (n=2376)	Unmatched cancers (n=404)	
		Rotterdam Study (n=288)	Netherlands Cancer Registry (n=116)
Head and neck	71 (91.0)	4 (5.1)	3 (3.8)
Oesophagus and gastric	141 (93.4)	5 (3.3)	5 (3.3)
Colorectal	393 (89.1)	30 (6.8)	18 (4.1)
Hepato-pancreato-biliary	121 (81.2)	26 (17.4)	2 (1.3)
Lung and mesothelioma	351 (82.4)	59 (13.8)	16 (3.8)
Bone and soft tissue	15 (71.4)	2 (9.5)	4 (19.0)
Breast	366 (89.5)	19 (4.6)	24 (5.9)
Female genital organs	101 (87.8)	10 (8.7)	4 (3.5)
Male genital organs	380 (90.0)	27 (6.4)	15 (3.6)
Unitary tract	176 (80.7)	28 (12.8)	14 (6.4)
Central nervous system	19 (73.1)	7 (26.9)	0
Haematological	165 (76.7)	46 (21.4)	4 (1.9)
Other	21 (67.7)	4 (12.9)	6 (19.4)
Unknown primary origin	56 (71.8)	21 (26.9)	1 (1.3)

Numbers are displayed in total number of cancer site (percentage per row).

DISCUSSION

In this study, we investigated the concordance of cancers in a prospective population-based cohort study, the Rotterdam Study, with the Netherlands Cancer Registry. There was a high concordance with regard to pathology-confirmed cancers (>95%), but the Rotterdam Study registered a higher number of non-pathology-confirmed cancers. Furthermore, there was a high accuracy with regarding to cancer site, but the accuracy with regard to date of diagnosis was lower in the Rotterdam Study than in the Netherlands Cancer Registry. These findings can help to identify the reasons for inaccurate cancer registration and emphasise that cancer registration by national cancer registries may complement population-based cohort studies and vice versa.

Completeness varying between 90 and 100% is considered as acceptable to estimate optimal cancer incidence, provided that there are no large differences regarding cancer site

Table 3 Overview of cancer sites according to correctly classified and misclassified cancers.

Cancer site	Correctly classified cancers (n=1965)	Misclassified cancers (n=411)*		
		Date of diagnosis (n=344)		ICD code (n=72)
		More than one month (n=324)†	More than one year (n=20)	
Head and neck	52 (73.2)	19 (26.8)	0	0
Oesophagus and gastric	124 (87.9)	13 (9.2)	0	4 (2.8)
Colorectal	361 (91.9)	25 (6.4)	1 (0.3)	6 (1.5)
Hepato-pancreato-biliary	88 (72.1)	24 (19.7)	1 (0.8)	9 (7.4)
Lung and mesothelioma	291 (82.2)	41 (11.6)	2 (0.6)	20 (5.6)
Bone and soft tissue	10 (66.7)	5 (33.3)	0	0
Breast	323 (88.3)	37 (10.1)	2 (0.5)	4 (1.1)
Female genital organs	89 (88.1)	9 (8.9)	1 (1.0)	2 (2.0)
Male genital organs	296 (77.9)	79 (20.8)	5 (1.3)	0
Unitary tract	136 (76.8)	38 (21.5)	0	3 (1.7)
Central nervous system	15 (78.9)	3 (15.8)	0	1 (5.3)
Haematological	130 (78.8)	27 (16.4)	8 (4.8)	0
Other	10 (47.6)	1 (4.8)	0	10 (47.6)
Unknown primary origin	40 (71.4)	3 (5.4)	0	13 (23.2)

Numbers are displayed in total number per cancer site (percentage per row). Cancers misclassified with regard to ICD code are classified according to the different cancer groups based on the ICD code of the Rotterdam Study.
** Five cancers were both misclassified with regard to date >1 month and <1 year and with regard to ICD code. Therefore, the number of misclassified cancers is lower than the total number of cancers in the different misclassification categories. † Difference in date of diagnosis more than one month and less than one year.*
ICD = International Classification of Diseases.

or age at cancer diagnosis between registered and unregistered cancers.[3] Completeness of pathology-confirmed cancers was comparable between the Rotterdam Study and the Netherlands Cancer Registry, but we found that the number of non-pathology-confirmed cancers, with lung and haematological cancers in particular, were underreported in the Netherlands Cancer Registry. This can be explained by the use of different sources of cancer registration, with the Rotterdam Study having access to the medical records of general practitioners in addition to notification of cancer diagnoses through the pathology database. Regarding the cancers missed by the Rotterdam Study, we observed that one-third of these

cancers were second primary cancers. It is often not well documented in discharge letters whether a second tumour is a recurrent cancer, metastasis, or second primary cancer, in contrast to the documentation in medical files in hospitals to which the Netherlands Cancer Registry has access. Although under-registration of second primary cancers within the same organ will not affect cancer statistics, because these cancers are not included in cancer incidence and survival estimations,[21] it may impact aetiological research questions.

Furthermore, we found that cancers coded by solely one registry occurred often in older persons, which has been observed in previous studies as well.[2,6,22] This observation can be explained because, compared to younger patients, pathological confirmation through biopsies can be limited in elderly patients due to poor clinical condition and prognosis.[23-25] Harms caused by histological tissue acquisition for pathological confirmation without consequences for cancer treatment may outweigh the benefit of knowing the diagnosis in these patients. Furthermore, older patients are less often referred to the hospital and are more likely to be treated (in nursing homes) by their general practitioner.[26] Such cancers will remain unnoticed in the Netherlands Cancer Registry, because there is no linkage with general practitioners.

Although the Rotterdam Study had a higher degree of completeness of non-pathology-confirmed cancers, the accuracy of calendar date of cancer diagnosis was lower than that of the Netherlands Cancer Registry. The Rotterdam Study aims to register the date of cancer diagnosis based on the date of biopsy (solid cancers) or laboratory assessment (haematological cancers). However, this information is not always documented in the hospital discharge letters and other medical files obtained from general practitioners. If the date of pathological confirmation is unavailable, a proxy is taken based on the date of hospital admission or the date of the medical letter. Most discrepancies regarding date of diagnosis were found for male genital organ cancer, mostly represented by prostate cancer, and haematological cancer. Prostate cancer is frequently detected by elevated prostate-specific antigen (PSA) levels. Since the long-term benefit of invasive treatment for prostate cancer is questionable,[27] treatment options such as watchful waiting and active surveillance are often applied for indolent localised prostate cancer. Monitoring of patients by measuring PSA levels limits the need for pathological confirmation of the cancer in contrast to cancer at other sites. Pathology can be obtained in case of cancer progression, which may occur months after the initial clinical diagnosis. The dates across these different clinical stages are not always accurately documented in medical letters, resulting in misclassification of the date of first diagnosis. Differences in the date of diagnosis of haematological cancers were explained by the different diagnostic examinations on which the date of diagnosis was based (peripheral blood versus bone marrow biopsy).

In addition, we showed that few of the registered cancers were misclassified with regard to

ICD code. We considered cancers with a different ICD code within the same organ system as correctly classified, because part of the misclassification is due to different coding rules. These different coding rules also explain the misclassified cancers with the ICD code for 'tumour of primary origin', with the Rotterdam Study being more lenient in coding cancers according to the most probably primary origin. Moreover, cancer diagnoses in the Rotterdam Study are coded independently by two physicians, whereas cancers in the Netherlands Cancer Registry are coded by one trained registration clerk, which could affect the accuracy of registered cancers as well.[28]

The main strength of this study is the independent case ascertainment method used to study the concordance between a large population-based cohort study and the nationwide cancer registry. Although the flow method may outperform the independent case ascertainment by having the advantage of measuring completeness during the registration process,[5] it does not appropriately describe the data when cancer registration begins with a delay, and is therefore not used in the Netherlands Cancer Registry. Data on cancer diagnoses were collected independently, partly from different sources, and with different aims, i.e., determining statistics on cancer incidence, prevalence, and survival by the Netherlands Cancer Registry while investigating aetiology by the Rotterdam Study. Although these aims are different, optimal cancer registration is fundamental for both purposes. However, it should be noted that the current study is conducted within persons aged 45 years and older and that these findings may differ among a younger population. Furthermore, we cannot rule out that cancers without pathological confirmation are actually benign. However, we classified cancers based on all available medical information, thereby limiting the number of false-positive diagnoses.

Based on our findings, we have identified the main limitations of both registries, which opens avenues for improvements. Date of diagnosis was misclassified in 11.8% in the Rotterdam Study compared to 4.8% in the Netherlands Cancer Registry. Since this information is not always documented in the medical files, we can improve the accuracy by standardised linkage with the Netherlands Cancer Registry. Regarding the Netherlands Cancer Registry, it is of the utmost importance to investigate the reason why some pathology-confirmed cancers are not captured. Therefore, continuous improvement of registration quality is necessary, especially regarding cancers in elderly and at specific cancer sites such as pancreas, lung, and haematological cancers. In addition, many non-pathology-confirmed cancers were not registered by the Netherlands Cancer Registry. Cancer diagnoses in the Netherlands Cancer Registry are primarily notified by the pathology laboratories and the national hospital discharge registry. However, outpatients are included in the national hospital discharge registry as of 2015, which is expected to improve notification of non-pathology-confirmed cancers to the Netherlands Cancer Registry. This effect is mainly visibly in lung cancer, for which the

proportion of non-pathology-confirmed cancers increased from 8% between 1989 and 2012 (the inclusion period of this study) to 13% between 2015 and 2017. Since this misclassification could result in an underestimation of cancer incidence, inclusion of these clinically diagnosed cancers may provide more accurate cancer statistics. However, cancers diagnosed by general practitioners or nursing home physicians without further diagnostics that include pathology or referral to a hospital are still not be captured by the Netherlands Cancer Registry.

In conclusion, our findings indicate that linkage of different cancer registries is needed to improve registration by identifying the reasons of inaccurate cancer registration. Cancer registration by national cancer registries may complement cancer registration by population-based cohort studies and vice versa. Combination of different sources is needed to provide reliable data on cancer incidence.

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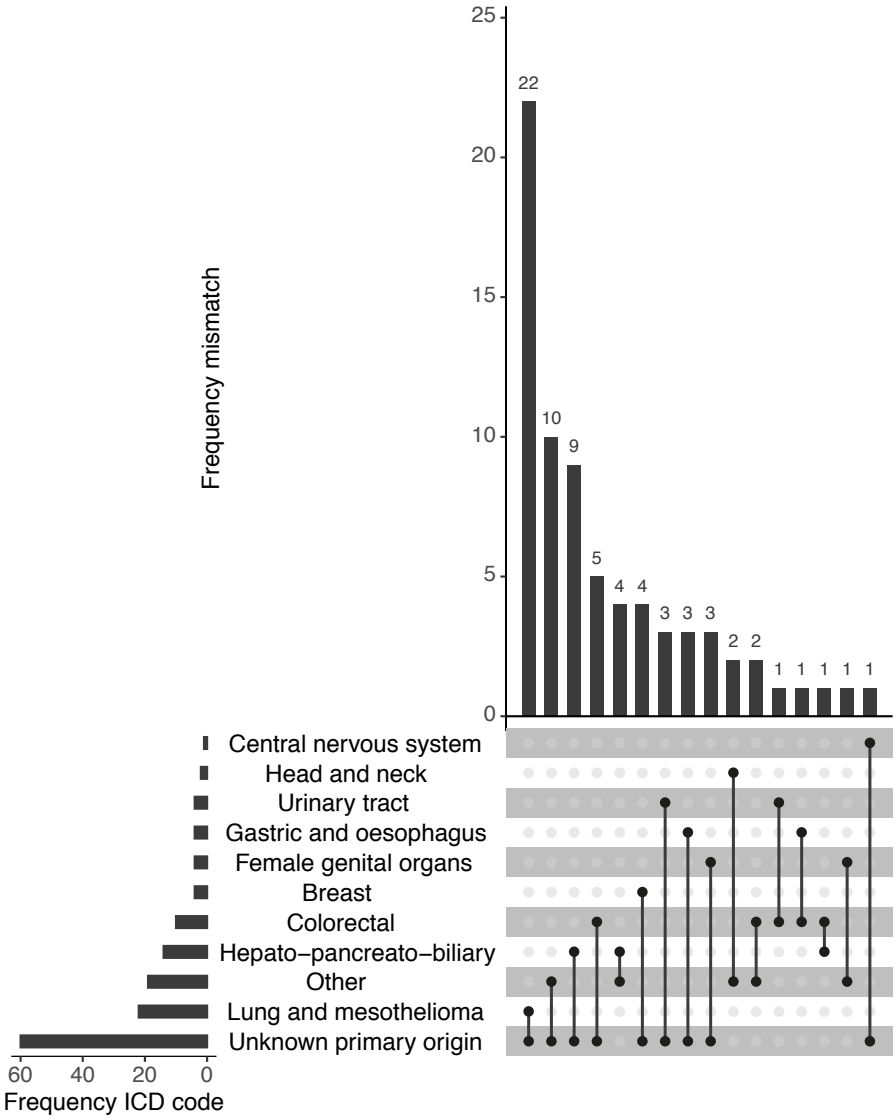
SUPPLEMENTARY MATERIAL



Supplementary Figure 1 Intersection plot of misclassified cancers with regard to ICD code.
The connecting dots represent the mismatched ICD codes per organ system. Tumour of unknown primary origin was the most common misclassified cancer type, especially in the match with cancers of the lung and mesothelioma. The term 'other' represents other and unspecified.
ICD = International Classification of Diseases.

Supplementary Table 1 Overview of the used ICD-10 codes per organ system.

Organ system	Corresponding ICD-10 code
Head and neck	C01-C14, C30, C32, C69, C73
Oesophagus and gastric	C15, C16
Colorectal	C18-C20
Hepato-pancreato-biliary	C22-C25
Lung and mesothelioma	C34, C45
Bone and soft tissue	C40, C41, C49
Breast	C50
Female genital organs	C51-C57
Male genital organs	C60-C63
Unitary tract	C64-C68
Central nervous system	C70-C72
Haematological	C81-C96
Other	C17, C21, C26, C37-C39, C48, C75, C76
Unknown primary origin	C80

ICD = International Classification of Diseases.

Supplementary Table 2 Overview of accuracy of misclassified cancers.

Registry	Misclassified cancers	
	Date of diagnosis more than one month difference (n=324)*	Date of diagnosis more than one year difference (n=20)
Date correctly classified by		
Rotterdam Study	57 (17.6)	6 (30.0)
New Rotterdam Study†	48 (14.8)	3 (15.0)
Netherlands Cancer Registry	219 (67.6)	11 (55.0)

Numbers are displayed in total number (percentage per column).
Original medical files were evaluated to determine the correct date of diagnosis.
** Difference in date of diagnosis more than one month and less than one year. † New Rotterdam Study indicates that date of diagnosis was originally not accurately registered in both registries, and was changed after evaluation of the original medical files of the corresponding participant.*
ICD = International Classification of Diseases.

Chapter 3

Pathology-confirmed versus non-pathology-confirmed cancer diagnoses: incidence, participant characteristics and survival

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ABSTRACT

Background Cancer diagnoses which are not confirmed by pathology are often under-registered in cancer registries compared to pathology-confirmed diagnoses. It is unknown how many patients have a non-pathology-confirmed cancer diagnosis, and whether their characteristics and survival differ from patients with a pathology-confirmed diagnosis.

Methods Participants from the prospective population-based Rotterdam Study were followed between 1989 and 2013 for the diagnosis of cancer. Cancer diagnoses were classified into pathology-confirmed versus non-pathology-confirmed (i.e., based on imaging or tumour markers). We compared participant characteristics and the distribution of cancers at different sites. In addition, we investigated differences in overall survival using survival curves adjusted for age and sex.

Results During a median (interquartile range) follow-up of 10.7 years (6.3 to 15.9), 2698 out of 14 024 participants were diagnosed with cancer, of which 316 diagnoses (11.7%) were non-pathology-confirmed. Participants with non-pathology-confirmed diagnoses were older, more often women, and had a lower education. Most frequently non-pathology-confirmed cancer sites included central nervous system (66.7%), hepato-pancreato-biliary (44.5%), and cancers of unknown primary origin (31.3%). Survival of participants with non-pathology-confirmed diagnoses after one year was lower than survival of participants with pathology-confirmed diagnoses (32.6% versus 63.4%; risk difference of 30.8% [95% confidence interval = 25.2% to 36.2%]).

Conclusions Pathological confirmation of cancer is related to participant characteristics and cancer site. Furthermore, participants with non-pathology-confirmed diagnoses have worse survival than participants with pathology-confirmed diagnoses. Missing data on non-pathology-confirmed diagnoses may result in an underestimation of cancer incidence and in an overestimation of survival in cancer registries, and may introduce bias in aetiological research.

INTRODUCTION

With ageing populations worldwide, the incidence of cancer is rising. In 2018, 17 million people were diagnosed with cancer and 9.6 million people died from cancer.[1] Accurate and complete registration of incident cancers is pivotal for cancer statistics. However, most cancer registries primarily rely on pathology databases. Although this limits the risk of false-positive diagnoses, it may result in under-registration of cancers that are diagnosed purely on the basis of other sources than pathology, such as imaging features or tumour markers.[2,3] This may lead to an underestimation of cancer incidence and to inaccurate estimates of survival. Furthermore, aetiological studies often only include patients with a pathology-confirmed cancer diagnosis, which may induce bias if pathological confirmation is related to patient or cancer characteristics.

Several studies have investigated characteristics of patients with unstaged cancer based on the Surveillance, Epidemiology and End Results (SEER) database[4-6] or state cancer registries in the United States.[7-9] It has been found that unstaged cancer occurs more often in patients with older age and in patients residing in nursing homes. Also, unstaged cancers were often cancers with a poor survival such as cancer in the oesophagus, liver, or pancreas.[6,10] Missing cancer stage was explained by different reasons such as failure of the registry system, refusal for diagnostic testing, or absence of therapeutic consequences of staging. However, tumour grade was known in the majority of the unstaged cancers, which suggests that the studied cancer population is a combination of patients with missing cancer stage, but with pathological confirmation of the cancer, and patients with missing both cancer stage and pathology. Therefore, the incident number of patients with a cancer diagnosis based on other sources than pathology and their characteristics remain largely unknown.

Patients with suspected cancer undergo an extensive diagnostic work-up that includes physical examination, laboratory assessments, imaging features, and pathology. In some patients, pathology is not included in the diagnostic work-up of cancer. In this study, we will refer to these cancer diagnoses as ‘non-pathology-confirmed’ diagnoses. If pathology is used to confirm the cancer diagnosis, we will use the term ‘pathology-confirmed’ diagnosis.

We hypothesised that pathology is more often omitted in older, vulnerable patients with impaired survival. Insight into the number of non-pathology-confirmed cancer diagnoses and identification of the reasons for omitting pathology in the diagnostic work-up of cancer could stimulate and facilitate cancer registries and aetiological research studies to capture these cancer diagnoses. In the current study, we therefore determined the number of participants with a non-pathology-confirmed cancer diagnosis, their characteristics, and their overall and

cancer-specific survival in the population-based Rotterdam Study.

METHODS

Study population

This study is embedded within the Rotterdam Study, a prospective population-based cohort designed to study the occurrence and determinants of age-related diseases in the general population. The objectives and design have been described in detail previously.[11] In 1989, all residents aged 55 years and over of the district Ommoord in Rotterdam, the Netherlands, were invited to participate. This initial cohort comprised 7983 participants (response of 78%) and was extended with a second subcohort in 2000 with 3011 participants (response of 67%) who had become 55 years of age or moved into the study district. In 2006, the cohort was further extended with 3932 participants (response of 65%) aged 45 years and over. In total, the Rotterdam Study comprises 14 926 participants aged 45 years and over. The current study includes all participants who provided informed consent for follow-up data collection without a history of cancer at study entry (N=14 024).

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl) and into the WHO International Clinical Trials Registry Platform (ICTRP; www.who.int/ictrp/network/primary/en/) under shared catalogue number NTR6831. All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

Assessment of incident cancer

Diagnosis of incident cancer was based on medical records of general practitioners (including hospital discharge letters) and furthermore through linkage with Dutch Hospital Data (Landelijke Basisregistratie Ziekenhuiszorg), histology and cytopathology registries in the region (PALGA), and the Netherlands Cancer Registry. Using different sources of cancer diagnoses, the Rotterdam Study aims to capture also the non-pathology-confirmed diagnoses. Incident cancer was defined as any primary malignant tumour, excluding non-melanoma skin cancer. Each primary malignant tumour was registered, so that participants could have been diagnosed with multiple cancers. Cancer diagnoses were coded independently by two physicians and

classified according to the International Classification of Diseases, 10th revision (ICD-10). In case of discrepancy, consensus was sought through consultation with a physician specialised in internal medicine. Level of uncertainty of diagnosis was established as: certain (pathology-confirmed), probable (e.g., based on imaging features or elevated tumour markers without pathological confirmation), and possible (e.g., based on symptoms and physical examination, without further analysis and without pathological confirmation). Date of diagnosis was based on date of biopsy (solid tumours), laboratory assessment (haematological tumours), or – if unavailable – date of hospital admission or hospital discharge letter. For non-pathology-confirmed cancers, we used the date of imaging, date of laboratory assessment, date of physical examination, or – if unavailable – the date of hospital admission or hospital discharge letter. Follow-up was completed up to January 1st, 2014. In case of multiple cancers within one participant, we only included the first diagnosis for analyses.

Assessment of mortality

Information on vital status was updated continuously. Date of death was obtained and verified through notification by the municipal administration. Cause of death was obtained through follow-up of records of general practitioners and hospital discharge letters, and was classified according to the ICD-10 by two research physicians independently. Thereafter, a medical expert in the field reviewed all coded events. Cancer-specific mortality was defined as mortality attributed to malignant neoplasms (ICD-10, C00 to C97).

Assessment of characteristics

During home interviews at study entry, participants provided information on marital status, educational level, smoking status, and alcohol use. Marital status was categorised as living with or without partner. Educational level was classified into primary education, lower (lower or intermediate general education, or lower vocational education), intermediate (intermediate vocational education or higher general education), or higher (higher vocational education or university). Smoking habits were categorised as never, current, or former smoker. Alcohol use was classified into any use or no use of alcohol. At the research centre, height and weight were measured from which the body mass index (BMI, kg/m^2) was computed. Hypertension was defined as a resting blood pressure exceeding 140/90 mm Hg or the use of blood pressure lowering medication.[12] Diabetes was defined as a fasting serum glucose level ≥7.1 mmol/L, random serum glucose level ≥11.1 mmol/L, or the use of antidiabetic medication.[13] History of stroke, coronary heart disease (myocardial infarction, percutaneous coronary intervention, or coronary artery bypass grafting), chronic obstructive pulmonary disease, and neurodegenerative disease (dementia and parkinsonism) was assessed by interview and

verified by reviewing medical records.[14-17]

Statistical analyses

We used the independent samples *t* test (for continuous variables with a normal distribution), the Wilcoxon signed-rank test (for continuous variables with a skewed distribution), or the chi-square test (for categorical variables) to investigate differences in characteristics between participants with pathology-confirmed diagnoses (certain cancer) and those with non-pathology-confirmed diagnoses (probable and possible cancer). Furthermore, we compared cancer site specific percentages. An overview of the different ICD-10 codes used for categorisation into different cancer sites is presented in **Supplementary Table 1**.

Next, we explored a potential trend of pathological confirmation of cancer diagnoses over the years by plotting the number of incident pathology-confirmed and non-pathology-confirmed diagnoses per calendar year. We formally tested the association between year of diagnosis and source of diagnosis (with or without pathological confirmation) using logistic regression models. This analysis was performed for all cancer sites combined and for the five most frequent non-pathology-confirmed cancer sites separately. We constructed two nested models: Model I was unadjusted; Model II was adjusted for age at diagnosis (continuous).

We used two different methods to estimate overall survival. First, time to event was defined as follow-up time starting from date of diagnosis until date of death or date of censoring (loss to follow-up or end of the study period [January 1st, 2014]), whichever came first). Second, differences in overall survival between participants with and without pathological confirmation of the cancer diagnosis were visualised by Kaplan-Meier curves and tested with a log-rank test. We additionally computed standardised survival curves to remove the influence of different distributions in age at diagnosis and sex between the groups.[18,19]

Standardised survival curves were created using a pooled logistic regression model for death including the following covariates: time (years), time squared (years), pathological confirmation of the diagnosis, age at diagnosis (continuous), and sex. Interactions between time and time squared with source of diagnosis were added to the model to allow for a flexible estimation of the baseline hazard. After fitting the pooled logistic model, we estimated the probability of death if all participants with cancer had a pathology-confirmed diagnosis, and the probability of death if all participants with cancer had a non-pathology-confirmed diagnosis at each time point. Subsequently we calculated the difference in survival probability at each time point by taking the cumulative product as with Kaplan-Meier method. Confidence intervals (CIs) were obtained by bootstrapping. In sensitivity analyses, we repeated the analyses for cancer-specific survival and explored effect modification by median age, sex, education, and marital status.

Statistical analyses were performed using IBM SPSS Statistics Version 24.0[20] and the packages 'survival'[21] and 'survminer'[22] from R software Version 3.3.2.

RESULTS

During a median (interquartile range) follow-up of 10.7 (6.3 to 15.9) years, 2698 out of 14 024 participants were diagnosed with cancer. The majority had a pathology-confirmed diagnosis (n=2382 [88.3%]). Of the participants with a non-pathology-confirmed diagnosis, 257 (9.5%) had a probable diagnosis and 59 (2.2%) had a possible diagnosis.

Characteristics of participants categorised into three groups, i.e., without cancer, with pathology-confirmed diagnosis, and with non-pathology-confirmed diagnosis, are presented in **Table 1**. Participants with non-pathology-confirmed diagnoses were older at diagnosis than participants with pathology-confirmed diagnoses (median age of 83.2 versus 74.2 years, *P*<.001). Furthermore, they were more often women (55.7% versus 47.6%, *P*=.007), lived more often without a partner (37.3% versus 25.1%, *P*<.001), and had lower educational levels (*P*=.002). Lastly, participants with non-pathology-confirmed diagnoses had more often hypertension (71.8% versus 49.8%, *P*<.001) and more frequently a history of stroke (12.0% versus 6.9%, *P*=.001), coronary heart disease (15.8% versus 12.7%, *P*<.001), chronic obstructive pulmonary disease (19.0% versus 13.7%, *P*=.011), and neurodegenerative disease (30.4% versus 14.8%, *P*<.001) at diagnosis than participants with pathology-confirmed diagnoses.

Most frequently diagnosed cancer sites that were non-pathology-confirmed included central nervous system (66.7% of all central nervous system cancers), hepato-pancreato-biliary (44.5%), cancers of unknown primary origin (31.3%), lung and mesothelioma (19.7%), and urinary tract (17.5%, **Table 2**). There was no statistically significant relation between pathological confirmation of these cancer sites with calendar year after adjustment for age at diagnosis, indicating that the number of participants with a pathology-confirmed diagnosis did not increase or decrease during the study period (**Supplementary Figure 1** and **Table 3**).

Table 1 Characteristics of study population stratified by cancer diagnosis.

Characteristic	Participants without cancer (N=11 326)	Participants with cancer (N=2698)	
		Pathology-confirmed (N=2382)	Non-pathology-confirmed (N=316)
Age, years, median (IQR)	62.4 (57.7 to 72.7)	65.0 (60.2 to 72.0)	72.0 (66.1 to 78.1)
Women, No. (%)	6912 (61.0)	1135 (47.6)	176 (55.7)
Living without partner, No. (%)	3036 (26.8)	597 (25.1)	118 (37.3)
Educational level, No. (%)			
Primary	2081 (18.4)	423 (17.8)	76 (24.1)
Lower	4393 (38.8)	948 (39.8)	131 (41.5)
Intermediate	2886 (25.5)	700 (29.4)	82 (25.9)
Higher	1718 (15.2)	283 (11.9)	20 (6.3)
Body mass index, kg/m^2, mean (SD)	26.9 (4.2)	26.7 (3.8)	26.3 (3.7)
Smoking, No. (%)			
Never	3838 (33.9)	629 (26.4)	104 (32.9)
Former	4950 (43.7)	1106 (46.4)	109 (34.5)
Current	2313 (20.4)	608 (25.5)	90 (28.5)
Alcohol use, No. (%)	7843 (69.2)	1683 (70.7)	188 (59.5)
Age at cancer diagnosis, No. (%)			
45-65 years		372 (15.6)	8 (2.5)
65-75 years		897 (37.7)	42 (13.3)
75-85 years		870 (36.5)	136 (43.0)
>85 years		243 (10.2)	130 (41.1)
Median (IQR)		74.2 (68.0 to 80.3)	83.2 (78.0 to 88.0)
Comorbidities at cancer diagnosis, No. (%)			
Stroke		164 (6.9)	38 (12.0)
Coronary heart disease		302 (12.7)	50 (15.8)
Hypertension		1186 (49.8)	227 (71.8)
Diabetes		324 (13.6)	37 (11.7)
Chronic obstructive pulmonary disease		326 (13.7)	60 (19.0)
Neurodegenerative disease		353 (14.8)	96 (30.4)

Characteristics are measured at entry in the Rotterdam Study except for age at cancer diagnosis and comorbidities. Missing values are not imputed and therefore numbers do not always sum up to 100%. IQR = Interquartile range, N = number of participants, SD = standard deviation.

Table 2 Overview of number of pathological confirmations per cancer type.

Cancer site	Pathology-confirmed diagnosis (N=2382)	Non-pathology-confirmed diagnosis (N=316)	All cancer diagnoses (N=2698)
Head and neck	83 (94.3)	5 (5.7)	88
Oesophagus and gastric	140 (97.9)	3 (2.1)	143
Colorectal	397 (96.6)	14 (3.4)	411
Hepato-pancreato-biliary	81 (55.5)	65 (44.5)	146
Lung and mesothelioma	314 (80.3)	77 (19.7)	391
Bone and soft tissue	19 (90.5)	2 (9.5)	21
Breast	341 (96.6)	12 (3.4)	353
Female genital organs	112 (94.9)	6 (5.1)	118
Male genital organs	387 (95.6)	18 (4.4)	405
Unitary tract	165 (82.5)	35 (17.5)	200
Central nervous system	9 (33.3)	18 (66.7)	27
Haematological	188 (90.0)	21 (10.0)	209
Other	80 (88.9)	10 (11.1)	90
Unknown primary origin	66 (68.8)	30 (31.3)	96

Numbers are displayed in total number (percentage per row).

Table 3 Association between calendar year of diagnosis and pathological confirmation of the cancer.

Cancer site	N pathology-confirmed diagnosis	N non-pathology-confirmed diagnosis	Model I	Model II
			OR (95% CI)	OR (95% CI)
All cancer sites	2382	316	1.01 (1.00 to 1.03)	1.01 (0.99 to 1.03)
Hepato-pancreato-biliary	81	65	0.97 (0.92 to 1.02)	0.95 (0.89 to 1.02)
Lung and mesothelioma	314	77	1.05 (1.01 to 1.09)	1.03 (0.99 to 1.08)
Unitary tract	165	35	0.96 (0.91 to 1.02)	0.96 (0.90 to 1.03)
Central nervous system	9	18	1.05 (0.93 to 1.19)	1.06 (0.94 to 1.21)
Unknown primary	66	30	1.06 (0.99 to 1.14)	1.05 (0.98 to 1.13)

Model I = unadjusted. Model II = adjusted for age at diagnosis (continuous).
CI = confidence interval, N = number of participants, OR = odds ratio.

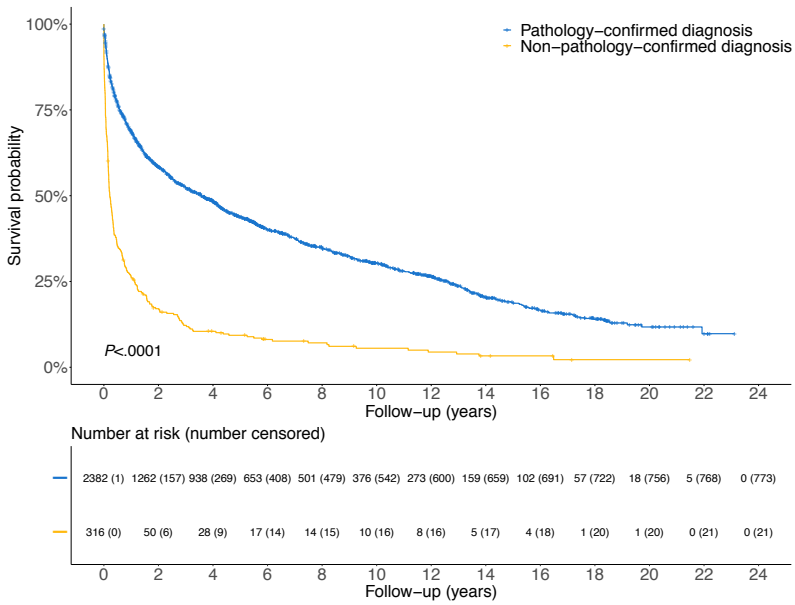

Figure 1 Kaplan-Meier curves for overall survival of participants with a pathology-confirmed diagnosis (blue) or a non-pathology-confirmed diagnosis (yellow).
Participants with a non-pathology-confirmed diagnosis had worse overall survival than those with a pathology-confirmed diagnosis (P of log-rank test <.0001). Participants were censored if they were lost to follow-up or at the end of the study period (January 1st, 2014), whichever came first.

Of the 2382 participants with a pathology-confirmed diagnosis, 1154 participants (48.4%) died from cancer and 455 participants (19.1%) died due to other causes, such as heart failure, dementia, and cardiac arrest. Among participants with non-pathology-confirmed diagnoses, 231 (73.1%) died from cancer, and 63 participants (19.9%) died from other causes. The overall survival of participants with non-pathology-confirmed diagnoses was lower than the overall survival of participants with pathology-confirmed diagnoses (*P* for log-rank test <.0001, **Figure 1**). After adjusting for age at diagnosis and sex, the overall survival of participants with non-pathology-confirmed diagnosis was 30.8% (95% CI = 25.2% to 36.2%) lower one year after diagnosis than the overall survival of participants with pathology-confirmed diagnoses (survival probability was 32.6% versus 63.4%, respectively, **Figure 2**). Two and five years after diagnosis, the difference in survival was respectively 29.3% (95% CI = 24.2% to 33.9%) and 22.5% (95% CI = 17.7% to 26.4%). Cancer-specific survival probability was comparable to overall survival probability, with a lower cancer-specific survival in participants with non-pathology-confirmed diagnoses (37.2%) than in participants with pathology-confirmed diagnoses (67.4%, **Supplementary Figure 2**). No significant effect modification was observed across different strata of median age, sex, education, and marital status.

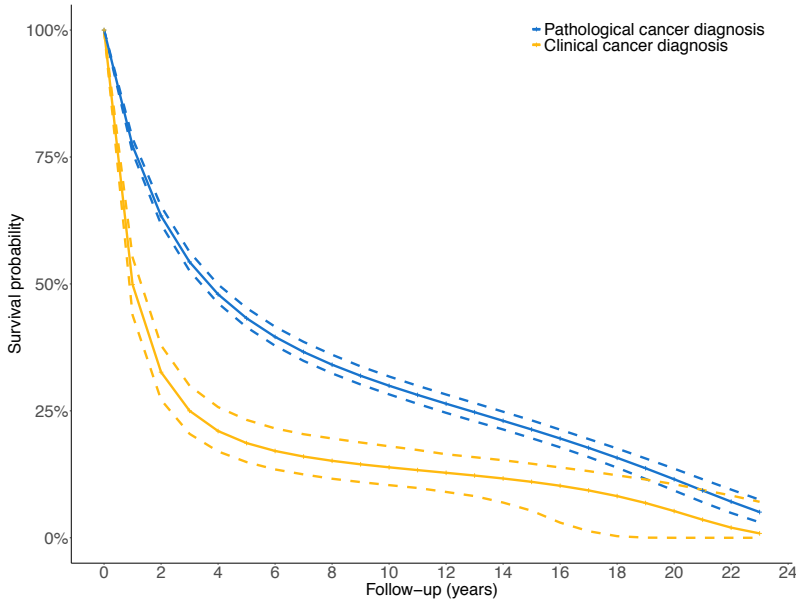

Figure 2 Standardised survival curves for overall survival of participants with a pathology-confirmed diagnosis (blue) or a non-pathology-confirmed diagnosis (yellow).
Dashed lines represent 95% confidence intervals. Survival curves are adjusted for age at diagnosis and sex. The risk difference of overall survival between participants with a non-pathology-confirmed and a pathology-confirmed diagnosis was 30.8% after one year, 29.3% after two years, and 22.5% after five years.

DISCUSSION

In a large population-based cohort study, we showed that non-pathology-confirmed diagnoses of cancer represent an additional ten percent of cancer diagnoses besides pathology-confirmed diagnoses. Pathological confirmation of cancer was associated with multiple participant characteristics, comorbidities, cancer site, and survival. The proportion of participants with pathology-confirmed diagnoses did not change over time.

In line with previous studies investigating characteristics of patients with unstaged cancer,[4-9] we found that participants with a non-pathology-confirmed diagnosis were on average older than those with a pathology-confirmed diagnosis. There are different reasons for this observation. First, older patients have more comorbidities that may be of greater health concern than a potential cancer diagnosis.[23] Therefore, the diagnostic cancer work-up may be partly omitted. Furthermore, older patients are sometimes more vulnerable, limiting the ability

to obtain pathology material for diagnosis through invasive procedures, such as endoscopic retrograde cholangiopancreatography (ERCP) for pancreatic cancer. In addition, age and comorbidities are associated with potentially less intensive treatment assignment including palliative radiotherapy and hormonal therapy.[24,25] Although pathological confirmation of the cancer is often preferred, it may not always be mandatory for these treatment regimens.[23,26]

Lack of therapeutic consequences of pathological confirmation may explain why cancers with a poor survival in particular, such as cancer of central nervous system, hepato-pancreato-biliary tract, and lung were often diagnosed without pathological confirmation. Cancers at these organ sites are often detected in a more advanced stage, limiting treatment options to palliative treatments. In addition, we found that participants with cancer of unknown primary origin often had no pathological confirmation, suggesting that these participants had metastatic disease and did not undergo further diagnostic testing.[27] Another explanation for this finding is that cancers at these sites are less accessible for obtaining tumour tissue, in particular regarding cancers of the central nervous system. Lastly, cancers in the urinary tract including renal cell carcinoma were often non-pathology-confirmed. These cancers can be diagnosed non-invasively with imaging modalities (renal cell carcinoma). Also, prostate cancer is sometimes diagnosed non-invasively based on tumour markers. Watchful waiting is increasingly being considered as an option for older, vulnerable patients with regard to prostate cancer,[28] which may result in a lower number of pathology-confirmed diagnoses.

Interestingly, we showed that participants with a non-pathology-confirmed diagnosis of cancer had worse overall and cancer-specific survival than participants with a pathology-confirmed diagnosis. Although the number of cancers with a poor survival was more frequently represented among non-pathology-confirmed diagnoses, this difference in cancer type distribution cannot completely explain the observed difference in survival. Therefore, the difference in survival may indicate that pathological confirmation is more often omitted in patients with a 'worse' cancer prognosis. In contrast, previous studies have found a better survival in patients with unstaged cancer. For instance, the survival of patients with unstaged colorectal cancer was higher than the survival of patients with distant-staged cancer.[5] Furthermore, non-pathology-confirmed early stage lung cancer patients had a better cancer-specific survival than patients with a pathology-confirmed diagnosis, due to the occurrence of benign lung nodules among the diagnosed cancers without pathological confirmation.[29] This misclassification of benign tumours may partly explain the discrepancy in survival between previous studies and the current study. Although we cannot exclude that we also classified benign tumours as non-pathology-confirmed cancers, the number of misclassified tumours is expected to be low because of the persistent poor cancer-specific survival of participants with a non-pathology-confirmed diagnosis.

We have previously shown that cancer registries primarily rely on pathology databases as signalling source of cancer diagnoses, resulting in under-registration of non-pathology-confirmed diagnoses.[30] The findings of our current study indicate that under-registration of such cancers may result in underestimation of the cancer incidence, and in overestimation of cancer survival. In addition, non-pathology-confirmed diagnoses were related to multiple characteristics including age, sex, smoking status, and education, and to cancer site. Most aetiological studies only include patients with a pathology-confirmed diagnosis, which may induce information bias and result in inaccurate estimates of association.[31] For these reasons, our results suggest that registries and research studies should also include patients with non-pathology-confirmed diagnoses for potential sensitivity analyses.

The main strength of this study is the unique setting of the Rotterdam Study in which cancer registration relies on medical letters and medical records from the general practitioners in addition to signalling of diagnoses through the nationwide pathology database as well as linkage to the Netherlands Cancer Registry. This allowed us to investigate also non-pathology-confirmed diagnoses not registered through the pathology database. Furthermore, we estimated survival by computing standardised survival curves in addition to the unadjusted Kaplan-Meier curves. Unfortunately, we could not adjust these survival curves for frailty. Although the Rotterdam Study started to collect data on frailty from 2009 onwards, including weight loss, physical activity, weakness, slowness, and fatigue to calculate the Fried frailty index,[32] this was not available for the majority of the participants (<20%), or – if available – was measured several years after cancer diagnosis. Another limitation is that the date of diagnosis is determined differently for non-pathology-confirmed and pathology-confirmed diagnoses. It is plausible that participants with non-pathology-confirmed diagnoses were diagnosed sooner, resulting in a slightly longer cancer-specific survival. Lastly, we cannot rule out that non-pathology-confirmed diagnoses are benign tumours. However, we classified cancers based on all the available information from medical letters and medical records, limiting the number of false-positive diagnoses. In addition, we showed that participants with non-pathology-confirmed diagnoses had worse cancer-specific survival persistent over time, suggesting that these cancers were malignant.

In conclusion, we show that purely non-pathology-confirmed diagnoses represent ten percent of the total number of diagnosed cancers, besides pathology-confirmed diagnoses. Pathological confirmation is associated with several characteristics and with worse overall and cancer-specific survival. Our findings suggest that missing data or exclusion of non-pathology-confirmed diagnoses may result in underestimation of the true cancer incidence, overestimation of survival, and potentially in biased aetiological research findings.

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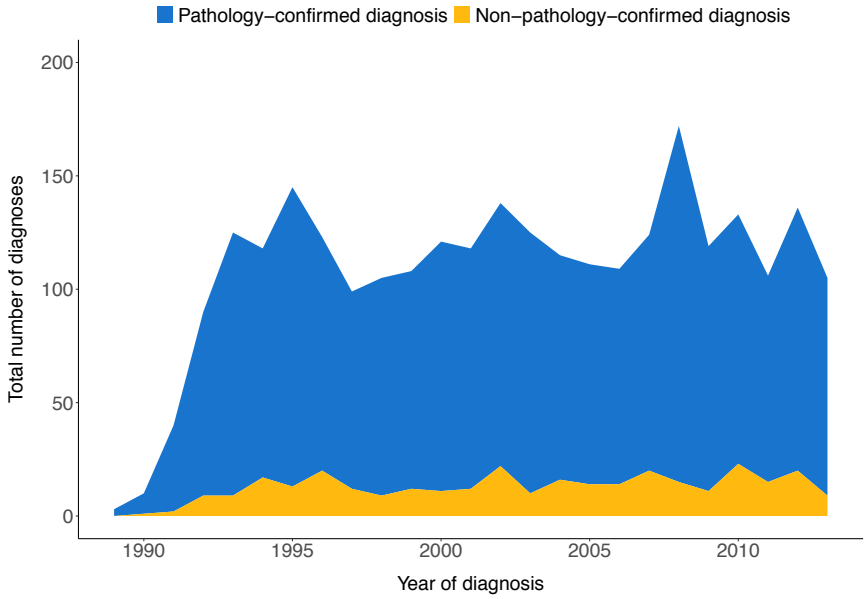
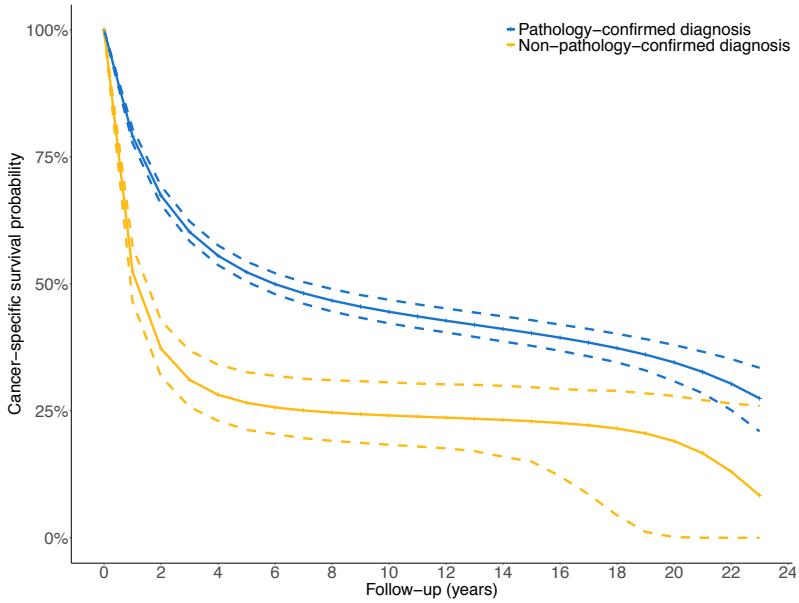
SUPPLEMENTARY MATERIAL



Supplementary Figure 1 Trends in cancer diagnoses per calendar year of cancer diagnosis. *Number of pathology-confirmed diagnoses (blue) and non-pathology-confirmed diagnoses (yellow) are shown as the total number of cancer diagnoses.*



Supplementary Figure 2 Standardised survival curves of individuals with a pathology-confirmed diagnosis (blue) or a non-pathology-confirmed diagnosis (yellow).
Dashed lines represent 95% confidence intervals. Survival curves are adjusted for age at diagnosis and sex. The risk difference of cancer-specific survival between participants with a non-pathology-confirmed and a pathology-confirmed diagnosis was 30.2% after one year, 29.1% after two years, and 24.3% after five years.

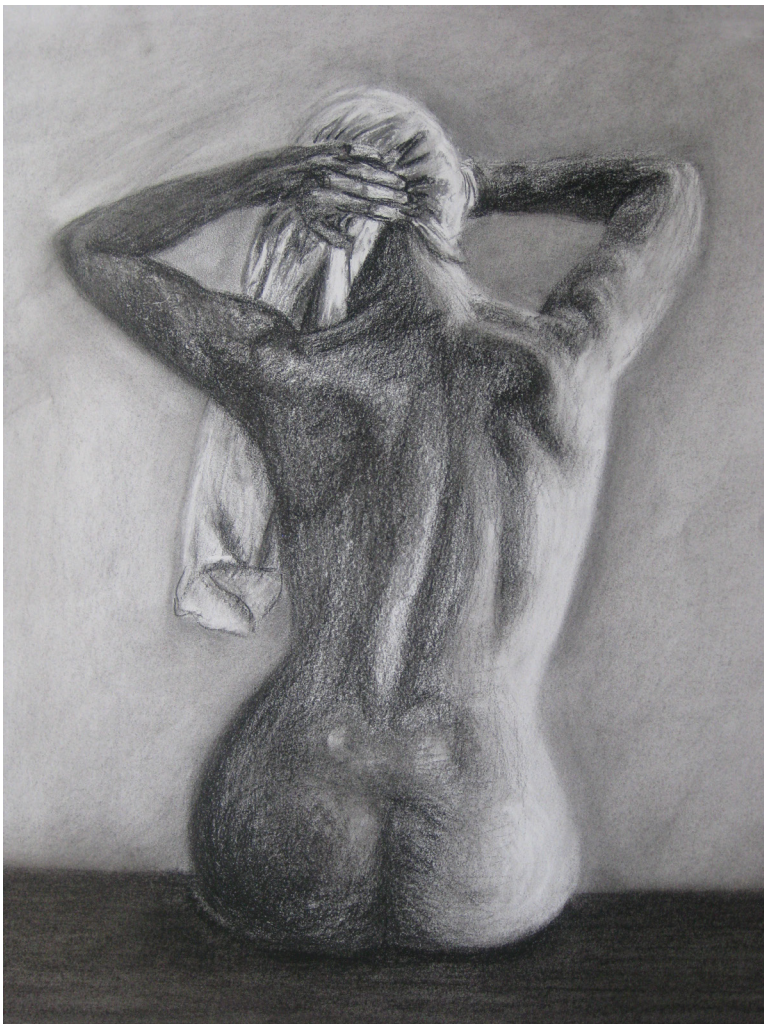
Supplementary Table 1 Overview of the used ICD-10 codes per organ system.

Organ system	Corresponding ICD-10 code
Head and neck	C01-C14, C30, C32, C69, C73
Oesophagus and gastric	C15, C16
Colorectal	C18-C20
Hepato-pancreato-biliary	C22-C25
Lung and mesothelioma	C34, C45
Bone and soft tissue	C40, C41, C49
Breast	C50
Female genital organs	C51-C57
Male genital organs	C60-C63
Unitary tract	C64-C68
Central nervous system	C70-C72
Haematological	C81-C96
Other	C17, C21, C26, C37-C39, C43, C48, C75, C76
Unknown primary origin	C80

ICD = International Classification of Diseases.

Part II

Cancer and cognition



Chapter 4

Trajectories of cognitive and motor function between ages 45 and 90 years

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* Both authors contributed equally to this study

ABSTRACT

Background To establish trajectories of cognitive and motor function, and to determine the sequence of change across individual tests in community-dwelling individuals aged 45 to 90 years.

Methods Between 1997 and 2016, we repeatedly assessed cognitive function with five tests in 9514 participants aged 45 to 90 years from the population-based Rotterdam Study. Between 1999 and 2016, we measured motor function with three tests in 8297 participants. All participants were free from dementia, stroke, and parkinsonism. We assessed overall and education-specific cognitive and motor trajectories using linear mixed models with age as time scale. Next, we determined the sequence of change across individual tests.

Results The number of assessments per participant ranged between one and six (mean [standard deviation [SD]] interval was 5.1 years [1.4]) for cognitive function, and one and four (5.4 years [1.4]) for motor function. Cognitive and motor trajectories declined linearly between ages 45 and 65 years, followed by steeper declines after ages 65 to 70 years. Lower educated participants had lower cognitive function at age 45 years (baseline), and declined faster on most cognitive, but not on motor tests than higher educated participants. Up to a 25-year age difference between the fastest and slowest declining test scores was observed.

Conclusions At a population-level, cognitive and motor function decline similarly. Compared to higher educated individuals, lower educated individuals had lower cognitive function at baseline, and a faster rate of decline thereafter. These educational-effects were not seen for motor function. These findings benefit the understanding of the natural course of cognitive and motor function during ageing, and highlight the role of education in the preservation of cognitive but not motor function.

INTRODUCTION

Understanding the natural course of cognitive and motor function during brain ageing is pivotal to determine deviations in function that may signal early stages of clinical neurodegenerative diseases.[1,2] Decline in both cognitive and motor function has been associated with an increased risk of dementia, Parkinson's disease, and stroke.[1-3] In addition, we have recently shown that individuals in whom decline in motor function precedes decline in cognitive function are at an increased risk of dementia.[3] Numerous studies have quantified the temporal relation of cognitive and motor function with advancing age,[4-17] yet little is known about the sequence of individual cognitive and motor tests in a population free from neurodegenerative diseases and stroke.

Comparing trajectories of cognitive and motor tests in the general population reveals whether decline in motor function precedes decline in cognitive function. In addition, it identifies the specific individual tests that have the earliest signs of decline. Such findings could inform clinicians about which cognitive and motor tests are most sensitive to detect change in cognitive or motor function. These trajectories can also be used to signal vulnerable patient groups that deviate from their expected course based on several key characteristics, such as age, sex, educational level, or genes. These characteristics significantly influence cognitive function and the rate of cognitive decline, but their effects on motor function beyond gait speed are less understood.[18,19]

Alike changes in brain structure, we hypothesise that change in cognitive and motor function accelerates with advancing age.[20] To model this non-linear change, we present trajectories of cognitive and motor function. In addition, we assess the effects of key determinants of cognitive and motor function, namely age, sex, education, and apolipoprotein E genotype (*APOE*) on these trajectories. Finally, we determine the sequence of change of individual cognitive and motor function tests.

METHODS

Study design

This study was embedded within the Rotterdam Study, a prospective population-based cohort designed to study the occurrence and determinants of age-related diseases in the general population.[21] In 1989, all inhabitants aged at least 55 years from Ommoord, a well-defined

district in Rotterdam, the Netherlands received an invitation to participate. This initial cohort comprised 7983 participants. In 2000, 3011 participants who had become 55 years of age or moved into the study district since the start of the study were additionally included in the cohort. In 2006, a further extension of the cohort was initiated in which 3932 participants aged at least 45 years participated. In total, the Rotterdam Study comprises 14 926 participants aged at least 45 years. The overall response rate across all three recruitment waves was 72%.

Standard protocol approvals, registrations, and patient consents

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

Study population

Of a total of 14 926 participants, we excluded those with a history of dementia (n=907), stroke (n=846), Parkinson's disease (n=300), or parkinsonism (n=20) at time of their first cognitive or motor assessment. Next, we excluded participants with insufficient data to determine whether they had a history of one or multiple of these diseases (n=1800). Baseline and follow-up ascertainment methods for dementia, stroke, Parkinson's disease, and parkinsonism have previously been described in detail.[22] In addition, five participants were excluded because they did not provide informed consent to access medical records and hospital discharge letters during follow-up. From the remaining 11 048 participants, 1494 participants were excluded because they did not have data available on any cognitive or motor test. Finally, we excluded assessments from participants after they had reached age 90 years in order to minimise the influence of leverage points on the trajectories of cognitive and motor function. This resulted in an additional exclusion of 33 participants who did not have any cognitive or motor function assessment at all before the age of 90 years, leaving 9521 participants with at least one cognitive or motor assessment. During follow-up, we excluded assessments of participants after the age of 90 years (n=1266) and of participants after a dementia, stroke, or Parkinson's disease diagnosis (n=3175). All of the included participants were thus free from neurodegenerative diseases and stroke at time of their test assessments. In total, 155 347 cognitive function assessments from 9514 participants and 62 545 motor function assessments from 8297 participants were available for analyses.

Assessment of cognitive function

Between 1997 and 2016, participants underwent cognitive assessments at the research centre using a neuropsychological test battery every three to six years.[6,21] This battery included the Word Fluency Test,[23] Letter-Digit Substitution Test,[24] and Stroop Test (Reading, Naming, and Interference subtask).[25] In 2002, the 15-Word Learning Test (Immediate recall, Delayed recall, and Recognition) was added to the test protocol.[26] This test protocol was further expanded with the Design Organisation Test in 2006.[27] Assessments of these cognitive tests have previously been validated and have a reasonable to good test-retest reliability.[28-31]

Word Fluency Test

In the Word Fluency Test, participants were asked to mention as many animals as possible within sixty seconds, thereby measuring semantic fluency.[23] The total number of correct answers was used as test score, with a maximum score of thirty in our study protocol.

Letter-Digit Substitution Test

The Letter-Digit Substitution Test is a modified version of the Symbol Digit Modalities Test for which participants were asked to write down as many numbers underneath the corresponding letters as possible within sixty seconds, following a key that shows correct combinations.[24] This test captures both information processing speed and aspects of executive function. The total number of correct answers was used as test score with a maximum attainable score of 125.

Stroop Test

The Stroop Test consists of three different subtasks, i.e., Reading, Naming, and Interference.[25] In the Stroop Reading subtask, participants were asked to read the printed colour names. For the Stroop Naming subtask, participants were asked to name the printed colour blocks. In the Interference subtask, participants were asked to name the ink colour of colour names printed in incongruous ink colours (information processing on an interference subtask). The time taken to complete the subtask was used as the outcome for each subtask separately and was adjusted for failures, i.e., total time plus for each failure the total time divided by the number of items, multiplied with 1.5.[32] Thus, a higher score indicates a worse performance. The Stroop Test assesses information processing speed and executive function.

Word Learning Test

The Word Learning Test comprises three subtasks: Immediate recall, Delayed recall, and Recognition.[26] For Immediate recall, participants were three times visually presented with

a sequence of 15 words and were subsequently asked to recall as many of these words as possible, measuring verbal learning. Free Delayed recall was tested approximately ten minutes after visual presentation, evaluating retrieval from verbal memory. Recognition was tested by visually presenting the participants a sequence of 45 words, followed by correctly recognising the 15 words presented during the Immediate recall while mixed with 30 other words. Outcome variables were the mean number of words of three trials immediately recalled (as a summary score for Immediate recall), after the delay of ten minutes (as a score for free Delayed recall), and the mean number of correctly recognised words during the recognition trial (as a score for Recognition), with a maximum score of 15 per subtask.

Design Organisation Test

The Design Organisation Test consists of square black-and-white grids with visual patterns, of which participants were asked to reproduce as many designs as possible in two minutes using a numerical code key. It measures visuospatial abilities and is based on and highly correlated to WAIS-III block design,[27] but is less dependent on motor skills. Test score on the Design Organisation Test has a range from 0 to 56 points for each individual, with higher scores indicating better performance.

Assessment of motor function

Participants repeatedly underwent motor tests every three to six years at the research centre between 1999 and 2016. This motor test battery included two tests to assess fine motor function and a quantitative gait assessment to assess gross motor function. From 1999 onwards, the Purdue Pegboard Test was implemented into the study protocol to assess manual dexterity. Assessment of fine motor function was further expanded in 2008 with the implementation of the Spiral Archimedes Test to assess manual precision. In 2009, a quantitative gait assessment using an electronic walkway at the research centre was implemented in the core study protocol.

Purdue Pegboard Test

For the Purdue Pegboard Test, participants were asked to place as many as possible cylindrical metal pegs into one of the 25 holes in a pegboard in thirty seconds in three separate trials, using their left hand only, right hand only, and both hands simultaneously, measuring fine motor function.[33] The test-retest reliability of assessments has been established previously. The outcome variable was the sum-score of Purdue Pegboard Test score of these three trials, with a maximum of 75 points.

Archimedes Spiral Test

The Archimedes Spiral Test measures fine motor function by requiring participants to trace a picture of a spiral template that was printed on paper attached to an electronic drawing board (WACOM Graphire Wireless Pen Tablet, model CTE-630BT).[7] Participants were instructed to trace the spiral as accurately and as fast as possible using a special pen with their dominant hand, starting in the middle (**Supplementary Figure 1**). Automatic quantitative analyses were done using custom-made software written in MatLab (version 8.1, The Mathworks, Natick, MA, USA), and processed and visually inspected by two trained physicians (S.L., S.K.L.D.) for analyses (intraclass correlation coefficient for interrater reliability for all test components >.95). A smoothly drawn spiral would have a length of drawing about 56 cm (the length of the template) with little deviation from the template, a low variability in speed, and no crossings (**Supplementary Figure 1**). The mean amplitude in deviation from the template to spiral drawing (cm) was used as outcome, since it is sensitive to capture small differences in fine motor function.[7] A higher deviation indicates worse performance.

Gait assessment

Gait was evaluated using a 5.79m long walkway (GAITRite Platinum, CIR systems, Sparta, NJ: 4.88m active area, 120-Hz sampling rate).[21] The reliability and validity of assessments obtained with this device have previously been established.[34] The standardised gait protocol comprises three walking conditions: normal walk, turning, and tandem walk. In the normal walk, participants walked at their usual pace across the walkway. In turning, participants walked at their usual pace, turned halfway, and returned to the starting position. In the tandem walk, participants walked heel-to-toe on a line across the walkway. Based on the recorded footfalls, the walkway software calculated thirty parameters, including 25 from the normal walk, two from turning, and three from the tandem walk. In **Supplementary Table 1**, we provide descriptions of the thirty gait parameters.

To summarise these thirty gait parameters into several independent domains, we log-transformed skewed gait parameters to obtain a normal distribution, and subsequently standardised all continuous gait parameters. Next, we conducted a principal component analysis with Varimax rotation to derive gait domains, as previously described.[35] This yielded seven gait domains with an eigenvalue > 1, which we labelled in accordance with the gait parameter that had the highest correlation coefficient with the corresponding domain: rhythm (step time), variability (standardised step length), phases (double support), pace (velocity), tandem (sum of step distance), turning (turning time), and base of support (stride width).[35] These gait domains are illustrated in **Supplementary Figure 2**. Higher values of the gait domains except 'pace', represent worse gait performance. Based on these seven gait

domains, the Purdue Pegboard Test and the Archimedes Spiral Test, a total of nine different facets of motor function were available for analysis.

Assessment of study population characteristics

During home interviews, educational level was assessed and categorised as primary education ('primary'), lower or intermediate general education, or lower vocational education ('lower'), intermediate vocational education or higher general education ('intermediate'), and higher vocational education or university ('higher'). Smoking and alcohol habits were assessed during the same home interviews. Participants were categorised as current, former, or never smokers. Alcohol habits were classified into any use or no use of alcohol. At the research centre, height and weight were measured from which the body mass index (BMI, kg/m^2) was computed. Blood pressure was measured twice in sitting position on the right arm using a random-zero sphygmomanometer, and the average of two measurements was used. In addition, non-fasting blood samples were collected and glucose levels were determined. In the initial subcohort, diabetes mellitus was defined as a random or post-load serum glucose concentration ≥11.1 mmol/L, or the use of drugs to lower blood glucose. In the first and second extension subcohorts, diabetes mellitus was defined as a fasting serum glucose concentration ≥7.0 mmol/L, a non-fasting serum glucose concentration ≥11.1 mmol/L (only if fasting serum was unavailable), or usage of blood glucose lowering drugs. *APOE* genotype was determined using polymerase chain reaction on coded DNA samples in the initial cohort and with a bi-allelic TaqMan assay in the two extensions.[36,37] *APOE* ε4 carrier status was defined as carrier of one or two *APOE* ε4 alleles.

Statistical analysis

We assessed trajectories of cognitive and motor function using linear mixed models with random intercepts and slopes. If models did not converge with both random intercepts and slopes, only a random intercept was used. Age of the participant at time of cognitive or motor function assessment was used as underlying time scale. To capture possible non-linearity, we included natural cubic splines of age with one, two, or three knots, depending on model performance determined by a likelihood ratio test ($P<.05$). Knots were defined at the median, tertiles, or quartiles for respectively one, two, or three knots. We only reported *P*-values for each of the age intervals, since appropriate interpretation of effect estimates is hindered by the inclusion of natural cubic splines in the models. Skewed test outcomes (i.e., Stroop Tests, Word Learning Test: Recognition subtask, Archimedes Spiral Test, and gait domains 'variability' and 'tandem') were natural log-transformed to reach an approximate normal distribution, and were back-transformed for visualisation. In addition, we visualised trajectories of cognitive

and motor function by sex, education, or both, using interaction terms on the additive scale between age and sex, age and educational level, and age with sex and educational level.

Missing data on educational level (1.1%) were imputed by chained equations with five iterations. We generated one imputed dataset based on age at baseline and sex. Furthermore, we assessed trajectories for *APOE* ε4 carriers and non-carriers separately by including an interaction term between age and *APOE ε4* status. This analysis was limited to the participants with known *APOE* ε4 status (N participants = 8986 for cognitive tests and N participants = 7835 for motor tests).

Next, we repeated these analyses by standardising the cognitive and motor test results to the test performance of the age of 45 years (study baseline) to investigate the temporal course of change across tests with ageing. Skewed test outcomes were natural log-transformed before standardisation. We depicted a threshold of decline in performance of 0.5 and 1.0 standard deviation (SD) compared to the test score at age 45 years. We subsequently assessed the age at which the test score had reached a decline of 0.5 and 1.0 SD compared to the test result at age 45 years.

Data were analysed with SPSS Statistics version 24.0 (IBM Corp., Armonk, NY) and R, CRAN version 3.4.3 'mice' and 'nlme' packages.[38,39]

RESULTS

Characteristics of the study population at time of study entry are presented in **Table 1**. A total of 9514 participants contributed to the cognitive function assessments. The mean (SD) age at first cognitive assessment was 64.7 years (9.5) and 5442 (57.2%) of the participants were women. Of all participants, 2058 (21.6%) had a single cognitive assessment, 4362 (45.8%) had two, 1174 (12.3%) had three, and 1920 (20.2%) had at least four cognitive assessments. The mean interval between tests was 5.1 years (1.4). During follow-up up to January 1st, 2016, 2977 out of 9514 participants (31.3%) died.

A total of 8297 participants contributed to the motor function assessments with a mean (SD) age at first assessment of 64.6 years (10.0), of whom 4737 (57.1%) were women (**Table 1**). Out of these participants, 2136 (25.7%) had a single motor function assessment, 4192 (50.5%) had two, 1091 (13.1%) had three, and 878 (10.6%) had four motor assessments with a mean (SD) test interval of 5.4 years (1.4). Out of 8297 participants, 1903 died (22.9%) during follow-up. The number of participants per cognitive and motor test is shown in **Supplementary Table 2**. **Supplementary Table 3** shows the characteristics of the excluded participants.

Overall, excluded participants were older at study entry, attained more often a lower level of education, and had a higher mean systolic blood pressure than included participants.

Trajectories of cognitive function

Performance on the cognitive tests declined with advancing age. Decline on cognitive tests was generally linear between ages 45 and 65 years, followed by a steeper, non-linear decline. Men had higher scores on most cognitive tests and generally declined less fast than women (*P*=.003 for Letter-Digit Substitution Test, *P*=.02 for Word Learning Test: Immediate recall, *P*=.05 for Word Learning Test: Delayed recall). These differences between men and women disappeared after assessing the trajectories per educational level, suggesting that sex-differences were largely attributable to differences in the level of attained education between men and women. As such, results from here onwards are presented per educational level for men and women combined.

For each higher level of attained education, participants showed better performance on all cognitive tests at age 45 years (**Figure 1**). Differences in trajectories of cognitive function between participants with 'primary' educational level and participants with other educational levels became larger with advancing age, albeit not statistically significant. In addition, participants with 'higher' education declined slower than those with 'primary' education over time on the Interference subtask of the Stroop Test (*P*=.002, **Figure 1E**) and the Word Learning Test: Recognition subtask (*P*=.017, **Figure 1H**). However, they declined faster than participants with 'primary' education on the Word Fluency Test (*P*=.048, **Figure 1A**) and the Word Learning Test: Delayed recall subtask (*P*=.007, **Figure 1G**).

Regarding *APOE* ε4 carrier status, carriers declined faster on all cognitive tests than non-carriers (*P* for interaction between age and *APOE* ε4 carrier status <.005), except on the Design Organisation Test that showed similar trajectories for carriers and non-carriers (**Supplementary Figure 3**).

Trajectories of motor function

Trajectories of decline in motor function varied across different motor tests (**Figure 1**) with the gait domain 'phases' and the Purdue Pegboard Test declining first at the age of 56 and 60 years, respectively. Performance on the gait domains 'rhythm', 'tandem', and 'base of support' remained largely stable over time. Significant differences between men and women were only found for trajectories of the domain 'tandem' and 'phases', with women performing increasingly worse over age than men (*P*=.005 for 'tandem' and *P*<.001 for 'phases').

Table 1 Characteristics of the study populations.

Characteristic	Analysis of cognitive function (N=9514)	Analysis of motor function (N=8297)
Age at study entry, years, mean (SD)	62.0 (7.9)	60.9 (7.4)
Age at first assessment, years, mean (SD)	64.7 (9.5)	64.6 (10.0)
Women, No. (%)	5442 (57.2)	4737 (57.1)
Educational level, No. (%)		
Primary	1160 (12.2)	886 (10.7)
Lower	3889 (40.9)	3375 (40.7)
Intermediate	2751 (28.9)	2422 (29.2)
Higher	1714 (18.0)	1614 (19.5)
Number of assessments*, No. (%)		
1	2058 (21.6)	2136 (25.7)
2	4362 (45.8)	4192 (50.5)
3	1174 (12.3)	1091 (13.1)
≥4	1920 (20.2)	878 (10.6)
Median number of assessments (range)	2 (1-6)	2 (1-4)
Test assessment interval, years, mean (SD)	5.1 (1.4)	5.4 (1.4)
Body mass index, kg/m^2, mean (SD)	27.0 (4.1)	27.1 (4.2)
Systolic blood pressure, mm Hg, mean (SD)	136 (20.8)	136 (20.6)
Diabetes mellitus, No. (%)	865 (9.1)	735 (8.9)
Smoking, n (%)		
Never	2941 (30.9)	2522 (30.4)
Former	4558 (47.9)	4063 (49.0)
Current	1944 (20.4)	1663 (20.0)
Alcohol use, No. (%)	7760 (81.6)	6928 (83.5)
APOE ε4 carrier, No. (%)	2539 (26.7)	2217 (26.7)

Characteristics were measured at study entry except for age at first assessment.
Missing values for all characteristics but educational level were not imputed and therefore numbers do not always sum up to 100%.
** Gait was considered as one assessment, because virtually all participants (95%) with an available gait assessment had complete values for all underlying gait parameters. Therefore, the presented number of motor assessments is independent from the number of underlying available gait parameters that were used to compute seven gait domains.*
APOE = apolipoprotein E, N = number of participants, SD = standard deviation.

In contrast to the effects of education on cognitive function, motor function trajectories were not associated with educational level (**Figure 1**), but those with a 'primary' educational level performed better over time on the Purdue Pegboard Test than participants with other

educational levels (*P*<.016, **Figure 1J**). In addition, they decreased less fast on the gait domains 'rhythm', 'phases', and 'turning' than participants with higher educational levels (*P* for all tests <.039, **Figures 1L**, **1N**, and **1Q**).

APOE ε4 carriers performed worse with advancing age than non-carriers on the Purdue Pegboard Test and on the gait parameters 'phases', and 'turning' (*P* for all tests <.034, **Supplementary Figure 3**).

Sequence of change in cognitive and motor function

Before the age of 75 years, most cognitive and motor test scores had reached a decline of 0.5 SD in standardised test score compared to test scores at age 45 years (**Figure 2**). Considering both cognitive and motor tests, the decline of 0.5 SD was first reached for the Stroop Test: Interference subtask at the age of 58 years. This was followed by the Design Organisation Test at the age of 59 years and the Stroop Test: Naming subtask at the age of 64 years. Of all motor tests, the gait domain 'phases' showed the fastest decline, reaching a 0.5 SD decrease in test score at the age of 58 years. Across all tests, the average time between the age of 45 years and the age at which 0.5 SD decrease in test score was reached, was shorter for cognitive tests than for motor tests (20.0 versus 24.7 years, respectively, *P*=.039). By contrast, the time between 0.5 SD and 1.0 SD decrease in test scores was longer for cognitive than for motor tests (11.2 years versus 8.9 years, respectively, *P*<.001).

DISCUSSION

In this population-based study, we showed that both cognitive and motor function generally decline linearly between the ages 45 and 65 years, followed by a steeper decline after the age of 65 to 70 years. Test scores for cognitive and motor function declined similarly, with high variation in the rate of decline across age for individual tests. Importantly, whereas a higher level of education was associated with higher cognitive function, there was no association between level of education and function on the majority of the motor tests.

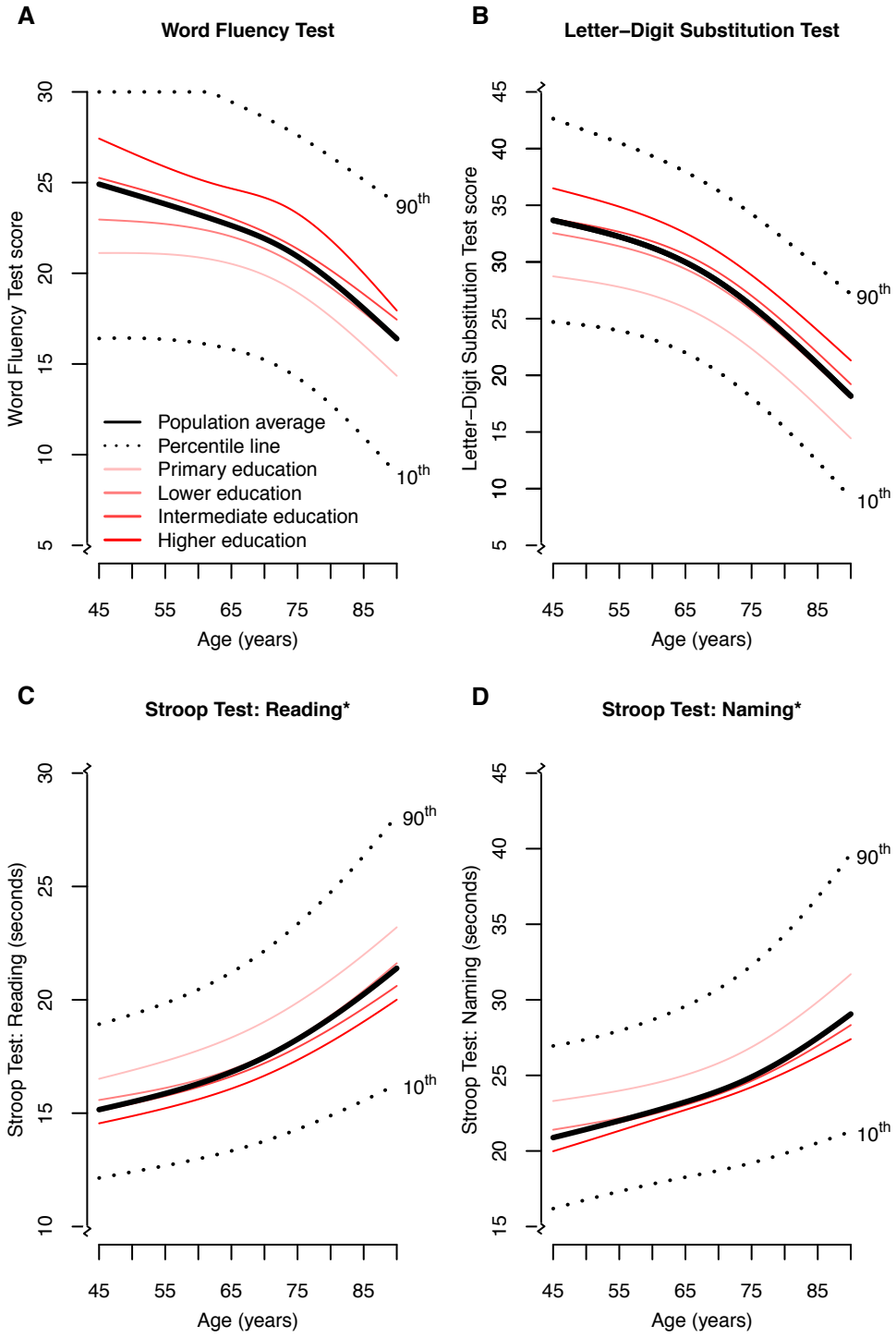
Various studies have reported changes in cognitive function with ageing, but evidence on the temporal relation between change in cognitive compared to motor function is limited. Most evidence has come from memory clinics,[11] or from studies that have solely relied on gait speed to assess motor function.[11,19,40-44] These studies have closely linked global cognitive function to gait speed. As yet, no studies have investigated differences in performance on specific cognitive tests nor studied other facets of motor function, such as fine motor skill.

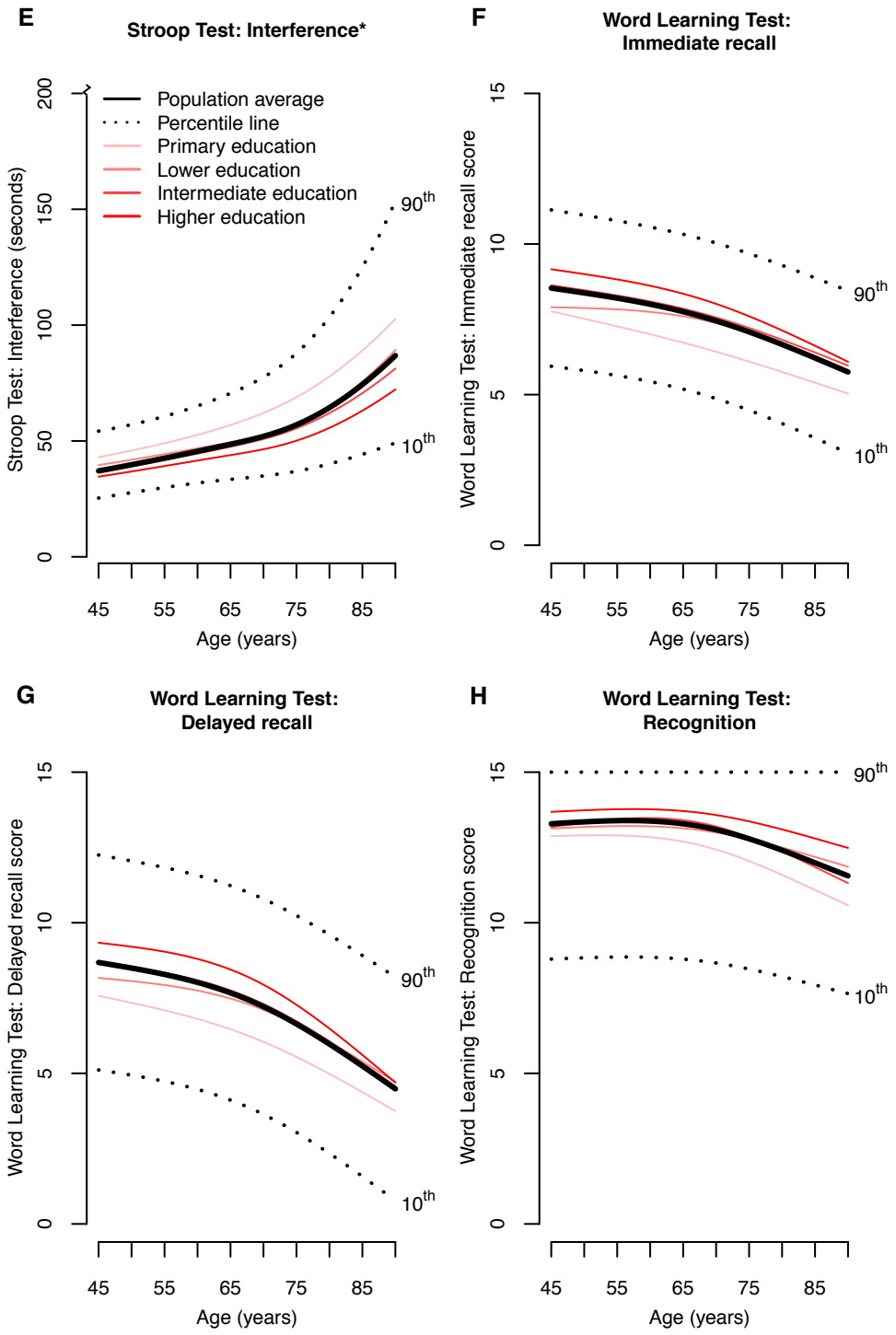
These knowledge gaps remain unaddressed since prior studies have found that decline of cognitive and motor function may vary, or that one may predate the other.[12,45-47] Most of these studies have been conducted in older participants (aged 70 years and older), with a limited sample size (varying between 488 and 2276 participants), or with relatively short follow-up (ranging from five to seven years). The current study is able to extend these findings by leveraging a detailed set of cognitive and motor tests among a broader age range (ages 45 to 90 years) in a larger, population-based sample (N≥8297) with up to six repeated assessments during a maximum follow-up of 19.4 years.

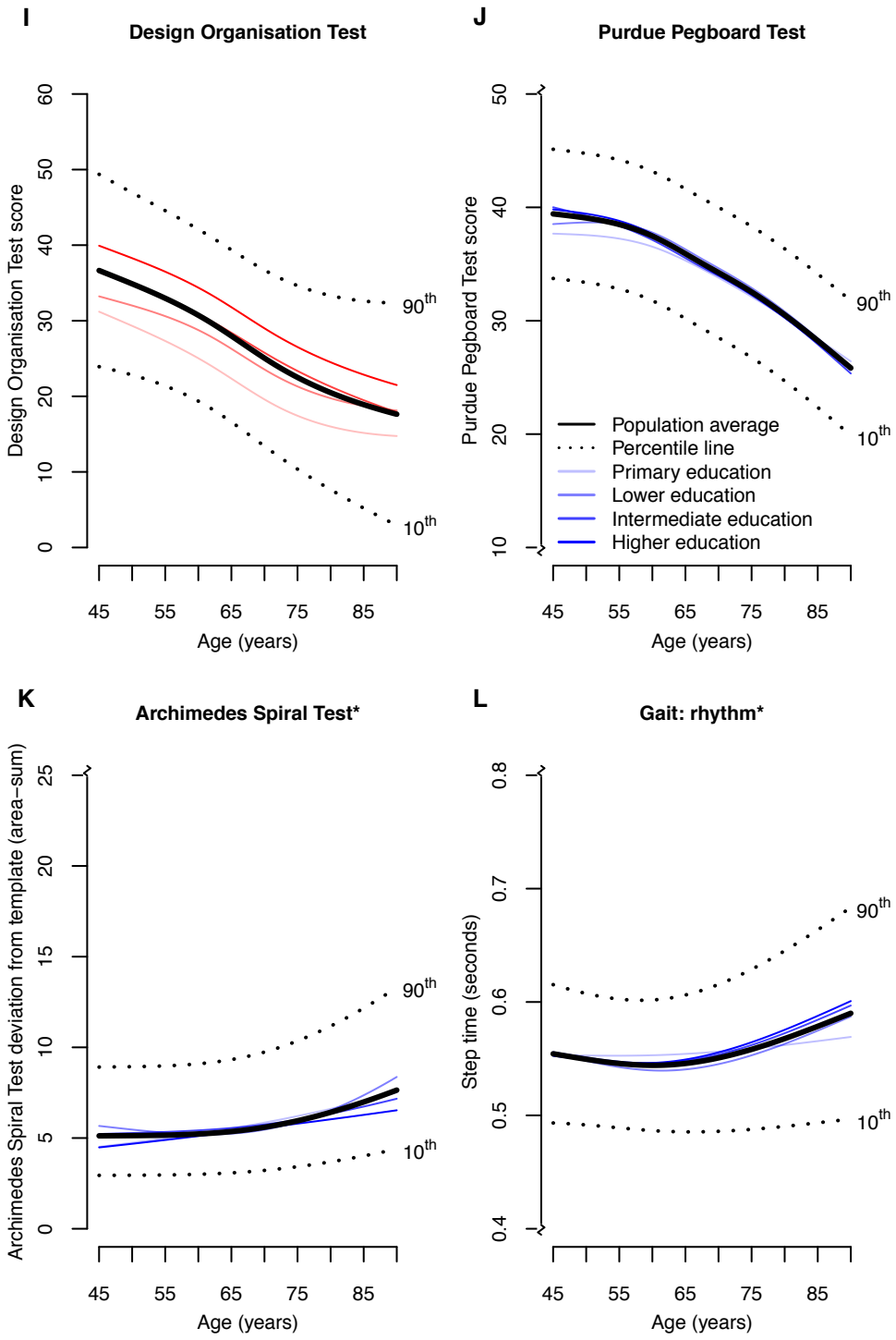
We did not find distinct patterns of an overall decline in cognitive function preceding motor function or vice versa, yet we observed large variability in test-specific decline. For instance, tendency to shuffle ('phases' gait domain) and fine motor function generally started to show initial signs of decline up to 25 years earlier than widely used cognitive (screening) tests, such as the Word Learning Tests: Delayed recall and Recognition.[11,40-42,48] These findings may be explained by accelerating changes in brain structure during ageing, with loss of white matter preceding loss of grey matter.[20,49] We indeed observed the earliest changes in cognitive and motor domains that depend on white matter integrity, including information processing speed, executive function, and the gait domain 'phases'.[20,50-52] In contrast, cognitive and motor domains related to alterations in grey matter volume (i.e., memory and the gait domain 'base of support') showed a later decline in function than those related to white matter integrity.[20,51-53]

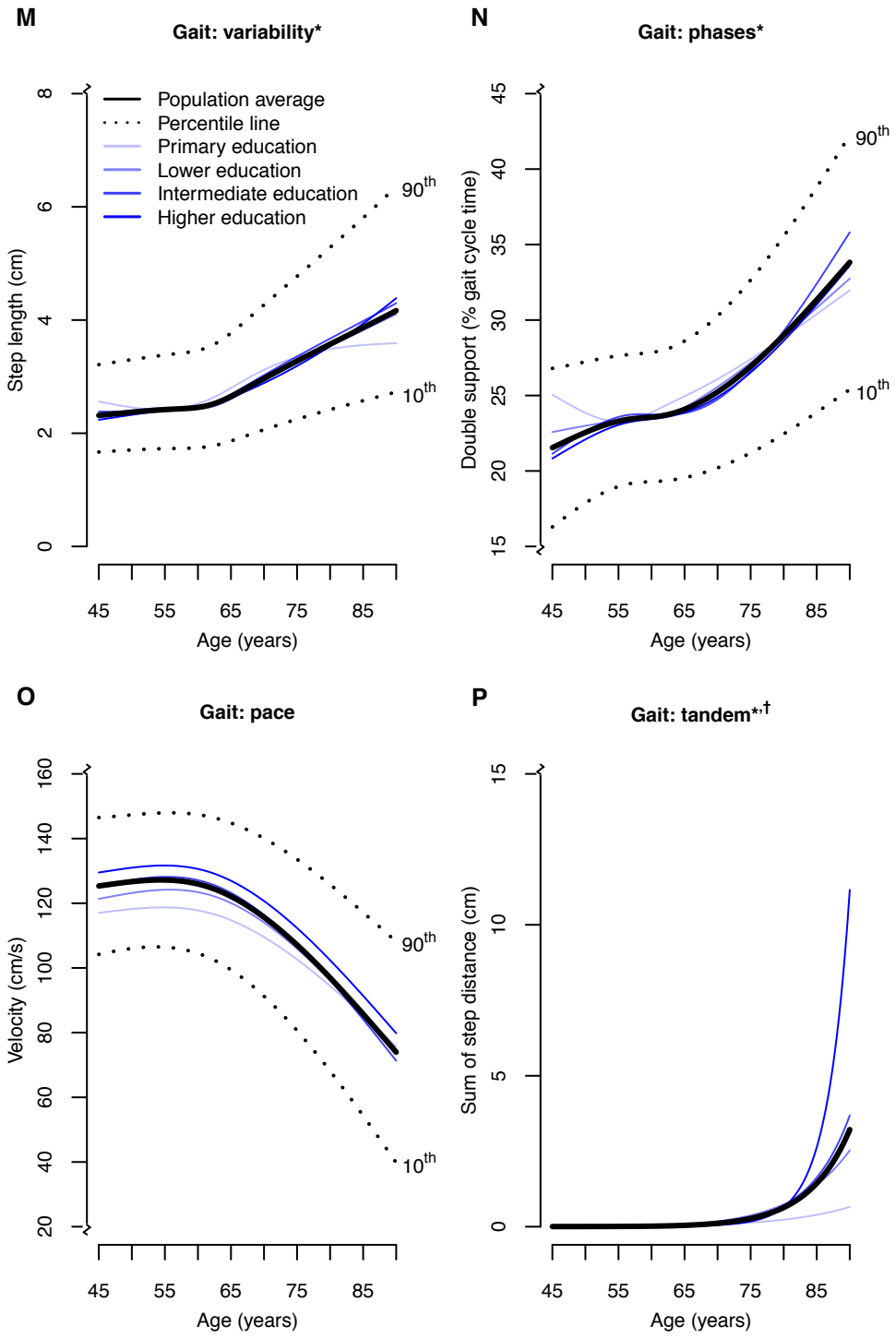
Variability in test-specific decline may also be explained by diseases and common comorbidities in these older adults, such as cardiovascular diseases, depression, respiratory diseases, cancer, or impairments in sensory organs.[54-57] These may differentially influence cognitive compared to motor function in some individuals. As an example, presence of peripheral artery disease or arthrosis limits walking speed, but does not directly influence executive functioning as assessed by the Stroop Task.[58] The contribution of these potentially modifiable determinants to sequence of test-specific decline and the shape of these trajectories was beyond the scope of the present study, and warrants further investigation using more advanced statistical models.

As expected, we found that participants with a higher educational level had higher baseline scores (scores at age 45 years) for cognitive tests than participants with a lower educational level. Regarding the rate of change in cognitive function, we found that participants with a 'primary' educational level declined faster on most tests than higher educated participants. The declines over time were largely similar among 'lower', 'intermediate', and 'higher' educated participants. This implies that higher educated individuals are generally older when they reach the same cognitive test performance than lower educated individuals. As an example, comparing performance between lower and higher educated participants on the


A
Word Fluency Test
Word Fluency Test score
Age (years)
90th
10th
Population average
Percentile line
Primary education
Lower education
Intermediate education
Higher education
B
Letter–Digit Substitution Test
Letter–Digit Substitution Test score
C
Stroop Test: Reading*
Stroop Test: Reading (seconds)
D
Stroop Test: Naming*
Stroop Test: Naming (seconds)


E
Stroop Test: Interference*
Population average
Percentile line
Primary education
Lower education
Intermediate education
Higher education
90th
10th
Stroop Test: Interference (seconds)
0
50
100
150
200
45
55
65
75
85
Age (years)
F
Word Learning Test: Immediate recall
Word Learning Test: Immediate recall score
0
5
10
15
90th
10th
45
55
65
75
85
Age (years)
G
Word Learning Test: Delayed recall
Word Learning Test: Delayed recall score
0
5
10
15
90th
10th
45
55
65
75
85
Age (years)
H
Word Learning Test: Recognition
Word Learning Test: Recognition score
0
5
10
15
90th
10th
45
55
65
75
85
Age (years)


I
Design Organisation Test
Design Organisation Test score
0
10
20
30
40
50
60
90th
10th
45
55
65
75
85
Age (years)
J
Purdue Pegboard Test
Purdue Pegboard Test score
10
20
30
40
50
90th
10th
Population average
Percentile line
Primary education
Lower education
Intermediate education
Higher education
45
55
65
75
85
Age (years)
K
Archimedes Spiral Test*
Archimedes Spiral Test deviation from template (area−sum)
0
5
10
15
20
25
90th
10th
45
55
65
75
85
Age (years)
L
Gait: rhythm*
Step time (seconds)
0.4
0.5
0.6
0.7
0.8
90th
10th
45
55
65
75
85
Age (years)


M
Gait: variability*
Step length (cm)
0
2
4
6
8
Population average
Percentile line
Primary education
Lower education
Intermediate education
Higher education
90th
10th
45
55
65
75
85
Age (years)
N
Gait: phases*
Double support (% gait cycle time)
15
20
25
30
35
40
45
90th
10th
45
55
65
75
85
Age (years)
O
Gait: pace
Velocity (cm/s)
20
40
60
80
100
120
140
160
90th
10th
45
55
65
75
85
Age (years)
P
Gait: tandem*,†
Sum of step distance (cm)
0
5
10
15
45
55
65
75
85
Age (years)

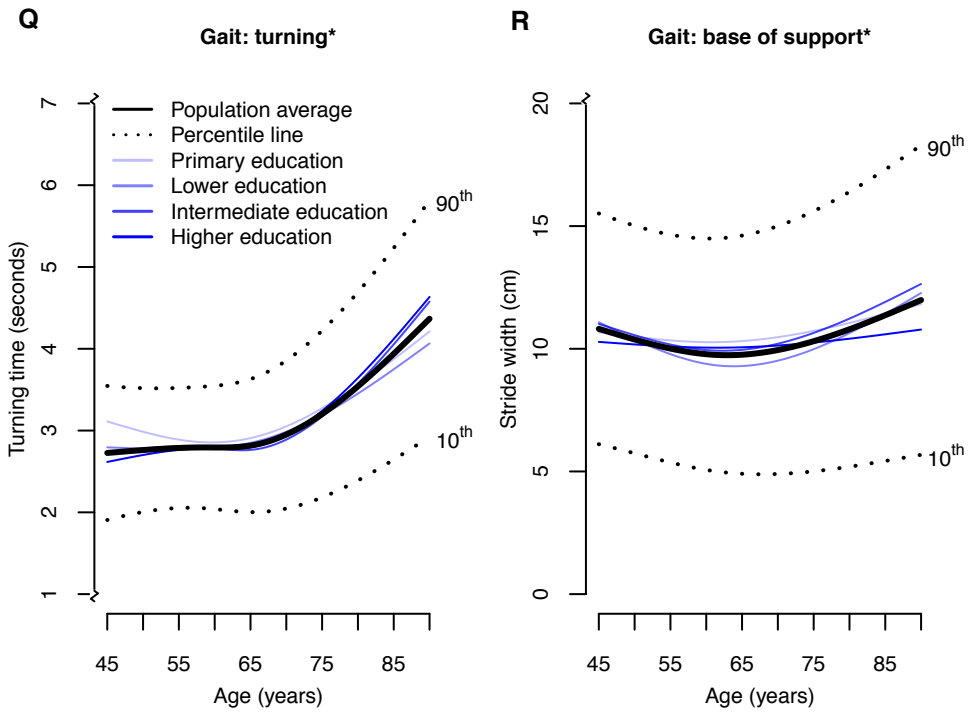
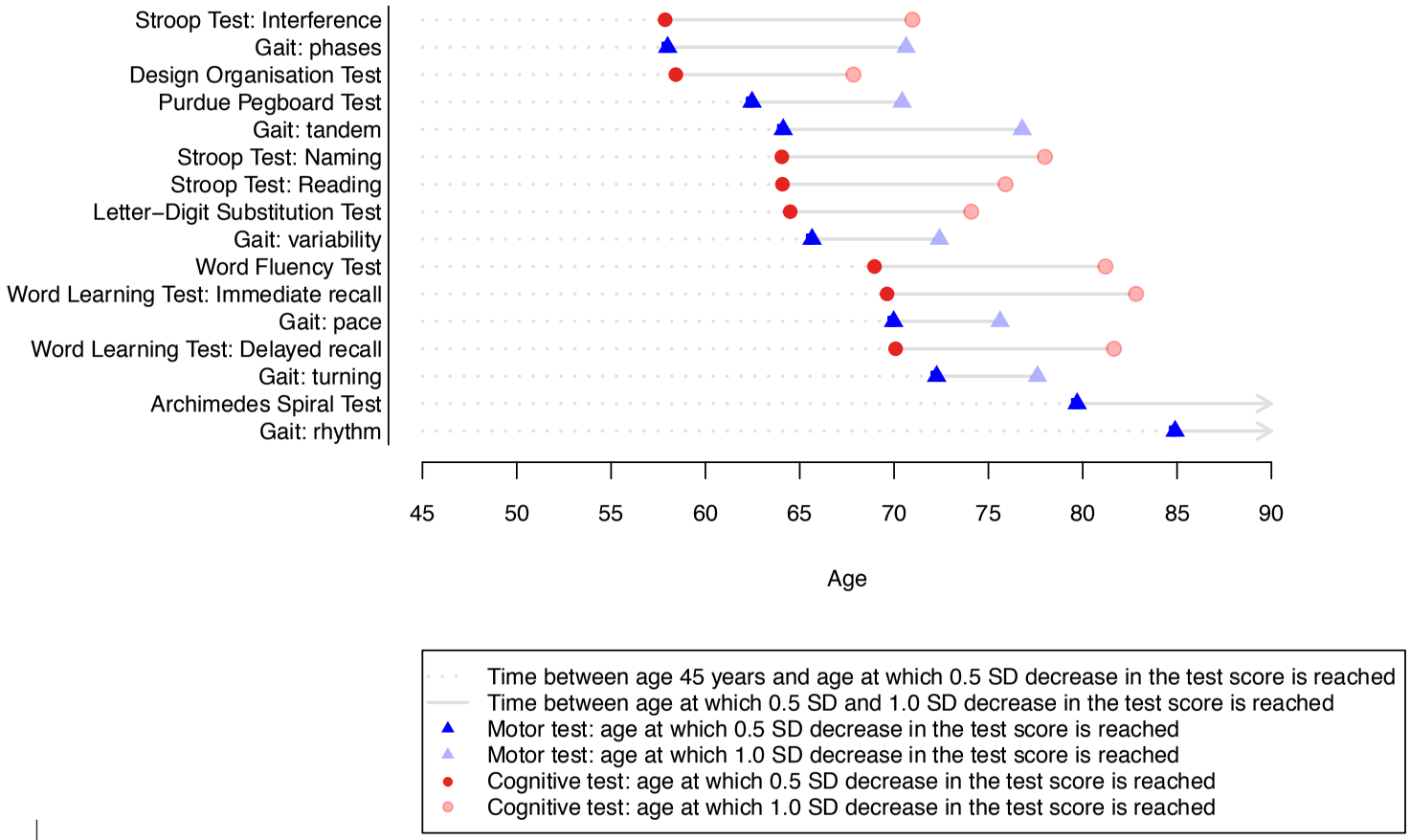


Figure 1 Trajectories of cognitive and motor function.
The thick black continuous line reflects the trajectory of performance for the total study population based on the results of the linear mixed model, the black dotted lines represent the 10th and 90th percentile curves. Test performance was visualised per educational level in red for cognitive tests and in blue for motor tests.
** Higher scores indicate worse performance. † Percentile curves could not be calculated and are therefore not shown, since the majority of the test scores was equal to 0.*

Figure 2 Sequence of decline of cognitive and motor function (right page).
Decline was defined as reaching an average population test score of 0.5 or 1.0 SD below the population mean of the test score at age 45 years. The circle or triangle is displayed at the age at which 0.5 SD (opaque) or 1.0 SD (transparent) lower score was reached with cognitive tests depicted in red circles and motor tests in blue triangles. The dotted line represents time between mean population test score at age 45 years and the age at which 0.5 SD decrease in that test score is reached. The continuous line denotes time between the age at which 0.5 SD decrease in the test score was reached and the age at which 1.0 SD in the test score was reached. The Word Learning Test: Recognition subtask and the gait domains 'tandem' and 'base of support' did not reach a score of 0.5 SD lower at a certain age than the score at age 45 years and are therefore not shown.
This sequence of decline was estimated based on the total study population. Note that not all participants had all cognitive and motor tests completed.
SD = standard deviation.


Stroop Test: Interference
Gait: phases
Design Organisation Test
Purdue Pegboard Test
Gait: tandem
Stroop Test: Naming
Stroop Test: Reading
Letter–Digit Substitution Test
Gait: variability
Word Fluency Test
Word Learning Test: Immediate recall
Gait: pace
Word Learning Test: Delayed recall
Gait: turning
Archimedes Spiral Test
Gait: rhythm
45
50
55
60
65
70
75
80
85
90
Age
Time between age 45 years and age at which 0.5 SD decrease in the test score is reached
Time between age at which 0.5 SD and 1.0 SD decrease in the test score is reached
Motor test: age at which 0.5 SD decrease in the test score is reached
Motor test: age at which 1.0 SD decrease in the test score is reached
Cognitive test: age at which 0.5 SD decrease in the test score is reached
Cognitive test: age at which 1.0 SD decrease in the test score is reached

Word Learning Test: Delayed recall subtask score, reveals that at age 45 years, the lowest educated individuals remembered on average eight of the 15 originally presented words after ten minutes. The highest educated individuals however attained this same score when they were on average 73 years. Yet, no association was found between educational level and motor function for the majority of the motor tests. These findings support emerging evidence that cognitive reserve, operationalised by for example educational attainment, could have long-lasting compensatory effects on cognitive but not on motor function, with the potential to postpone cognitive decline and thereby the clinical diagnosis of dementia.[59-61]

This study has several limitations. First, given that participants underwent most cognitive tests at the research centre, we cannot exclude that selection bias may have influenced our results, with those who are considered less healthy being less likely to participate. Therefore, the presented test scores on cognitive and motor function may be an overestimation of the true performance in the general population, especially for those at older ages.[62] Second, repetitive administering of cognitive tests can lead to learning effects, which could have led to overestimating performance with increasing age. However, these effects are expected to be limited, since the median test interval was 5.1 years for cognitive assessments and 5.4 years for motor assessments. Third, in the early nineties, the completed level of education was determined by several factors including sex and social economic status. As such, educational attainment in this study may not be a proper proxy for cognitive reserve in women. Lastly, we estimated trajectories of cognitive and motor function at a population-level, yet deviations from this pattern on an individual level may signal an under recognised group at high risk for neurodegenerative diseases and stroke. Strengths of this study include the large sample size and the repeated and simultaneous assessments of cognitive and motor function in a single, community-dwelling population.

In this study, we present trajectories of decline of both cognitive and motor functioning among individuals aged 45 to 90 years in the general population. Such data are essential to understand the natural course of cognitive and motor function during ageing. Cognitive and motor function decline similarly during ageing, characterised by a linear decline between the ages 45 and 65, and a steeper decline thereafter. Higher educational attainment was related to higher cognitive function at baseline and to a slower rate of subsequent decline, but it did not affect motor function. In the sequence of decline across individual tests, up to a 25-year age difference between the fastest and slowest declining test scores was observed.

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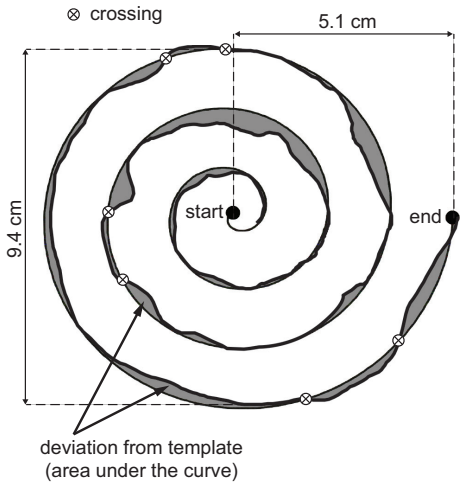
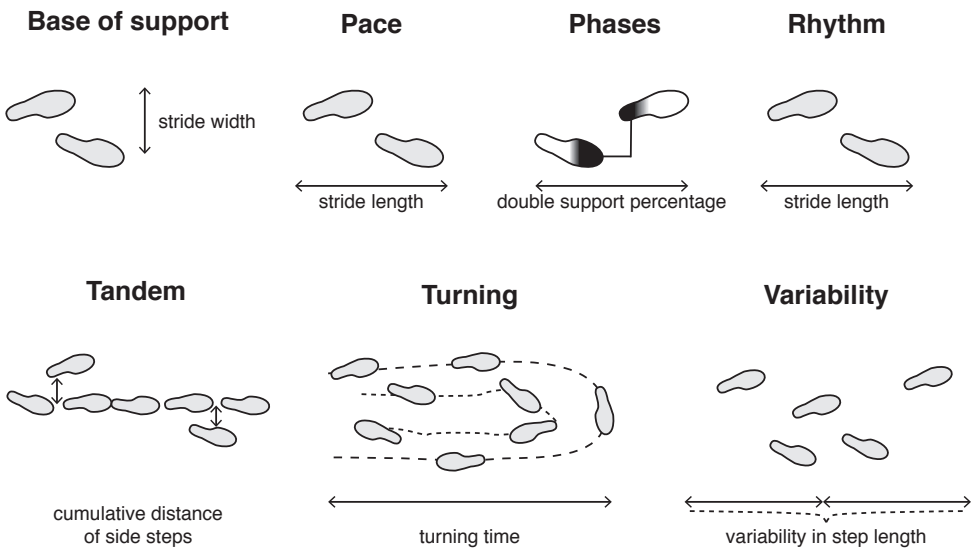
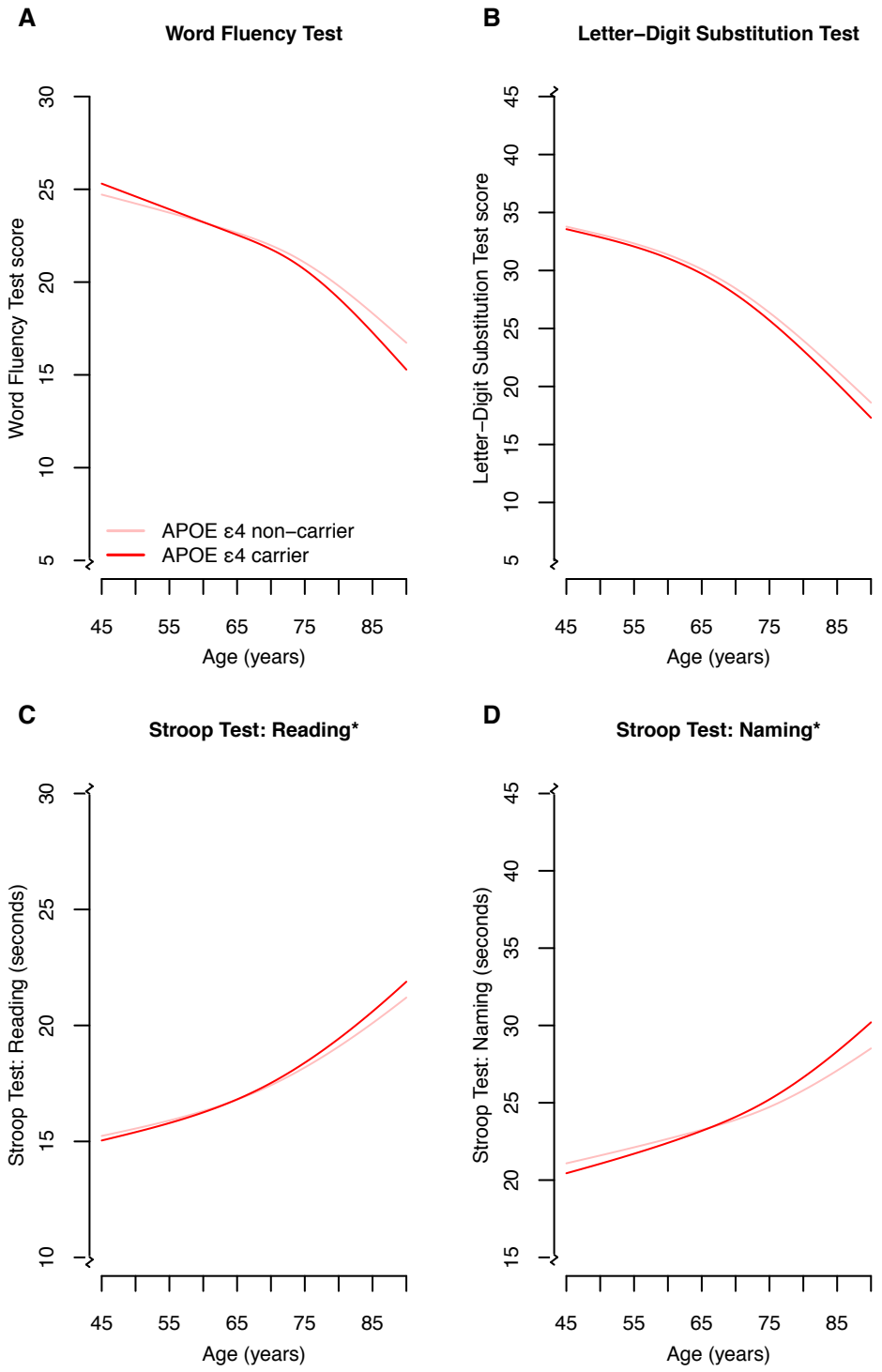
SUPPLEMENTARY MATERIAL

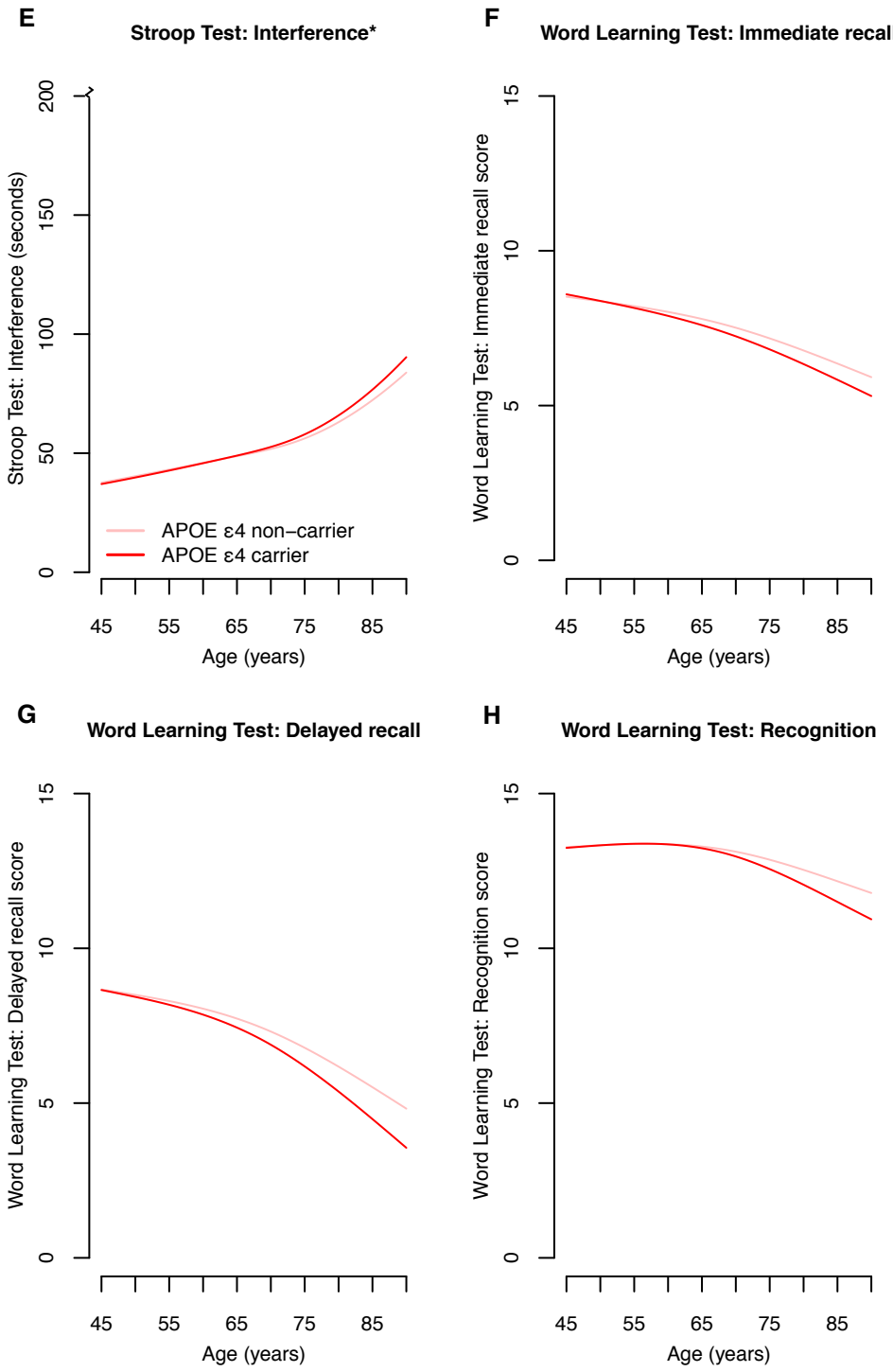


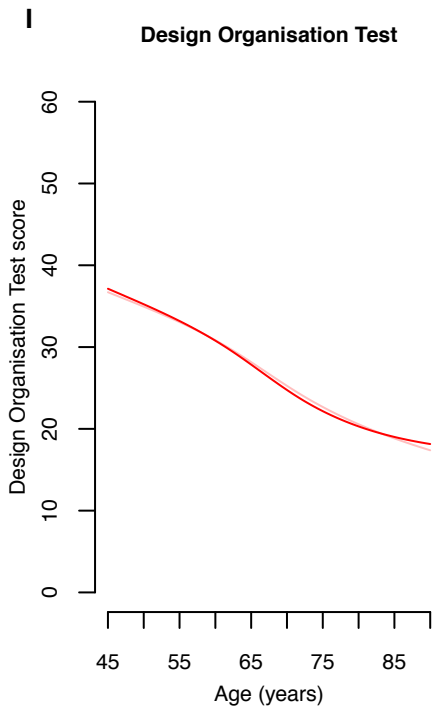
Supplementary Figure 1 Spiral test.
Example of a spiral-drawing quantification, showing an example of the calculation of quantitative measures of fine motor skills. The start and endpoint are indicated by a dot. The Figure explains how deviation from template and crossings are defined.

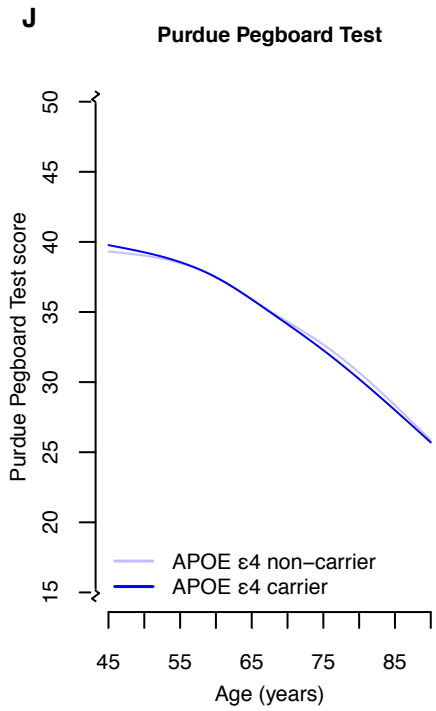


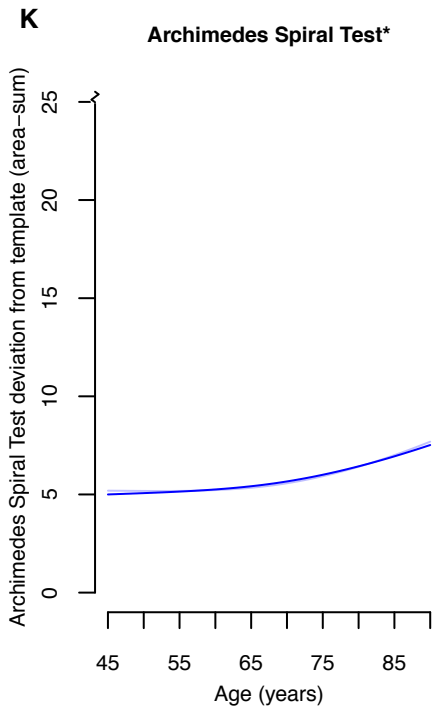
Supplementary Figure 2 Gait domains.
To summarise gait parameters into independent domains, we conducted a principal component analysis. This yielded seven independent gait domains: 'base of support', 'pace', 'phases', 'rhythm', 'tandem', 'turning', and 'variability'. For each gait domain, a single gait parameter that has high correlation with the domain is illustrated.

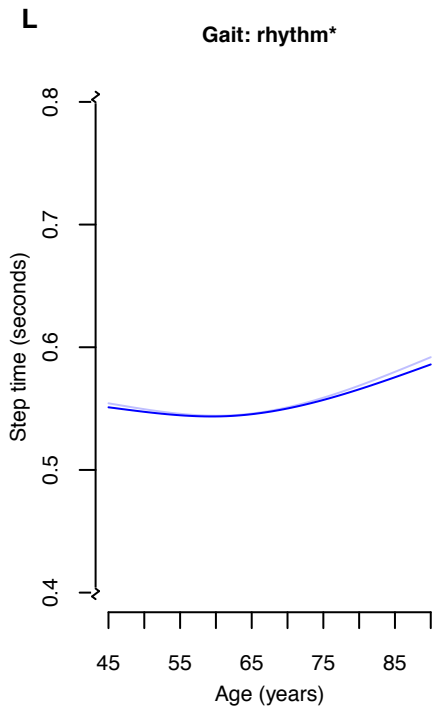

A
Word Fluency Test
Word Fluency Test score
5
10
15
20
25
30
APOE ε4 non−carrier
APOE ε4 carrier
45
55
65
75
85
Age (years)
B
Letter−Digit Substitution Test
Letter−Digit Substitution Test score
5
10
15
20
25
30
35
40
45
45
55
65
75
85
Age (years)
C
Stroop Test: Reading*
Stroop Test: Reading (seconds)
10
15
20
25
30
45
55
65
75
85
Age (years)
D
Stroop Test: Naming*
Stroop Test: Naming (seconds)
15
20
25
30
35
40
45
45
55
65
75
85
Age (years)

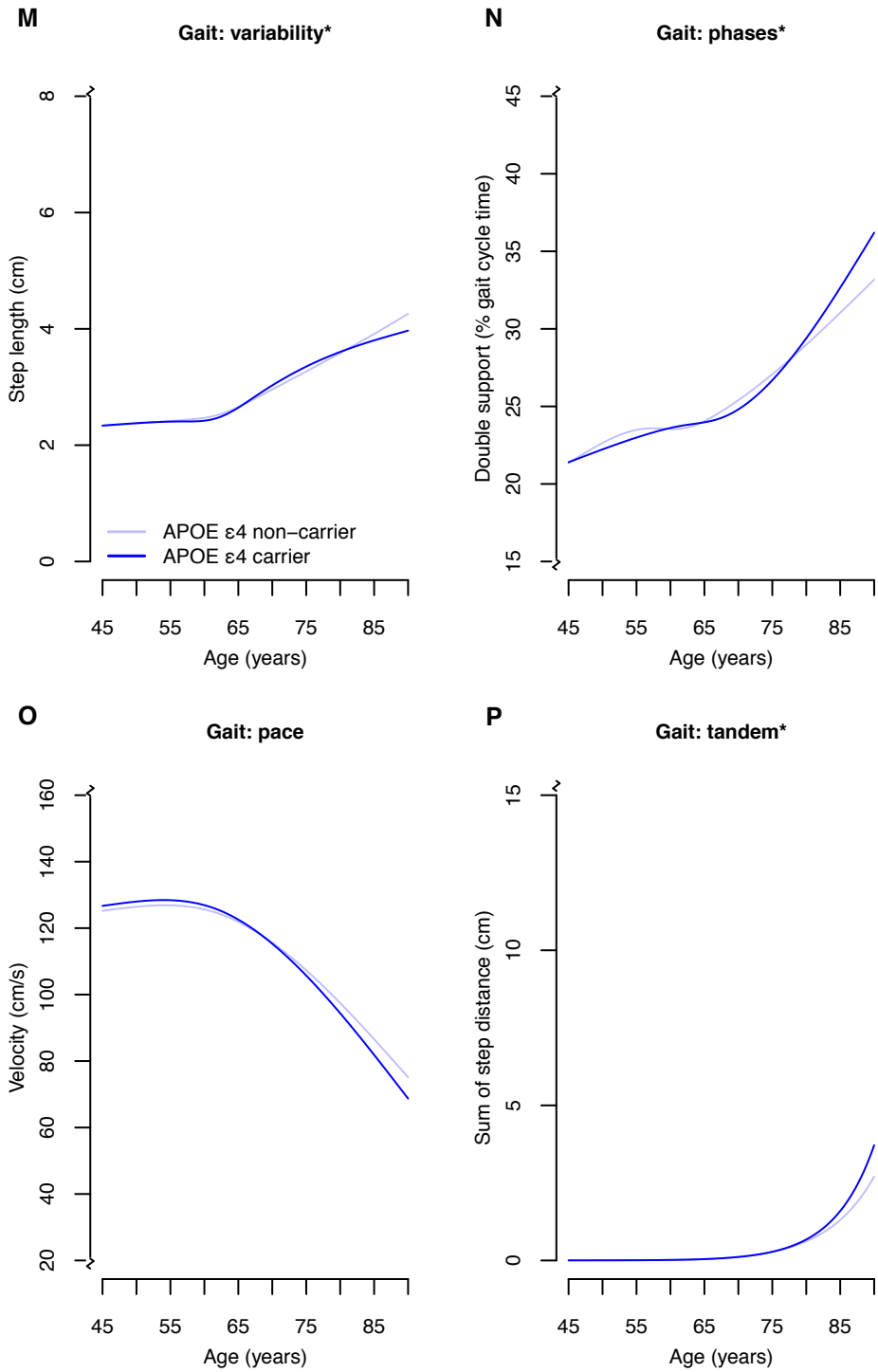

E
Stroop Test: Interference*
Stroop Test: Interference (seconds)
0
50
100
150
200
APOE ε4 non−carrier
APOE ε4 carrier
45
55
65
75
85
Age (years)
F
Word Learning Test: Immediate recall
Word Learning Test: Immediate recall score
0
5
10
15
45
55
65
75
85
Age (years)
G
Word Learning Test: Delayed recall
Word Learning Test: Delayed recall score
0
5
10
15
45
55
65
75
85
Age (years)
H
Word Learning Test: Recognition
Word Learning Test: Recognition score
0
5
10
15
45
55
65
75
85
Age (years)

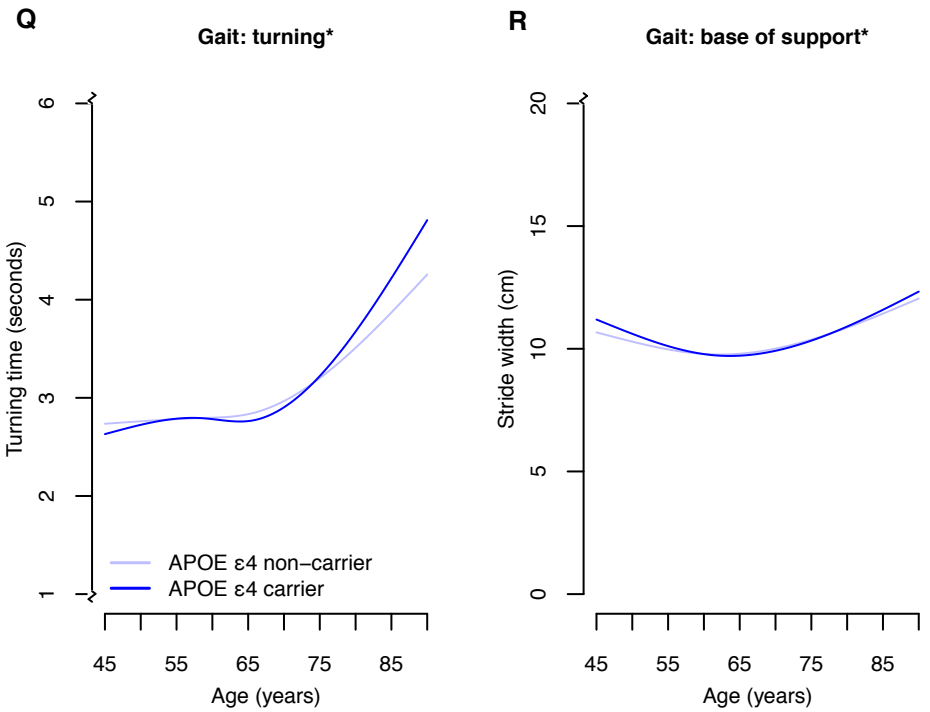

I
Design Organisation Test
Design Organisation Test score
0
10
20
30
40
50
60
45
55
65
75
85
Age (years)


J
Purdue Pegboard Test
Purdue Pegboard Test score
15
20
25
30
35
40
45
50
APOE ε4 non−carrier
APOE ε4 carrier
45
55
65
75
85
Age (years)


K
Archimedes Spiral Test*
Archimedes Spiral Test deviation from template (area−sum)
0
5
10
15
20
25
45
55
65
75
85
Age (years)


L
Gait: rhythm*
Step time (seconds)
0.4
0.5
0.6
0.7
0.8
45
55
65
75
85
Age (years)


M
Gait: variability*
Step length (cm)
0
2
4
6
8
APOE ε4 non−carrier
APOE ε4 carrier
45
55
65
75
85
Age (years)
N
Gait: phases*
Double support (% gait cycle time)
15
20
25
30
35
40
45
45
55
65
75
85
Age (years)
O
Gait: pace
Velocity (cm/s)
20
40
60
80
100
120
140
160
45
55
65
75
85
Age (years)
P
Gait: tandem*
Sum of step distance (cm)
0
5
10
15
45
55
65
75
85
Age (years)



Supplementary Figure 3 Trajectories of cognitive and motor function stratified by APOE ε4 carrier status.

Test performance was visualised for APOE ε4 carrier status level in red for cognitive tests and in blue for motor tests. Participants with unknown APOE ε4 carrier status were excluded for analysis regarding APOE ε4 carrier status (528 out of 9514 participants for cognitive tests and 462 out of 8297 participants for motor tests).

** Higher scores indicate worse performance.*

Supplementary Table 1 Description of original gait parameters.

Parameter	Description	Indication of "worse" gait	Main underlying domain
Single support time	The time elapsed between the last contact of the opposite foot and the first contact of the next footfall of the opposite foot when a foot touches the ground	Higher	Rhythm
Swing time	The time elapsed between the last contact of the current footfall to the first contact of the next footfall on the same foot in seconds	Higher	Rhythm
Step time	The time elapsed between the first contact of one foot and the first contact of the opposite foot	Higher	Rhythm
Stride time	The elapsed time between the first contacts of two consecutive footfalls of the same foot in seconds	Higher	Rhythm
Cadence	The number of steps/minute	Lower	Rhythm
Stance time	The time elapsed between the first contact and the last contact of two consecutive footfalls on the same foot in seconds. It is initiated by heel contact and ends with the toe off of the same foot	Higher	Rhythm
Stride length SD	The standard deviation in the stride length in centimetres	Higher	Variability
Step length SD	The standard deviation in the step length in centimetres	Higher	Variability
Stride velocity SD	The standard deviation in the stride velocity (stride length/stride time) in centimetres/second	Higher	Variability
Stride time SD	The standard deviation in the stride time in seconds	Higher	Variability
Step time SD	The standard deviation in the step time in seconds	Higher	Variability
Stance time SD	The standard deviation in the stance time in seconds	Higher	Variability
Swing time SD	The standard deviation in the swing time in seconds	Higher	Variability
Single support time SD	The standard deviation in the single support time in seconds	Higher	Variability
Double support time SD	The standard deviation in the double support time in seconds	Higher	Variability
Single support (%GC)	The single support time as a percentage of the stride time	Lower	Phases

Supplementary Table 1 Description of original gait parameters (continued).

Parameter	Description	Indication of "worse" gait	Main underlying domain
Swing (%GC)	The swing time as a percentage of the stride time	Lower	Phases
Stance (%GC)	The stance time as a percentage of the stride time	Higher	Phases
Double support (%GC)	The double support time as a percentage of the stride time	Higher	Phases
Double support time	The amount of time that two feet are on the ground at the same time within one footfall in seconds	Higher	Phases
Stride length	The distance between the heel points of two consecutive footprints of the same foot on the line of progression in centimetres	Lower	Pace
Step length	The distance between the heel points of two consecutive opposite footprints on the line of progression in centimetres	Lower	Pace
Velocity	The velocity in centimetres/second	Lower	Pace
Sum of feet surface	The sum of the surfaces of the side steps* as a percentage of the surface of a normal step	Higher	Tandem
Sum of step distance	The sum of the distances of the side steps* from the line on the walkway in centimetres	Higher	Tandem
Double step	A double-step was a step with one foot, followed by a step with the same foot, where both feet were on the line of the walkway	Higher	Tandem
Turning step count	The number of steps used within the turning time	Higher	Turning
Turning time	The turning time was defined as the time between the last contact of the second foot before the first turn foot and the first contact of the second foot with a normal angle coming out of the turn. In which the first turn foot is defined as the first foot deviating from the normal angle of the feet (subject dependent)	Higher	Turning
Stride width SD	The standard deviation in the stride width in centimetres	Higher	Base of support
Stride width	The distance from heel centre of one footprint to the line of progression formed by two footprints of the opposite foot in centimetres	Lower	Base of support

**A sidestep was defined as a step next to the line on the walkway, which was followed by a step with the same foot or a step with the other foot.*
SD = standard deviation, %GC = as a percentage of the stride time.

Supplementary Table 2 Overview of number of participants per cognitive and motor test.

Cognitive or motor test	Analysis of cognitive function (N=9514)	Analysis of motor function (N=8297)
Word Fluency Test	9458 (99.4)	
Letter-Digit Substitution Test	9419 (99.0)	
Stroop Test: Reading	9311 (97.9)	
Stroop Test: Naming	9300 (97.8)	
Stroop Test: Interference	9281 (97.6)	
Word Learning Test: Immediate recall	7875 (82.8)	
Word Learning Test: Delayed recall	7875 (82.8)	
Word Learning Test: Recognition	7882 (82.8)	
Design Organisation Test	5561 (58.5)	
Purdue Pegboard Test		8225 (99.1)
Archimedes Spiral Test		5424 (65.4)
Gait assessments		4154 (50.1)

Numbers are presented as number of participants per cognitive or motor test (% of total number of participants for cognitive or motor tests).
N = number of participants.

Supplementary Table 3 Characteristics of excluded participants.

Characteristic	Excluded participants*		
	History of dementia, stroke, or Parkinson's disease at time of first assessment† (N=3873)	No cognitive or motor assessments (N=1494)	Aged ≥90 years at time of first assessment (N=33)
Age‡, years, mean (SD)	73.0 (13.7)	72.6 (9.3)	88.2 (4.7)
Women, No. (%)	2509 (64.8)	844 (56.5)	19 (57.6)
Educational level, No. (%)			
Primary	1087 (28.1)	466 (31.2)	11 (33.3)
Lower	1327 (34.3)	575 (38.5)	10 (30.3)
Intermediate	802 (20.7)	334 (22.4)	6 (18.2)
Higher	341 (8.8)	90 (6.0)	3 (9.1)
Body mass index, kg/m^2, mean (SD)	26.6 (4.0)	26.4 (4.1)	25.6 (2.9)
Smoking, No. (%)			
Never	1367 (35.3)	486 (32.5)	18 (54.5)
Former	1403 (36.2)	567 (38.0)	11 (33.3)
Current	805 (20.8)	399 (26.7)	1 (3.0)
Alcohol use, No. (%)	1663 (42.9)	781 (52.3)	17 (51.5)
Systolic blood pressure, mm Hg, mean (SD)	143 (22.9)	144 (23.6)	145 (18.9)
Diabetes mellitus, No. (%)	527 (13.6)	211 (14.1)	5 (15.2)
APOE ε4 carrier, No. (%)	714 (18.4)	364 (24.4)	6 (18.2)

Characteristics are measured at study entry.
Missing values for all characteristics are not imputed and therefore numbers do not always sum up to 100%.
** In total, 5405 out of 14 926 participants were excluded. Characteristics of participants without consent for follow-up (n=5) are not shown. † Also comprises participants with insufficient data to determine a history of one of these diseases. ‡ Age at study entry, not age at first assessment.*
APOE = apolipoprotein E, N = number of participants, SD = standard deviation.

Chapter 5

Trajectories of cognitive function prior to cancer diagnosis

van der Willik KD, Hauptmann M, Jóźwiak K, Vinke EJ, Ruiter R, Stricker BHCh, Compter A, Ikram MA, Schagen SB

ABSTRACT

Background An emerging body of research suggests that non-central nervous system (CNS) cancer may negatively impact the brain apart from effects of cancer treatment. However, studies assessing cognitive function in newly diagnosed cancer patients cannot exclude selection bias and psychological effects of cancer diagnosis. To overcome these limitations, we investigated trajectories of cognitive function in patients before cancer diagnosis.

Methods Between 1989 and 2013, 2059 participants from the population-based Rotterdam Study were diagnosed with non-CNS cancer. Cognitive assessments were performed every three to six years using a neuropsychological battery. The general cognitive factor was composed of individual cognitive tests to assess global cognition. Using linear mixed models we compared change in cognitive function in cancer cases before diagnosis with cognitive change in age-matched cancer-free controls (1:2). In addition, we performed sensitivity analyses by discarding assessments of controls five years before the end of follow-up to exclude effects from potential undiagnosed cancer. All statistical tests were two-sided.

Results The Word Learning Test: Immediate recall declined faster among cases than among controls (-0.05 [95% confidence interval = -0.09 to -0.01] versus 0.01 [95% confidence interval = -0.01 to 0.03], *P* for difference = .003). However, this difference was not statistically significant in sensitivity analyses. Furthermore, no statistically significant differences were observed in change in other individual cognitive tests and in the general cognitive factor.

Conclusions In this study we evaluated cognitive function in a large group of cancer patients prior to diagnosis, thereby excluding the psychological impact of cancer diagnosis and biased patient selection. In contrast to previous studies shortly after cancer diagnosis, we found no difference in change in cognitive function between cancer patients and controls.

INTRODUCTION

About 20 to 30% of the patients with non-central nervous system (CNS) cancer report cognitive problems following cancer diagnosis and cancer treatment that can persist into the survivorship period.[1-3] Whereas most studies have focused on the effects of chemotherapy on the brain, more recent evidence has shown that newly diagnosed cancer patients may already perform lower than expected on cognitive tests prior to cancer treatment, including surgery.[4-9] Although these patients have just been confronted with a cancer diagnosis, cognitive impairment persisted after statistical correction for psychological distress and fatigue. This suggests that shared risk factors for both cancer and cognitive impairment, such as genetic susceptibility, ageing, and lifestyle could contribute to the development of cognitive impairment in cancer patients.[10] Also, tumour growth itself may cause cognitive impairment, for instance through inflammatory or vascular processes.[5,11]

If the previously reported cognitive impairment in newly diagnosed cancer patients is related to shared risk factors for both cancer and cognitive impairment or to a growing, yet undiagnosed, cancer, it is conceivable that future cancer patients would already demonstrate altered cognitive function compared to cancer-free controls some time before cancer diagnosis. Based on this reasoning, it is expected that cancer patients' cognitive function declines faster prior to cancer diagnosis than cognitive function in controls.

Understanding the origin of cognitive impairment in cancer patients is essential for prevention and treatment. We aimed to contribute to this understanding by evaluating cognitive function in cancer patients longitudinally prior to the clinical manifestation of the disease. We evaluated the longitudinal change in cognitive function to learn about the effect of shared risk factors and cancer itself as determinants of cognition. This approach is superior to a cross-sectional comparison of absolute cognition levels prior to diagnosis because it includes all available assessments. Using the unique context of a population-based cohort, we compared cognitive trajectories between individuals prior to cancer diagnosis and individuals who remained cancer-free during follow-up.

METHODS

Setting

We used data from the Rotterdam Study, a Dutch population-based prospective cohort. The initial cohort (RS-I) started in 1989 with 7983 participants aged 55 years and older who reside in the district Ommoord in Rotterdam, the Netherlands. The cohort was expanded with 3011 participants in 2000 (RS-II), followed by an additional inclusion of 3392 participants aged 45 years and older in 2006 (RS-III).

Participants were interviewed at home by a trained research assistant, followed by two visits to the research facility for laboratory assessments, imaging, and physical examinations. Follow-up examinations are aimed to take place every three to six years. The design of the Rotterdam Study has been previously described in detail.[12]

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus Medical Centre and by the Ministry of Health, Welfare and Sport of the Netherlands. Written informed consent was obtained from all participants.

Study population

Of the 14 926 participants of the Rotterdam Study, we excluded those with a history of cancer at study entry (n=566), prevalent dementia at study entry (n=521), those who were insufficiently screened for prevalent dementia (n=601), a history of stroke (n=341), and those without informed consent to access medical records for follow-up (n=165), leaving 12 732 eligible participants (**Figure 1**).

Cases

Of the 2308 participants who were diagnosed with cancer during follow-up (between 1989 and 2013), we excluded those with primary CNS cancer (n=15), dementia before cancer diagnosis (n=62), stroke before cancer diagnosis (n=99), and participants without cognitive test results prior to cancer diagnosis (n=73), resulting in 2059 cases.

Controls

From participants who remained cancer-free during follow-up (n=10 424), we excluded cognitive test results after dementia or stroke diagnosis. Although this exclusion resulted in a lower number of assessments, it did not change the number of cancer-free participants, because participants with a history of dementia or stroke at study entry were already excluded (**Supplementary Table 1**). Subsequently we excluded participants without cognitive test

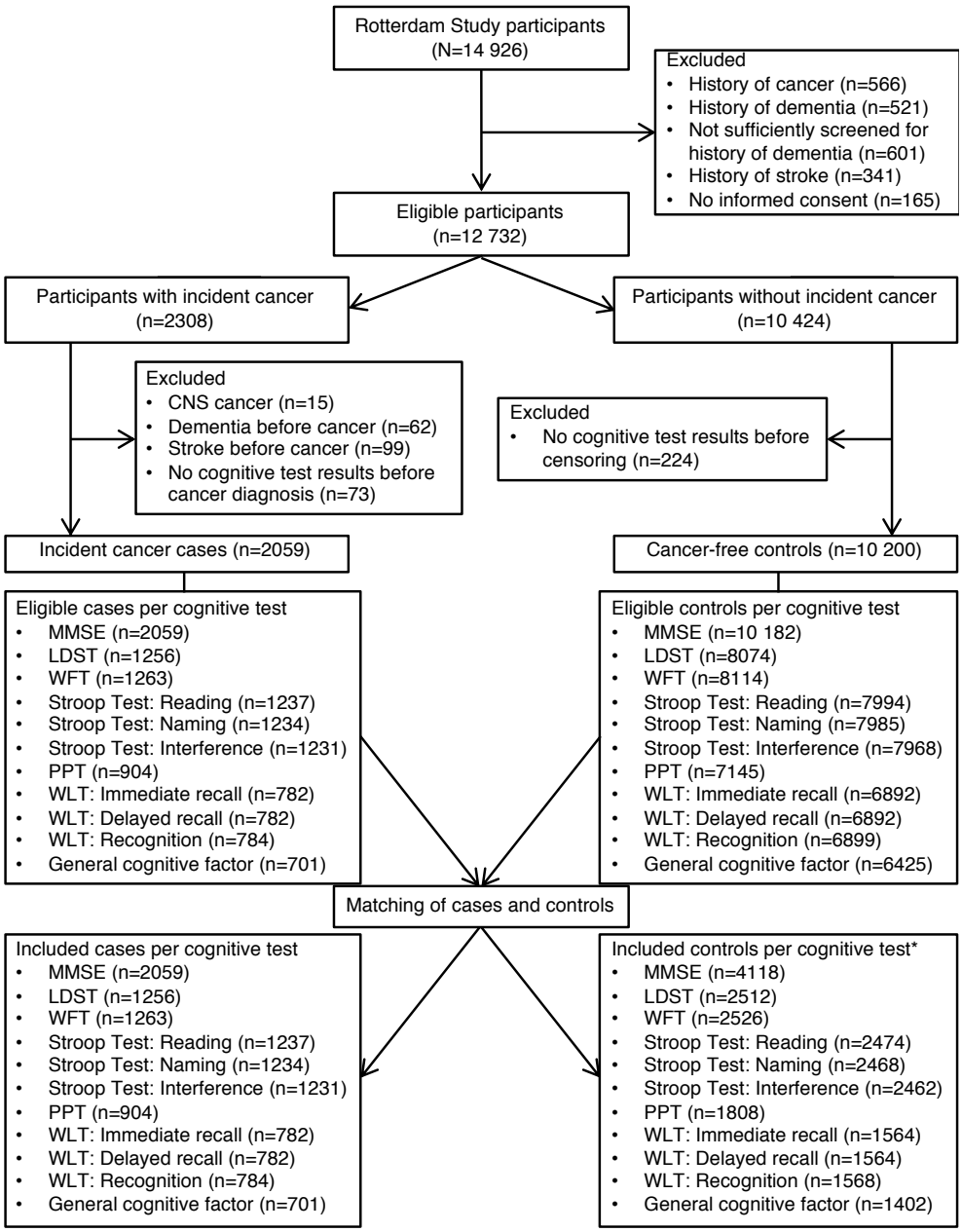


Figure 1 Flowchart of study population, separately for cancer cases and cancer-free controls per cognitive test.

** The number of assessments discarded after dementia or stroke diagnosis and after matching are presented in Supplementary Table 1.*

CNS = central nervous system, LDST = Letter-Digit Substitution Test, MMSE = Mini-Mental State Examination, PPT = Purdue Pegboard Test, WFT = Word Fluency Test, WLT = Word Learning Test.

results before end of follow-up (n=224), leaving 10 200 participants as eligible controls.

Matching procedure

Each case was individually matched to two randomly selected cancer-free controls at the age of diagnosis of the case (index age). A participant was eligible as control if he or she had at least one cognitive assessment before index age and no dementia or stroke diagnosis prior to index age. To avoid overmatching, we only matched on index age.[13] Assessments of controls after the index age were discarded (**Supplementary Table 1**). Matching started with the oldest case and was performed without replacement for each cognitive test separately.

Ascertainment of cancer

Cancer incidence up to January 1st, 2014 was based on medical records of general practitioners (including hospital discharge letters) and through linkage with Dutch Hospital Data, Netherlands Cancer Registry, and histology and cytopathology registries in the region. Incident cancer was defined as any primary malignant tumour, excluding non-melanoma skin cancer. Diagnoses were coded independently by two physicians according to the International Classification of Diseases, tenth revision (ICD-10).[14] In case of discrepancy, consensus was sought through consultation with a physician specialised in internal medicine. Date of diagnosis was based on date of biopsy (solid tumours) and laboratory assessment (haematological tumours), or – if unavailable – date of hospital admission or discharge letter. Level of uncertainty of diagnosis was defined as follows: certain (pathology-confirmed), probable (clinic-based on imaging or elevated tumour markers), and possible (clinic-based, suspicion based on symptoms or physical examination). Only pathology-confirmed cancers were included in the primary analysis. In sensitivity analyses, we included cases with probable or possible cancer and excluded controls who had probable or possible cancer.

Cognitive function assessment

Cognitive function was assessed by a neuropsychological test battery during research centre visits. Up to 2013, the following tests were administrated: Mini-Mental State Examination, Letter-Digit Substitution Test, Word Fluency Test, Stroop Test (Reading, Naming, and Interference), Purdue Pegboard Test (right, left, and both hands), and 15-Word Learning Test (Immediate recall, Delayed recall, and Recognition).[15-20]

A measure of global cognitive function was established by the general cognitive factor based on the Letter-Digit Substitution Test, Word Fluency Test, Stroop Test: Interference subtask, sum-score of individual Purdue Pegboard Test subtasks, and Word Learning Test: Delayed recall and was identified as the first unrotated component of a principal component

analysis which explained at least 46.1% of the total variance in individual cognitive tests.[21] The general cognitive factor was only computed if all five individual tests were completed.

The total number of individuals differed per cognitive test because of different moments of implementation of cognitive tests in the examination program or because of missing data (**Figure 1**). All available cognitive test results prior to index age were included for analysis. An overview of the cognitive tests is provided in **Supplementary Table 2**.

Measurement of covariates

During the home interview, we assessed educational level (primary: primary education; lower: lower or intermediate general education, or lower vocational education; intermediate: intermediate vocational education or higher general education; or higher: higher vocational education or university), smoking status (never, current, or former), and alcohol use (yes or no). Symptoms of depression were evaluated with the Centre for Epidemiologic Studies Depression scale (CES-D), which was converted to a sum-score.[22] Body mass index (BMI, kg/m^2) was computed from measurements of height and weight.

Statistical analysis

Each cognitive test was modelled with a two-level linear mixed model with the test result as the outcome and each observation representing one individual cognitive test result. Cognitive test results were transformed, if necessary, to reach an approximate normal distribution. When a transformation did not change the statistical significance, results were reported based on untransformed values for interpretation purposes.

Covariates were case-control status (cancer = 1 for cases, cancer = 0 for controls) and time of cognitive assessments expressed as time to index age (e.g. time = 0 for time at index age, time = -5 for 5 years prior to index age). An interaction term between these two variables reflects whether the change in cognitive function over time differs between cases and controls. Other covariates related to both change in cognitive function[23-28] and cancer[29-31] were age at first test (continuous), sex (women or men), educational level (primary, lower, intermediate, or higher), smoking status (never, current, or former), alcohol use (yes or no), CES-D sum-score (continuous), and BMI (continuous). In case of time-varying covariates – that is smoking status, alcohol use, CES-D sum-score, and BMI – values of covariates measured closest to the date of cognitive assessment were used.

Missing data on covariates were generally between 0% and 2%, except for the CES-D sum-score which was missing in 16% of the total study population. Missing values were replaced with mean (continuous) or mode (categorical) values of the observed data (cases and controls combined). Using linear mixed models, we modelled the jth cognitive test result of

participant i as:

$$\text{Cognitive test result}_{ij} = (\beta_{00} + u_{0i}) + (\beta_1 + u_{1i})\text{Time}_{ij} + \beta_2\text{Cancer}_i + \beta_3\{\text{Time}_{ij}*\text{Cancer}_i\} + \beta_4\text{Age}_i + \beta_5\text{Sex}_i + \beta_6\text{Education}_i + \beta_7\text{Smoking}_i + \beta_8\text{Alcohol}_i + \beta_9\text{CESD}_i + \beta_{10}\text{BMI}_i + \varepsilon_{ij}$$

The β parameters are fixed effects, while the u parameters are random effects, allowing variation of the intercept and slope of time between subjects. A random intercept $\beta_{00} + u_{0i}$ was used to allow for differences in cognitive test results at time of index age ($\text{Time}_{ij} = 0$). A random slope of time $\beta_1 + u_{1i}$ allowed the change in cognitive function over time to vary between participants if the model fit improved based on the Bayesian Information Criterion (BIC). This criterion penalises -2 log likelihood by the number of parameters multiplied by the logarithm of the sample size. The model with the lowest BIC represents the best fitting model.[32] The residual term ε was modelled with the autocorrelation structure of the variance-covariance matrix,[33] and the general positive-definite matrix was used for the random part. The average change in cognitive test result per year for controls before index age was equal to β_1, whereas the change for cases was equal to $\beta_1 + \beta_3$, i.e., β_3 indicates whether the change differs between cases and controls. Sensitivity analyses comparing models with a random intercept only and with a random intercept and slope showed that the choice of models has no effect on β_3 (data not shown). Furthermore, we evaluated non-linearity in time by determining whether including time squared improved the model fit. The normality assumption of the residuals was checked by visual inspection of the QQ-plots.

We performed separate analyses for the most frequent cancer sites (breast, prostate, colorectal, and lung) for which the matching procedure was repeated, with breast cancer cases matched to female controls, and prostate cancer cases to male controls only. In addition, we investigated whether change in cognitive function was different in cases who had metastatic cancer at diagnosis compared to controls, excluding cases with unknown tumour stage (n=718 out of 2059).

To investigate the robustness of our findings, we conducted two sensitivity analyses: (i) including cases and excluding controls with probable or possible cancer (i.e., cancer not confirmed by pathology); and (ii) discarding assessments of controls less than five years before the end of follow-up to minimise effects of potential undiagnosed cancer.

All statistical tests were two-sided and a *P*-value of less than .05 was considered statistically significant. Multiple testing for individual cognitive tests was accounted for by using the Bonferroni method so that a *P*-value of less than .005 was considered statistically significant.

Statistical analyses were performed using SPSS[34] and the 'nlme' package from R software Version 3.3.2.[35]

RESULTS

Characteristics of participants

At the first cognitive assessment, cases were older than controls and were more often men and current smokers (**Table 1**). Furthermore, controls had more often a higher education than cases. Mean (standard deviation [SD]) age at cancer diagnosis was 73.8 years (8.3). Most frequently diagnosed cancer sites were prostate (31.7% among men), breast (29.2% among women), colorectal (16.0%), and lung (12.1%). Of the cases with a known tumour stage (n=1341), 280 had metastatic cancer at diagnosis (20.9%). More details are presented in **Supplementary Table 3**.

Change in cognitive function prior to cancer diagnosis

The Word Learning Test: Immediate recall declined among cases by 0.05 units per year prior to index age (95% CI = -0.09 to -0.01), whereas it increased by 0.01 units per year among controls (95% CI = -0.01 to 0.03; *P* for difference = .003, **Table 2**). The difference was statistically significant after correction for multiple testing and corresponds to 2.4 years of age, given a decline in the Word Learning Test: Immediate recall of 0.25 units per ten years.[21] Although the change over time was different, there was no statistically significant difference between cases and controls at index age. Furthermore, no statistically significant difference in change between cases and controls was observed for the other nine individual cognitive test scores. Also on the general cognitive factor no statistically significant difference was found between cases and controls (*P*=.613, **Figure 2**): cases decreased by 0.02 units per year (95% CI = -0.05 to 0.00) versus 0.03 units per year among controls (95% CI = -0.04 to -0.02).

Separate analyses by cancer site revealed relatively homogeneous cognitive trajectories of both individual cognitive tests and the general cognitive factor (**Table 3**). Also no statistically significant differences in cognitive change were observed between cancer cases with metastatic disease at cancer diagnosis and cancer-free controls.

Sensitivity analyses

After inclusion of cases (n=143) and exclusion of eligible controls with probable or possible cancer, cases still declined faster on the Word Learning Test: Immediate recall than controls (0.04 versus 0.00 units per year, *P*=.009). No statistically significant differences were observed for other cognitive test scores (**Supplementary Table 4**).

Table 1 Characteristics of cases and their matched cancer-free controls at time of first cognitive assessment.

Characteristic	Study population		*P*†
	Cases (n=2059)	Controls (n=7403)*	
Age, years, median (IQR)	64.7 (60.2 to 71.5)	62.5 (58.3 to 70.6)	<.001
Women, No. (%)	980 (47.6)	4446 (60.1)	<.001
Educational level, No. (%)			<.001
Primary	349 (16.9)	1067 (14.4)	
Lower	847 (41.1)	3155 (42.6)	
Intermediate	607 (29.5)	2040 (27.6)	
Higher	256 (12.4)	1141 (15.4)	
Smoking status, No. (%)			<.001
Never	452 (22.0)	2149 (29.0)	
Former	1107 (53.8)	3796 (51.3)	
Current	500 (24.3)	1458 (19.7)	
Alcohol use, No. (%)	1685 (81.8)	6059 (81.8)	.99
CES-D sum-score, median (IQR)	3 (0 to 7)	4 (1 to 8)	<.001
BMI, kg/m^2, median (IQR)	26.7 (24.5 to 29.2)	27.0 (24.6 to 29.7)	<.001
Age at time of cancer diagnosis, years, mean (SD)	73.8 (8.3)		

** Controls were matched to cases per individual cognitive test. Some controls were matched to cases for different cognitive tests, whereas other controls were only matched to cases for one cognitive test. The controls in this Table represent all individual controls used for the different cognitive test analyses.*
† Two-sided P-values were calculated using the independent samples t test (for continuous variables with a normal distribution), the Wilcoxon signed-rank test (for continuous variables with a skewed distribution), and the chi-square test (for categorical variables) to investigate differences in characteristics between cases and controls. A P-value of less than .05 was considered as statistically significant.
BMI = body mass index, CES-D = Centre for Epidemiologic Studies Depression Scale, IQR = interquartile range, SD = standard deviation.

After discarding assessments of controls less than five years before the end of follow-up to exclude effects of potentially undiagnosed cancer, there was no statistically significant difference in change on the Word Learning Test: Immediate recall between cases and controls (*P*=.872, **Supplementary Table 5**). The score of the Word Learning Test: Delayed recall declined by 0.04 units per year (95% CI = -0.12 to 0.04) among cases compared with an increase by 0.03 units per year among controls (95% CI = -0.02 to 0.08). However, this difference was not statistically significant after correction for multiple testing (*P*=.042).

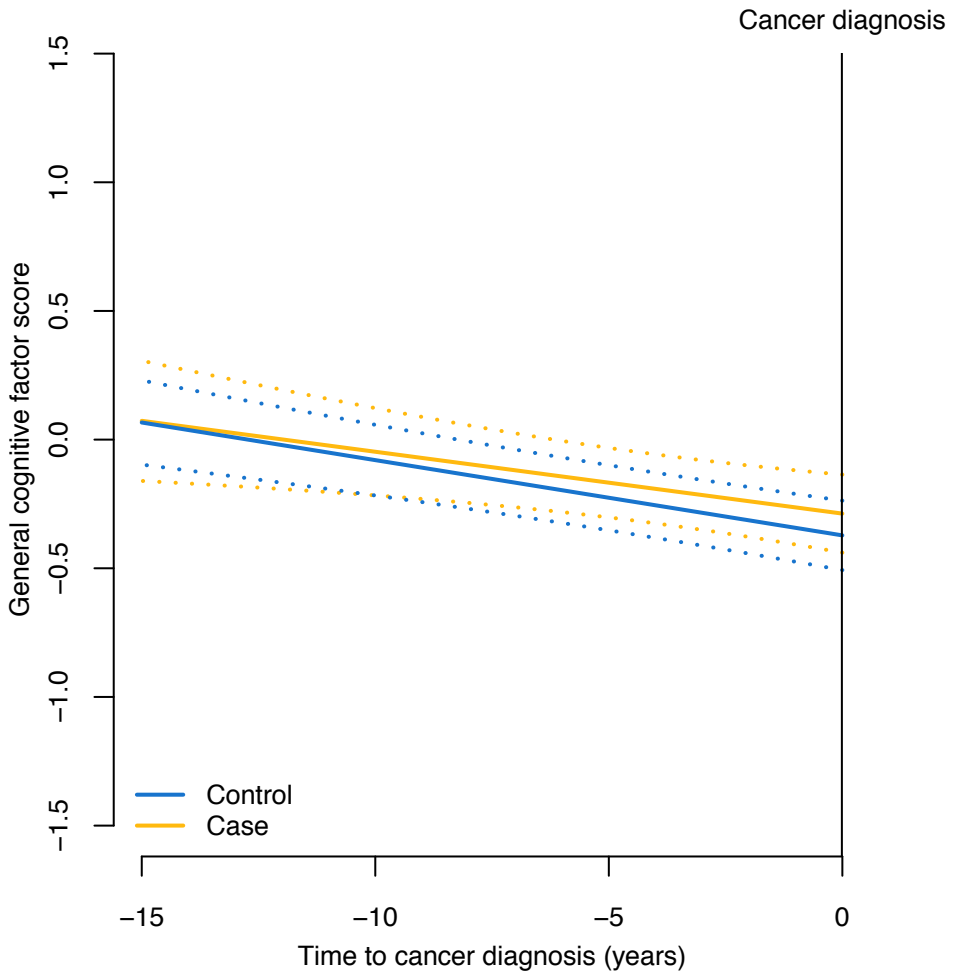


Figure 2 Trajectories of the general cognitive factor scores reflecting global cognitive function for cases (in yellow, prior to cancer diagnosis) and controls (in blue, prior to end of follow-up).

DISCUSSION

This study investigated the change in cognitive function among non-CNS cancer patients prior to cancer diagnosis using the unique setting of a large population-based study. There is a key need to understand the causes of cognitive impairment after non-CNS cancer. Cases performed less well over time in their ability to learn a list of words than cancer-free controls.

Table 2 Change in cognition by case-control status for individual cognitive tests and the general cognitive factor.*

Cognitive test	Cases (No.)	Controls (No.)	Assess-ments (No.)	Cases		Controls		*P* for difference in change§	Difference at index age‖	95% CI	*P* for difference at index age¶
				Change/ year†	95% CI	Change/ year‡	95% CI				
Mini-Mental State Examination	2059	4118	14 926	-0.04	-0.05 to -0.02	-0.03	-0.04 to -0.03	.71	0.11	-0.01 to 0.22	.07
Letter-Digit Substitution Test	1256	2512	7064	-0.29	-0.35 to -0.23	-0.31	-0.33 to -0.28	.43	1.07	0.58 to 1.55	<.001
Word Fluency Test	1263	2526	7045	-0.11	-0.17 to -0.05	-0.12	-0.14 to -0.09	.81	0.52	0.08 to 0.96	.02
Stroop Test: Reading#	1237	2474	6809	0.14	0.09 to 0.19	0.11	0.09 to 0.13	.16	-0.23	-0.57 to 0.10	.17
Stroop Test: Naming#	1234	2468	6839	0.15	0.10 to 0.21	0.15	0.13 to 0.18	.99	-0.68	-1.16 to -0.20	.006
Stroop Test: Interference#	1231	2462	6792	0.67	0.38 to 0.95	0.64	0.51 to 0.77	.84	-2.52	-4.71 to -0.33	.02
Purdue Pegboard Test	904	1808	4229	-0.33	-0.40 to -0.25	-0.32	-0.35 to -0.29	.78	0.64	0.19 to 1.09	.005
Word Learning Test: Immediate recall	782	1564	3350	-0.05	-0.09 to -0.01	0.01	-0.01 to 0.03	.003	-0.18	-0.40 to 0.04	.11
Word Learning Test: Delayed recall	782	1564	3395	-0.03	-0.09 to 0.02	-0.02	-0.04 to 0.01	.56	0.00	-0.29 to 0.30	.99
Word Learning Test: Recognition	784	1568	3380	-0.03	-0.08 to 0.01	-0.01	-0.03 to 0.01	.33	-0.09	-0.33 to 0.14	.43
General cognitive factor	701	1402	2754	-0.02	-0.05 to 0.00	-0.03	-0.04 to -0.02	.61	0.08	-0.03 to 0.20	.13

** Results based on the model: Cognitive test result$_{ij}$ = ($\beta_{00} + u_{0j}$) + ($\beta_1 + u_{1j}$)Time$_{ij}$ + β_2Cancer$_i$ + β_3(Time$_{ij}$*Cancer$_i$) + β_4Age$_i$ + β_5Sex$_i$ + β_6Education$_i$ + β_7Smoking$_i$ + β_8Alcohol$_i$ + β_9CESD$_i$ + β_{10}BMI$_i$ + ε_{ij} for participant i and repeated measure j, assuming autocorrelation structure of the variance-covariance matrix of residuals and the general positive-definite matrix of the random part. † $\beta_1 + \beta_3$, ‡ β_1, § Test of H_0: $\beta_3 = 0$ using the linear mixed model, for Bonferroni adjusted conclusions about individual tests, two-sided P<.05/10 = .005 is statistically significant, ‖ β_2 = difference between cases and controls at time = 0 (at index age), ¶ Test of H_0: $\beta_2 = 0$ using the linear mixed model, for Bonferroni adjusted conclusions about individual tests, two-sided P<.05/10 = .005 is statistically significant, # Better performance corresponds to lower scores.*

BMI = body mass index, CES-D = Centre for Epidemiologic Studies Depression Scale.

However, this difference disappeared in sensitivity analyses when excluding the effects of potentially undiagnosed cancer. We therefore found no evidence in the current study that cognitive function changes differently over time between individuals who will be diagnosed with cancer and individuals who will remain free of cancer.

The hypothesis that cancer outside the CNS does impact the brain resulting in alterations of cognitive function has been posed by several studies that have investigated cognitive function in cancer patients after diagnosis[36] and prior to any type of treatment including surgery. Five out of six studies have observed cognitive impairment in patients compared to either study-specific controls or normative data.[4-9] These observations, differentially explained by inflammation processes triggering neurotoxic cytokine responses, vascular changes, or oxidative stress,[1,5,37] have been supported by preclinical studies showing that tumour-bearing, treatment-naive rodents can have impaired declarative memory.[38-40] However, at this moment, we do not exactly know if and in what way processes may affect cognitive function and if specific cognitive domains may be particularly vulnerable.

How can we explain the disconnect between the current results and these previous findings? First, although most studies carried out after diagnosis and prior to subsequent treatment have tried to adjust for the psychological impact of being recently confronted with a cancer diagnosis, residual confounding can still be a concern.[41-43] This confounder is non-existent in the current study. Second, recruiting patients who have been diagnosed recently with cancer can be challenging, resulting in small sample sizes and susceptibility for selection bias, whereas our study consisted of a large unselected group of both cases and controls.[44,45] Third, our results may not be directly comparable to previous studies because of differences in study design. In the current study, we looked at cognitive changes over time in the years preceding a cancer diagnosis, whereas the other studies have measured cognitive function only once shortly after diagnosis. Also in preclinical studies, cognitive function has been assessed within a short time frame after the tumour had reached a certain size.[38] Fourth, cancer patients in our study were somewhat older (mean age at diagnosis was 73.8 years) than patients in previous studies (mean age generally ranged between 48.6 and 60.5 years,[4-8] only in one study was the mean age 79.8 years[9]). It is questionable, however, whether this difference in age contributes to the discrepant results, because it would require the mechanism of cognitive impairment in cancer patients to be dependent on age.

Since we did not observe statistically significant differences in trajectories of cognitive function between cancer patients and controls prior to cancer diagnosis, a strong role of shared risk factors for both cancer and cognitive impairment prevalent in our study population is less plausible. However, cancer itself could still be considered as a potential underlying cause for subtle cognitive impairment before diagnosis, because we have not evaluated

Table 3 Estimated change in cognitive function by case-control status for individual cognitive tests and the general cognitive factor by cancer site.*

Cognitive test	Prostate cancer			Breast cancer			Colorectal cancer		
	Change/ year cases†	Change/ year controls‡	*P* for difference in change§	Change/ year cases†	Change/ year controls‡	*P* for difference in change§	Change/ year cases†	Change/ year controls‡	*P* for difference in change§
Mini-Mental State Examination	-0.03	-0.03	.84	-0.04	-0.04	.92	-0.02	-0.05	.03
Letter-Digit Substitution Test	-0.34	-0.32	.83	-0.19	-0.33	.09	-0.29	-0.35	.41
Word Fluency Test	-0.02	-0.07	.56	-0.10	-0.14	.65	-0.15	-0.09	.40
Stroop Test: Reading‖	0.15	0.14	.81	0.13	0.07	.30	0.10	0.11	.90
Stroop Test: Naming‖	0.15	0.18	.61	0.14	0.18	.51	0.05	0.17	.16
Stroop Test: Interference‖	0.26	0.70	.06	0.93	0.69	.51	0.48	0.32	.68
Purdue Pegboard Test	-0.40	-0.43	.71	-0.30	-0.25	.57	-0.25	-0.26	.88
Word Learning Test: Immediate recall	-0.06	-0.02	.29	0.03	-0.01	.58	-0.11	-0.01	.03
Word Learning Test: Delayed recall	0.03	-0.02	.41	0.05	-0.02	.44	-0.06	0.00	.28
Word Learning Test: Recognition	-0.07	-0.04	.61	0.06	0.03	.63	-0.07	0.02	.05
General cognitive factor	-0.03	0.02	.12	-0.04	-0.02	.41	0.07	0.06	.74

**Results based on the model:* $Cognitive\ test\ result_{ij} = (\beta_{00} + u_{0i}) + (\beta_1 + u_{1i})Time_{ij} + \beta_2 Cancer_i + \beta_3(Time_{ij}{*}Cancert_i) + \beta_4 Age_i + \beta_5 Sex_i + \beta_6 Education_i + \beta_7 Smoking_i + \beta_8 Alcohol_i + \beta_9 CESD_i + \beta_{10} BMI_i + \varepsilon_{ij}$ *for participant i and repeated measure j, assuming autocorrelation structure of the variance-covariance matrix of residuals and the general positive-definite matrix of the random part. Models were run for each different cancer site separately.* β_5 *was not used for breast and prostate cancer.* † $\beta_1 + \beta_3$, ‡ β_1, § *Test of* H_0: $\beta_{13} = 0$ *using the linear mixed model, for Bonferroni adjusted conclusions about individual tests, two-sided P<.05/10 = .005 is statistically significant,* ‖ *Better performance corresponds to lower scores.*

BMI = body mass index, CES-D = Centre for Epidemiologic Studies Depression Scale.

Table 3 Estimated change in cognitive function by case-control status for individual cognitive tests and the general cognitive factor by cancer site (continued).*

Cognitive test	Lung cancer			Metastatic cancer		
	Change/ year cases†	Change/ year controls‡	*P* for difference in change§	Change/ year cases†	Change/ year controls‡	*P* for difference in change§
Mini-Mental State Examination	-0.04	-0.03	.49	-0.04	-0.04	.86
Letter-Digit Substitution Test	-0.31	-0.27	.58	-0.32	-0.26	.29
Word Fluency Test	-0.10	-0.15	.51	-0.07	-0.10	.66
Stroop Test: Reading‖	0.17	0.10	.32	0.09	0.11	.69
Stroop Test: Naming‖	0.24	0.11	.06	0.16	0.14	.71
Stroop Test: Interference‖	1.54	0.73	.01	1.03	0.92	.70
Purdue Pegboard Test	-0.32	-0.35	.80	-0.33	-0.32	.88
Word Learning Test: Immediate recall	-0.07	0.02	.10	0.06	0.02	.46
Word Learning Test: Delayed recall	-0.08	-0.01	.35	0.07	0.02	.43
Word Learning Test: Recognition	-0.02	-0.03	.83	0.02	-0.00	.64
General cognitive factor	0.02	0.02	.96	-0.03	-0.02	.76

**Results based on the model: Cognitive test result$_{ij}$ = (β_{00} + u_{0i}) + (β_1 + u_{1i})Time$_{ij}$ + β_2Cancer$_i$ + β_3(Time$_{ij}$*Cancer$_i$) + β_4Age$_i$ + β_5Sex$_i$ + β_6Education$_i$ + β_7Smoking$_i$ + β_8Alcohol$_i$ + β_9CESD$_i$ + β_{10}BMI$_i$ + ε_{ij} for participant i and repeated measure j, assuming autocorrelation structure of the variance-covariance matrix of residuals and the general positive-definite matrix of the random part. Models were run for each different cancer site separately. β_5 was not used for breast and prostate cancer. † β_1 + β_3, ‡ β_1, § Test of H_0: β_{13} = 0 using the linear mixed model, for Bonferroni adjusted conclusions about individual tests, two-sided P<.05/10 = .005 is statistically significant, ‖ Better performance corresponds to lower scores.*
BMI = body mass index, CES-D = Centre for Epidemiologic Studies Depression Scale.

cognitive function repeatedly within a short timeframe, for example in the weeks directly preceding cancer diagnosis.

This latter point is one of several limitations that we faced. Because of the design of the Rotterdam Study in which cognition is intended to be assessed every three to six years, we could not investigate cognitive function within smaller timeframes directly preceding cancer diagnosis. In addition, by using linear mixed models we assumed a linear change in cognitive function, which may have led to model misspecification if cognitive change is not linear. However, including time squared in the model did not improve the model fit and resulted in less power to detect differences in cognitive change. Therefore, we have chosen not to include non-linear parameters in the model. Also, we investigated the average cognitive trajectories by which we may not have been able to identify subgroups of patients who do have steeper cognitive declines prior to cancer diagnosis. Lastly, we did not have information about the location of metastases at time of cancer diagnosis and could therefore not exclude participants with brain metastases. However, we did not observe that cognitive function changed differently among cases with metastatic disease than among controls.

In addition, our study has multiple and unique strengths. It is a population-based cohort with standardised ascertainment of cognitive function and cancer incidence, providing the opportunity to investigate change in cognitive function prior to cancer diagnosis. We studied an unselected sample of cases and controls, thereby minimising the effects of selection bias. By using linear mixed models, we were able to investigate the change in cognitive function per year. Also, our study has by far the largest number of participants in this research area. Most previous studies have included 56 up to 174 patients,[4-8] and even the largest study with 341 patients[9] is much smaller than the current study. This enabled us to investigate cognitive trajectories for different cancer sites. Lastly, we investigated the trajectory of the general cognitive factor in addition to the trajectories of the individual cognitive tests, because we did not have an indication for a specific cognitive domain to be affected.

In conclusion, we found no evidence that cognitive function declines differently over time among individuals who will be diagnosed with cancer prior to disease manifestation than among individuals who will remain cancer-free. Our results suggest that the role of shared risk factors for both cancer and cognitive impairment on cognitive function in cancer patients is limited. Future research needs to confirm our findings and to evaluate cognitive function within a short period before cancer diagnosis to estimate the effects of undiagnosed cancer on cognitive function more accurately.

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Supplementary Table 1 Overview of number of discarded assessments of included controls.

Cognitive test	N included controls	Total N assessments of controls*	N assessments discarded after a dementia diagnosis	N assessments discarded after a stroke diagnosis	N assessments discarded after the matched index age	Included N assessments of controls†
Mini-Mental State Examination	4118	11 055	73	367	511	10 104
Letter-Digit Substitution Test	2512	5151	14	122	78	4937
Word Fluency Test	2526	5180	20	132	95	4933
Stroop Test: Naming	2474	5005	25	136	99	4745
Stroop Test: Reading	2468	5023	18	132	94	4779
Stroop Test: Interference	2462	4955	13	128	76	4738
Purdue Pegboard Test	1808	3040	5	54	34	2947
Word Learning Test: Immediate recall	1564	2383	4	38	25	2316
Word Learning Test: Delayed recall	1564	2434	6	39	28	2361
Word Learning Test: Recognition	1568	2409	5	37	23	2344
General cognitive factor	1402	2092	3	27	152	1910

** Total number of assessments of controls prior to discarding assessments after dementia diagnosis, stroke diagnosis, and after the matched index age. † Total number of included assessments of controls after discarding assessments after dementia diagnosis, stroke diagnosis, and after the matched index age.*
N = number.

Supplementary Table 2 Overview of neuropsychological tests.

Neuropsychological test	Acronym	Test element	Domain assessed	Outcome measured	Range	Score good performance	Implemented round RS
Mini-Mental State Examination	MMSE		Global cognition, dementia screener	Total correct answers	0 to 30	High	RS-1-1
Letter-Digit Substitution test	LDST		Information processing and executive functioning	Number of correctly substituted letters	0 to 125	High	RS-1-3
Word Fluency Test	WFT		Executive functioning	Number of animals mentioned within one minute	≥0	High	RS-1-3
Stroop Test		1st subtask Reading; word card	Information processing	Seconds needed to complete the first four lines	≥0	Low	RS-1-3
		2nd subtask Naming; colour card	Information processing	Idem	≥0	Low	RS-1-3
		3rd subtask Interference; colour-word card	Executive functioning	Idem	≥0	Low	RS-1-3
Purdue Pegboard Test	PPT	Sum-score of right, left, and both hands	Motor function	Number of pins inserted in the board within 1 minute for right, left, and both hands separately	0 to 75	High	RS-2-1
15-Word Learning Test	WLT	Immediate recall	Memory	Number of words remembered immediately after each trial	0 to 15	High	RS-4-1
		Delayed recall	Memory	Number of words remembered after 20 minutes	0 to 15	High	RS-4-1
		Recognition	Memory	Number of words recognized	0 to 30	High	RS-4-1
General cognitive factor			Global cognitive function	Compound score of LDST, WFT, Stroop 3rd subtask, PPT, and WLT: Delayed recall	-4 to 4	High	RS-4-1

RS = Rotterdam Study, LDST = Letter-Digit Substitution Test, MMSE = Mini-Mental State Examination, PPT = Purdue Pegboard Test, WFT = Word Fluency Test, WLT = Word Learning Test.

Supplementary Table 3 Overview of cognitive test scores, number of assessments, time between assessments, and time between assessment and cancer diagnosis.

Cognitive test	Number of participants		Mean test score at first assessment (SD)*		Number of cognitive assessments per participant					
					Cases			Controls		
	Cases	Controls	Cases	Controls	1	2	≥3	1	2	≥3
Mini-Mental State Examination	2059	4118	28 (27 to 29)	28 (27 to 29)	646	651	762	2059	1235	1189
Letter-Digit Substitution Test	1256	2512	28 (7)	29 (7)	620	451	185	1256	898	886
Word Fluency Test	1263	2526	22 (5)	22 (6)	649	428	186	1263	919	891
Stroop Test: Reading	1237	2474	17 (15 to 19)	17 (15 to 19)	635	426	176	1237	935	879
Stroop Test: Naming	1234	2468	23 (21 to 27)	23 (21 to 27)	633	425	176	1234	917	861
Stroop Test: Interference	1231	2462	50 (41 to 61)	49 (41 to 62)	632	424	175	1231	929	851
Purdue Pegboard Test	904	1808	35 (5)	35 (5)	578	274	52	904	903	671
Word Learning Test: Immediate recall	782	1564	7 (2)	7 (2)	581	158	43	782	884	632
Word Learning Test: Delayed recall	782	1564	7 (3)	7 (3)	581	158	43	782	854	648
Word Learning Test: Recognition	784	1568	14 (12 to 15)	14 (12 to 15)	582	160	42	784	862	658
General cognitive factor	701	1402	-0.1 (1)	0.0 (1)	563	133	5	701	914	468

** Values are presented as median (interquartile range) for the Mini-Mental State Examination, Stroop Test: Reading, Stroop Test: Naming, Stroop Test: Interference, and Word Learning Test: Recognition.*
SD = standard deviation.

Supplementary Table 3 Overview of cognitive test scores, number of assessments, time between assessments, and time between assessment and cancer diagnosis (continued).

Cognitive test	Median time between assessments (IQR)		Median time between last assessment and index age (IQR)	
	Cases	Controls	Cases	Controls
Mini-Mental State Examination	3.3 (1.8 to 4.6)	4.3 (2.4 to 5.4)	-2.4 (-4.3 to -1.1)	-2.7 (-5.4 to -1.1)
Letter-Digit Substitution Test	4.4 (3.7 to 4.8)	4.7 (4.2 to 6.3)	-2.6 (-4.4 to -1.2)	-2.4 (-5.1 to -1.1)
Word Fluency Test	4.4 (3.6 to 4.9)	4.7 (4.2 to 6.3)	-2.6 (-4.4 to -1.2)	-2.3 (-4.9 to -1.0)
Stroop Test: Reading	4.4 (3.5 to 4.8)	4.7 (4.2 to 6.3)	-2.7 (-4.5 to -1.2)	-2.4 (-5.1 to -1.1)
Stroop Test: Naming	4.4 (3.4 to 4.8)	4.8 (4.2 to 6.3)	-2.7 (-4.5 to -1.2)	-2.4 (-5.1 to -1.0)
Stroop Test: Interference	4.4 (3.5 to 4.8)	4.8 (4.3 to 6.3)	-2.7 (-4.5 to -1.2)	-2.4 (-5.0 to -1.0)
Purdue Pegboard Test	4.3 (4.0 to 6.2)	5.6 (4.2 to 6.4)	-2.8 (-4.8 to -1.3)	-2.5 (-5.0 to -1.1)
Word Learning Test: Immediate recall	5.6 (3.2 to 6.5)	6.3 (5.6 to 6.5)	-2.6 (-4.5 to -1.2)	-2.2 (-4.6 to -1.1)
Word Learning Test: Delayed recall	5.6 (3.2 to 6.5)	6.3 (5.6 to 6.6)	-2.6 (-4.5 to -1.2)	-2.2 (-4.4 to -1.0)
Word Learning Test: Recognition	5.7 (3.2 to 6.5)	6.3 (5.6 to 6.5)	-2.6 (-4.5 to -1.2)	-2.3 (-4.3 to -1.0)
General cognitive factor	6.3 (3.8 to 6.5)	6.4 (5.9 to 6.6)	-2.9 (-4.9 to -1.3)	-2.6 (-5.2 to -1.1)

IQR = interquartile range.

Supplementary Table 4 Estimated change of cognitive function by case-control status. Cases with cancer not confirmed by pathology (probable or possible cancer) were included.*

Cognitive test	Cases (No.)	Controls (No.)	Assessments (No.)	Cases		Controls		*P* for difference in change§	Difference at index age‖	95% CI	*P* for difference at index age¶
				Change/year†	95% CI	Change/year‡	95% CI				
Mini-Mental State Examination	2202	4404	16 199	-0.03	-0.05 to -0.02	-0.04	-0.04 to -0.03	.77	0.12	0.01 to 0.23	.03
Letter-Digit Substitution Test	1334	2668	7600	-0.30	-0.35 to -0.24	-0.31	-0.33 to -0.29	.59	0.75	0.28 to 1.23	.002
Word Fluency Test	1343	2686	7589	-0.12	-0.18 to -0.06	-0.11	-0.13 to -0.08	.56	0.69	0.27 to 1.10	.001
Stroop Test: Reading#	1314	2628	7335	0.12	0.08 to 0.17	0.12	0.10 to 0.14	.16	-0.18	-0.50 to 0.14	.27
Stroop Test: Naming#	1311	2622	7315	0.15	0.10 to 0.20	0.15	0.12 to 0.17	.74	-0.64	-1.10 to -0.18	.006
Stroop Test: Interference#	1308	2616	7285	0.64	0.38 to 0.90	0.76	0.65 to 0.88	.30	-2.76	-4.93 to -0.60	.01
Purdue Pegboard Test	963	1926	4564	-0.32	-0.39 to -0.25	-0.32	-0.35 to -0.29	.92	0.75	0.32 to 1.18	.001
Word Learning Test: Immediate recall	848	1696	3678	-0.04	-0.08 to -0.01	-0.00	-0.02 to 0.02	.009	-0.12	-0.32 to 0.09	.26
Word Learning Test: Delayed recall	848	1696	3670	-0.03	-0.08 to 0.02	-0.02	-0.04 to 0.00	.71	-0.01	-0.30 to 0.27	.92
Word Learning Test: Recognition	850	1700	3725	-0.04	-0.08 to 0.00	-0.01	-0.03 to 0.01	.11	-0.11	-0.34 to 0.12	.34
General cognitive factor	748	1496	2965	0.05	0.03 to 0.06	0.05	0.04 to 0.05	.65	-0.07	-0.16 to 0.02	.12

** Results based on the model: Cognitive test result*$_{ij} = (\beta_{00} + u_{0j}) + (\beta_1 + u_{1j})Time_{ij} + \beta_2 Cancer_i + \beta_3(Time_{ij}*Cancer_i) + \beta_4 Age_i + \beta_5 Sex_i + \beta_6 Education_i + \beta_7 Smoking_i + \beta_8 Alcohol_i + \beta_9 CESD_i + \beta_{10} BMI_i + \varepsilon_{ij}$ *for participant i and repeated measure j, assuming autocorrelation structure of the variance-covariance matrix of residuals and the general positive-definite matrix of the random part. † $\beta_1 + \beta_3$, ‡ β_1, § Test of H_0: $\beta_3 = 0$ using the linear mixed model, for Bonferroni adjusted conclusions about individual tests, two-sided P<.05/10 = .005 is statistically significant, ‖ β_2 = difference between cases and controls at time = 0 (at index age), ¶ Test of H_0: $\beta_2 = 0$ using the linear mixed model, for Bonferroni adjusted conclusions about individual tests, two-sided P<.05/10 = .005 is statistically significant, # Better performance corresponds to lower scores.*

BMI = body mass index, CES-D = Centre for Epidemiologic Studies Depression Scale.

Supplementary Table 5 Estimated change of cognitive function by case-control status. Assessments of controls within five years of the end of follow-up were discarded.*

Cognitive test	Cases (No.)	Controls (No.)	Assess-ments (No.)	Cases		Controls		*P* for difference in change§	Difference at index age‖	95% CI	*P* for difference at index age¶
				Change/ year†	95% CI	Change/ year‡	95% CI				
Mini-Mental State Examination	2059	4118	15 083	-0.03	-0.05 to -0.02	-0.04	-0.05 to -0.04	.10	0.08	-0.03 to 0.20	.16
Letter-Digit Substitution Test	1256	2512	6436	-0.29	-0.36 to -0.22	-0.35	-0.39 to -0.31	.05	-0.01	-0.50 to 0.48	.97
Word Fluency Test	1263	2526	6361	-0.11	-0.19 to -0.03	-0.17	-0.21 to -0.13	.08	0.40	-0.05 to 0.84	.08
Stroop Test: Reading#	1237	2474	6224	0.12	0.06 to 0.18	0.18	0.15 to 0.22	.02	-0.36	-0.72 to -0.00	.05
Stroop Test: Naming#	1234	2468	6183	0.15	0.08 to 0.22	0.13	0.09 to 0.17	.54	-0.07	-0.56 to 0.42	.77
Stroop Test: Interference#	1231	2462	6130	0.63	0.23 to 1.02	0.82	0.61 to 1.03	.27	0.16	-1.95 to 2.27	.88
Purdue Pegboard Test	904	1808	3631	-0.32	-0.43 to -0.22	-0.34	-0.40 to -0.28	.69	-0.33	-0.80 to 0.14	.17
Word Learning Test: Immediate recall	782	1564	2793	-0.05	-0.10 to 0.01	-0.05	-0.08 to -0.01	.87	-0.07	-0.30 to 0.16	.54
Word Learning Test: Delayed recall	782	1564	2805	-0.04	-0.12 to 0.04	0.03	-0.02 to 0.08	.04	-0.07	-0.30 to 0.16	.10
Word Learning Test: Recognition	784	1568	2797	-0.04	-0.10 to 0.03	-0.07	-0.11 to -0.03	.16	0.02	-0.24 to 0.28	.89
General cognitive factor	701	1402	2318	0.02	-0.02 to 0.06	0.01	-0.01 to 0.04	.74	0.12	-0.02 to 0.25	.08

** Results based on the model: Cognitive test result$_{ij}$ = (β_{00} + u_{0i}) + (β_1 + u_{1i})Time$_{ij}$ + β_2Cancer$_i$ + β_3(Time$_{ij}$*Cancer$_i$) + β_4Age$_i$ + β_5Sex$_i$ + β_6Education$_i$ + β_7Smoking$_i$ + β_8Alcohol$_i$ + β_9CESD$_i$ + β_{10}BMI$_i$ + ε_{ij} for participant i and repeated measure j, assuming autocorrelation structure of the variance-covariance matrix of residuals and the general positive-definite matrix of the random part. † β_1 + β_3, ‡ β_1, § Test of H_0: β_3 = 0 using the linear mixed model, for Bonferroni adjusted conclusions about individual tests, two-sided P<.05/10 = .005 is statistically significant, ‖ β_2 = difference between cases and controls at time = 0 (at index age), ¶ Test of H_0: β_2 = 0 using the linear mixed model, for Bonferroni adjusted conclusions about individual tests, two-sided P<.05/10 = .005 is statistically significant, # Better performance corresponds to lower scores.*

BMI = body mass index, CES-D = Centre for Epidemiologic Studies Depression Scale.

Chapter 6

Brain structure prior to non-central nervous system cancer diagnosis

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ABSTRACT

Background Many studies have shown that patients with non-central nervous system (CNS) cancer can have brain abnormalities, such as reduced grey matter volume and cerebral microbleeds. These abnormalities can sometimes be present even before start of treatment, suggesting a potential detrimental effect of non-CNS cancer itself on the brain. In these previous studies, psychological factors associated with a cancer diagnosis and selection bias may have influenced results. To overcome these limitations, we investigated brain structure with magnetic resonance imaging (MRI) prior to cancer diagnosis.

Methods Between 2005 and 2014, 4622 participants from the prospective population-based Rotterdam Study who were free of cancer, dementia, and stroke, underwent brain MRI and were subsequently followed for incident cancer until January 1st, 2015. We investigated the association between brain MRI measurements, including cerebral small vessel disease, volumes of global brain tissue, lobes, and subcortical structures, and global white matter microstructure, and the risk of non-CNS cancer using Cox proportional hazards models. Age was used as time scale. Models were corrected for e.g. sex, intracranial volume, educational level, body mass index, hypertension, diabetes mellitus, smoking status, alcohol use, and depression sum-score.

Results During a median (interquartile range) follow-up of 7.0 years (4.9 to 8.1), 353 participants were diagnosed with non-CNS cancer. Results indicated that persons who develop cancer do not have more brain abnormalities before clinical manifestation of the disease than persons who remain free of cancer. The largest effect estimates were found for the relation between presence of lacunar infarcts and the risk of cancer (hazard ratio [HR] 95% confidence interval [CI] = 1.39 [0.97 to 1.98]) and for total brain volume (HR [95% CI] per standard deviation increase in total brain volume = 0.76 [0.55 to 1.04]).

Conclusions We did not observe associations between small vessel disease, brain tissue volumes, and global white matter microstructure, and subsequent cancer risk in an unselected population. These findings deviate from previous studies indicating brain abnormalities among patients shortly after cancer diagnosis.

INTRODUCTION

Patients with non-central nervous system (CNS) cancer frequently report cognitive problems during and after cancer treatment.[1-3] Whereas most research has focused on the effects of cancer treatment (e.g. chemotherapy) on brain health and cognitive function, several studies have shown that cancer patients can have impaired cognitive function even before start of cancer treatment.[4-12] This pretreatment cognitive impairment can sometimes persist after adjustment for psychological factors, suggesting that non-CNS cancer may impact the brain apart from cancer treatment, for instance through inflammatory or vascular processes.[1,8,13-15] This hypothesis has further been supported by preclinical studies showing that tumour-bearing, treatment-naive rodents can have impaired memory function.[16-18]

Understanding the underlying causes of cognitive impairment in non-CNS cancer patients is pivotal to develop prevention and intervention strategies. Several neuroimaging studies have performed brain magnetic resonance imaging (MRI) to investigate the neural underpinnings of cognitive impairment in cancer patients from pre- to posttreatment.[19-21] These studies have shown subtle changes in grey and white matter volumes and frontal lobe hyperactivation before start of treatment, and various brain abnormalities after treatment, including reductions in grey matter volume, cerebral microbleeds, and decreased white matter microstructure.[5-7,12,20,22-28]

However, these studies are challenged by the effects of psychological factors accompanying a cancer diagnosis, including stress, depression, and anxiety, which may influence brain structure.[29,30] Also, the feasibility of a baseline assessment after diagnosis but before subsequent treatment is limited, resulting in high rates of non-participation and selection bias.[31] These limitations can be overcome by studying brain structure and function of cancer patients before cancer diagnosis, with the underlying assumption that cancer is already present, yet not diagnosed.

Within the unique setting of the prospective population-based Rotterdam Study, we have previously shown that the trajectory of cognitive function prior to cancer diagnosis did not differ between participants who developed cancer and those who remained cancer-free during follow-up.[32] Since in general, changes in brain structure correlate only moderately with cognitive function,[33] absence of accelerated change in cognitive function before cancer diagnosis does not preclude presence of abnormalities in brain structure.

Here, we studied the association between brain MRI measurements of cerebral small vessel disease, brain tissue volumes, and white matter microstructure prior to the clinical manifestation of cancer, and the subsequent risk of different types of non-CNS cancer. Such associations may reflect whether there are differences in brain structure between participants

who are diagnosed with cancer during follow-up and those who remain cancer-free. This study population is defined by the availability of brain MRI scans. Therefore, the study is conducted in a slightly different sample than the sample in which we found no indication of impaired cognitive function before cancer diagnosis.[32] For this reason, we also explored the association between cognitive function (self-reported and tested) and the risk of cancer in the current sample.

METHODS

Setting

This study was embedded in the Rotterdam Study, an ongoing population-based prospective cohort study that investigates determinants and occurrence of chronic diseases in the middle-aged and elderly population. The design of the Rotterdam Study has been described in detail previously.[34] Briefly, the initial cohort started in 1989 with 7983 participants aged 55 years and over residing in the district Ommoord in Rotterdam, the Netherlands. The cohort was expanded with 3011 participants in 2000, followed by an additional inclusion of 3392 participants aged at least 45 years in 2006. From 2005 onwards, brain MRI was implemented into the study protocol of the Rotterdam Study.[35]

Participants were interviewed at home by a trained research assistant, followed by two visits to the research facility for different examinations including laboratory assessments and imaging. Follow-up examinations take place every three to five years.

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus Medical Centre and by the Ministry of Health, Welfare and Sport of the Netherlands. Written informed consent was obtained from all participants.

Study population

Out of the 14 926 participants of the Rotterdam Study, 5766 had at least one brain MRI scan acquired between 2005 and 2014. Of the 5766 participants, we excluded those without informed consent to access medical files during follow-up (n=30), with a history of dementia (n=57) or who were not sufficiently screened for history of dementia (n=43), with a history of stroke (n=198), with a history of cancer (n=464), and those without any cognitive test result (n=9), resulting in 4965 eligible participants. Subsequently, we excluded participants who had MRI scans with artefacts and unreliable tissue segmentation (n=121), without FreeSurfer segmentation (n=37), with poor FreeSurfer segmentation quality (n=94), and with MRI-defined

cortical infarcts (n=91), ending up with 4622 participants. For diffusion tensor imaging (DTI) analyses we additionally excluded participants who had MRI scans, but no available DTI data (n=268), resulting in 4354 participants for DTI analyses (**Figure 1**). If a participant had multiple MRI scans, we included only the first obtained scan for analyses to avoid bias because of the prospective cohort design.

MRI acquisition and processing

Brain MRI was performed on a 1.5-tesla MRI scanner with a dedicated 8-channel head coil (General Electric Healthcare, Milwaukee, USA). The scan protocol and sequence details have been described in detail previously and are summarised in **Supplementary Table 1**.[35] Scans for brain volumetry included T1-weighted (voxel size 0.49 x 0.49 x 1.6 mm^3), proton density-weighted (voxel size 0.6 x 0.98 x 1.6 mm^3), and fluid-attenuated inversion recovery (FLAIR, voxel size 0.78 x 1.12 x 2.5 mm^3) sequences which were used for automated segmentation of supratentorial grey matter volume, white matter volume, cerebrospinal fluid, and white matter hyperintensities.[36,37] Pre-processing included co-registration, correction of non-uniformity, and variance scaling. Before segmentations, the brain is extracted from the scan using a manually segmented brain mask that is non-rigidly registered to the T1-weighted image using Elastix.[38] We used the k-nearest neighbour segmentation to classify scans into brain tissue volumes and cerebrospinal fluid.[39] All segmentations were inspected and manually corrected if necessary using a dedicated tool that has been developed in MevisLab that can visualize the original scan with the image processing results.[35] Editing tools were available to adjust segmentations if necessary. Manual editing of errors was needed in less than ten percent.

Markers of cerebral small vessel disease included white matter hyperintensity volume (mL), presence of cerebral microbleeds, and presence of infarcts. Cerebral microbleeds were rated on a 3-dimensional, T2*-weighted gradient-recalled echo MRI scan (voxel size 0.78 x 1.12 x 1.6 mm^3) as focal areas of very low signal intensity. Infarcts were categorised as cortical infarcts (infarcts with involvement of cortical grey matter which were excluded for analyses for reliability of tissue segmentations) and lacunar infarcts (focal lesions between 3 and 15 mm in non-cortical tissue with signal intensity similar to that of cerebrospinal fluid on all sequences, and, when located supratentorially, with a hyperintense rim on the FLAIR sequence).[40,41]

Total brain volume (mL) was defined as the sum of grey matter volume (mL) and white matter volume (mL, sum of normal appearing white matter and white matter hyperintensity volume). Intracranial volume (mL) was the sum of total brain volume and cerebrospinal fluid. Although these volumes were restricted to the supratentorial region, we refer to these volumes as total brain volume and intracranial volume. Lobar volumes were segmented by using an atlas in which the lobes were manually outlined.[42] This atlas was subsequently

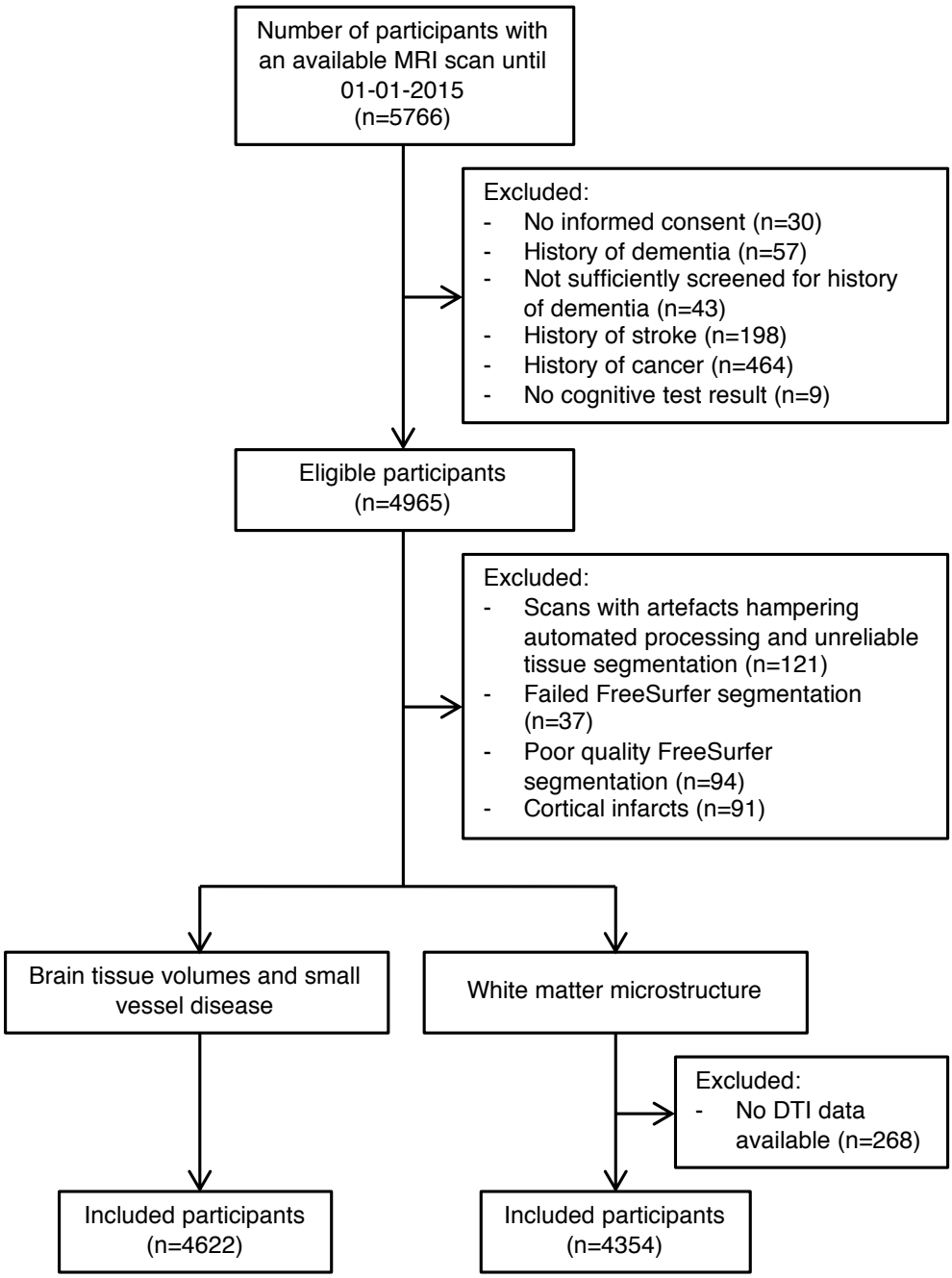

Figure 1 Flowchart of study population.
DTI = diffusion tensor imaging, MRI = magnetic resonance imaging.

non-rigidly transformed to each brain to obtain the volume of each lobe.[35] Lobar volumes included both grey matter and white matter. T1-weighted MR images were processed using FreeSurfer (version 6.0) to calculate cortical thickness (mm), cortical surface area (mm^2), and subcortical volumes (mL) of the hippocampus, amygdala, caudate, putamen, thalamus, and pallidum (FreeSurfer is freely available for download online at http://surfer.nmr.mgh.harvard.edu/). For quality assessment, we have randomly selected a subset of scans that we visually inspected. Next, we identified a cut-off on our automated quality assessment metric which allowed us to exclude unusable FreeSurfer data.[43] This cut-off has subsequently been applied to the remaining data and all scans below this cut-off were excluded. We have confirmed that several metrics (e.g., cortical thickness) have no significant correlation with the automated quality assessment metric after the exclusions have been performed.[44]

Measurements of white matter microstructure were obtained from DTI (supratentorially, voxel size 3.3 x 2.2 x 3.5 mm^3), which was embedded in the protocol of the Rotterdam Study from March 2006 onwards.[37,45] Echo-planar imaging (EPI) was used as readout module. Normal appearing white matter was distinguished from white matter hyperintensities using an automatic post-processing step based on the FLAIR image and the tissue segmentation.[36] Next, the segmentation of white matter hyperintensities was mapped into DTI image space using boundary-based registration performed on the white matter segmentation and the T1-weighted image.[46] Co-registrations of the DTI to the T1-weighted image were visually inspected to ensure a good fit and that DTI measures did not include grey matter or cerebrospinal fluid partial volumes. This co-registration partly corrected potential non-linear changes induced by the EPI readout module. DTI data were pre-processed using a standardised pipeline that included correction for subject motion and Eddy currents, estimation of the diffusion tensor, and registration to tissue segmentation matter.[45] Diffusion tensors were estimated using a non-linear Levenberg-Marquardt estimator (available in Explore DTI),[47] from which global mean fractional anisotropy (FA) and mean diffusivity (MD, 10^{-3} mm^2/s) in the normal appearing white matter were obtained. FA reflects the degree of diffusion directionality of water molecules.[48] MD represents the average diffusion of water molecules. Lower FA and higher MD are indications of lower white matter microstructure. DTI images were manually inspected for registration and segmentation and corrected where possible. Between February 2007 and May 2008, 1169 participants were scanned with the phase and frequency encoding directions swapped for the diffusion acquisition due to a technical issue. We have therefore included phase encoding direction as covariate in the analyses (see statistical analysis).[49]

Cognitive function assessment

Cognitive function was assessed by a neuropsychological test battery administered at the

research centre. Assessments took place between 2002 and 2014. The cognitive assessment corresponding to the same visit round as the visit round of the MRI scan was used, with a median (interquartile range [IQR]) time between cognitive function assessment and MRI scan of -0.13 years (-0.31 to -0.08). Up to 2015, the following cognitive tests were administered: Mini-Mental State Examination (MMSE), Word Fluency Test (WFT), Letter-Digit Substitution Test (LDST), Stroop Test (Reading, Naming, Interference), Purdue Pegboard Test (PPT, right, left, both hands), and 15-Word Learning Test (WLT, Immediate recall, Delayed recall, Recognition).[50-55]

Global cognitive function was assessed by the general cognitive factor based on WFT, LDST, Stroop Test: Interference, sum-score of individual PPTs, and WLT: Delayed recall and was identified as the first unrotated component of a principal component analysis, which explained at least 48.0% of the total variance in individual cognitive tests.[56] The general cognitive factor was only computed if all five individual tests were completed.

Self-reported memory complaints were measured with three yes/no questions: (i) 'Do you have more problems remembering things than before?'; (ii) 'Has there been an increase in the times that you forgot what you were up to?'; and (iii) 'Do you have more word-finding problems than before?'.

Ascertainment of cancer

Diagnoses of cancer were based on medical records of general practitioners (including hospital discharge letters) and through linkage with Dutch Hospital Data, Netherlands Cancer Registry, and histology and cytopathology registries in the region.[34] Incident cancer was defined as any primary malignant tumour, excluding non-melanoma skin cancer. Diagnoses were coded independently by two physicians according to the International Classification of Diseases, tenth revision (ICD-10). In case of discrepancy, consensus was sought through consultation with a physician specialised in internal medicine. Date of diagnosis was based on date of biopsy (solid tumours) and laboratory assessment (haematological tumours), or – if unavailable – date of hospital admission or discharge letter. Only pathology-confirmed cancers were included in the analysis. Follow-up of cancer registration was completed up to January 1st, 2015.

Measurement of covariates

During home interviews, participants provided information on educational level, smoking status, and alcohol use. Educational level was classified into primary, lower (lower or intermediate general education, or lower vocational education), intermediate (intermediate vocational education or higher general education), or higher (higher vocational education

or university). Smoking was categorised as never, current, or former. Alcohol use was classified into any use or no use of alcohol. At the research centre, height and weight were measured from which the body mass index (BMI, kg/m^2) was computed. Furthermore, systolic and diastolic blood pressures were measured twice on the right arm with a random-zero sphygmomanometer of which the mean was used for analyses. Hypertension was defined as a systolic blood pressure of ≥140 mm Hg, a diastolic blood pressure of ≥90 mm Hg, or use of antihypertensive medication.[57] Diabetes mellitus was defined as fasting serum glucose level ≥7.1 mmol/L, a random serum glucose level ≥11.1 mmol/L, or use of glucose-lowering medication.[58] Symptoms of depression were evaluated with the Centre for Epidemiologic Studies Depression scale (CES-D), which was converted to a sum-score.[59]

Statistical analysis

We investigated the association between brain MRI measurements including cerebral small vessel disease, brain tissue volumes, and white matter microstructure, and the risk of cancer using Cox proportional hazards models.[60] Cox proportional hazards models are semiparametric regression models for survival data and can be used to obtain hazard ratios (HRs) and 95% confidence intervals (95% CIs). The hazard is the instantaneous risk of an event at time *t*, given that the event has not occurred until time *t*. In the current study, we are interested in cancer as the event. For interpretation purposes and to facilitate comparisons across different MRI measurements, we standardised continuous brain MRI measurements (i.e., white matter hyperintensity volume, brain tissue volumes, and white matter microstructure) by creating Z-scores (individual value minus population mean, divided by population standard deviation [SD]). Therefore, the HR for continuous variables indicates the change in the risk of cancer if the brain MRI measurement of interest rises by one SD.[61] A HR above one indicates that the risk of cancer increases for every SD increase in the brain MRI measurement. For categorical variables (i.e., cerebral microbleeds and lacunar infarcts) the hazard ratio can be interpreted as the ratio of the hazard for cancer at time *t* for participants with microbleeds or infarcts to the hazard for cancer at *t* for those without microbleeds or infarcts. A HR above one indicates that participants with microbleeds or infarcts have a higher risk of cancer than participants without microbleeds or infarcts.

White matter hyperintensity volume was transformed using the natural logarithm to reach a normal distribution. For volumes of the lobes and subcortical structures we used the average of the left and right hemisphere. We explored non-linear associations by categorising global brain volumes into quantiles. For each MRI measurement, we constructed two nested models. Covariates were selected based on previous literature[62] on the relation between cancer, brain abnormalities, and cognitive function. In Model I, the effect of each MRI measurement was

adjusted for sex and intracranial volume. In a middle-aged to elderly population, correcting for intracranial volume is preferred over correcting for total brain volume to better estimate the extent of global atrophy or atrophy between different regions.[63,64] In addition to these adjustments for all MRI measurements, the effect of grey matter volume was adjusted for total white matter volume (i.e., normal appearing white matter volume plus white matter hyperintensity volume), and analyses for measurements of white matter microstructure were adjusted for normal appearing white matter volume, white matter hyperintensity volume, and phase encoding direction. Model II was Model I plus additional adjustment for educational level (primary, lower, intermediate, or higher), BMI (continuous), hypertension (yes or no), diabetes mellitus (yes or no), smoking status (never, current, or former), alcohol use (yes or no), and CES-D sum-score (continuous). An overview of the distributions of the continuous determinants and covariates used in the models is provided in **Supplementary Figure 1**.[65] Ethnicity was not used as a covariate since nearly all participants (97.0%) were of European descent. Age was used as the underlying time scale in all Cox models to control for the confounding effects of age and to allow a non-parametric age effect.[66,67] Follow-up time was measured from the date of first MRI scan until the date of cancer diagnosis, death, loss to follow-up, or January 1st, 2015, whichever came first. Participants with CNS cancer were censored at date of diagnosis (i.e., follow-up was terminated at date of CNS cancer diagnosis), because mechanisms underlying brain abnormalities differ between non-CNS and CNS cancers, given that CNS cancer can cause direct damage to the brain.[68] Multicollinearity was checked by calculating the Variance Inflation Factor (VIF). None of the covariates had a VIF above ten.[69] The proportional hazards assumption was checked by visual inspection of the Schoenfeld residuals.[70]

Given that cortical grey matter volume is approximated by the product of cortical thickness and cortical surface area, we explored whether any association between grey matter volume and risk of cancer may be driven by one of these features. Cortical surface area is the main determinant of variation in cortical grey matter volumes between individuals.[71] Cortical thickness and surface area decrease both during aging, but it has been shown that reduced cortical thickness is probably the main driver of decreasing cortical grey matter volume.

Next, to investigate the robustness of our findings, we conducted sensitivity analyses in which we limited the analyses to a shorter follow-up time by censoring all participants two years after MRI scan. Cancer might indirectly affect the brain through inflammatory or vascular processes.[72] Tumour progression has been associated with inflammation and vascular changes.[73] We therefore hypothesised that if growing, yet undiagnosed cancer affects the brain, brain abnormalities will become more apparent closer to the date of cancer diagnosis.

Next, we analysed effects separately for the most frequent cancer types (breast, prostate,

colorectal, or lung) and cancer stage (local versus metastatic). In addition, we studied effect modification for sex by stratifying. We adjusted these models for the same covariates that were used in Model II. Participants were censored at time of cancer diagnosis if they were diagnosed with another type of cancer than the cancer type of interest.

We subsequently investigated the relation between tested cognitive function and self-reported memory function, and the risk of cancer. A Cox model with a particular cognitive test result included also information on sex, educational level, BMI, hypertension, diabetes mellitus, smoking status, alcohol use, and CES-D sum score. The cognitive test results were standardised by creating Z-scores to facilitate comparisons across the different measures.

Lastly, we repeated analyses using the MRI scan closest to cancer diagnosis in a matched cohort design by matching each participant with cancer to three cancer-free participants based on age, sex, and follow-up time. These analyses provided similar findings to those obtained from the original cohort design using the first available MRI scan and are therefore not reported separately.

Multiple imputation was used for missing covariates (maximum of 0.9%) based on determinants, outcome, and covariates. The missing values were imputed five times, resulting in five datasets. Rubin's method was used to estimate pooled HRs and 95% CIs from these five datasets.[74] A two-sided *P*-value of <.05 was considered statistically significant. We did not correct for multiple testing, because the brain MRI measurements were not independent from each other and the analyses were exploratory. Correction for multiple testing may therefore be too conservative.[75] In total, 36 Cox proportional hazards models were run for the main analyses, six to explore non-linear associations by categorising global brain tissue volumes, 18 for analyses stratified by sex, 90 for analyses stratified by cancer type, 18 for sensitivity analyses, and 14 for analyses on cognitive function. All analyses were performed using the 'survival' package from R software Version 3.4.1.[76]

RESULTS

Characteristics of participants at time of MRI scan are presented in **Table 1**. During a median (IQR) follow-up of 7.0 years (4.9 to 8.1), 353 out of 4622 participants (7.6%) were diagnosed with cancer. The most frequently diagnosed cancer types were prostate (16.1%), female breast (13.0%), colorectal (17.8%), and lung (10.5%). The median time (IQR) between MRI scan and cancer diagnosis was 3.3 years (1.7 to 5.6), with a mean (SD) age of 70.6 years (9.0) at diagnosis.

Table 1 Baseline characteristics of total study population.

Characteristic	All participants (N=4622)
Age, years, median (IQR)	61.6 (55.5 to 71.7)
Women, No. (%)	2574 (55.7)
Educational level, No. (%)	
Primary	390 (8.4)
Lower	1743 (37.7)
Intermediate	1368 (29.6)
Higher	1080 (23.4)
Body mass index, kg/m^2, mean (SD)	27.4 (4.1)
Hypertension, No. (%)	2823 (61.1)
Diabetes mellitus, No. (%)	315 (6.8)
Smoking, No. (%)	
Never	1426 (30.9)
Former	2436 (52.7)
Current	734 (15.9)
Alcohol use, No. (%)	4072 (88.1)
CES-D sum score, median (IQR)	4.0 (3.0 to 8.0)
Cerebral small vessel disease	
White matter hyperintensity volume, mL, median (IQR)	2.8 (1.6 to 5.7)
Microbleeds, No. (%)	840 (18.2)
Lacunar infarcts, No. (%)	283 (6.1)
Global brain tissue volume, mL, mean (SD)	
Intracranial volume	1138.9 (116.1)
Total brain volume	939.9 (100.6)
Grey matter	530.6 (55.4)
Normal appearing white matter	403.8 (60.9)
Lobar brain tissue volume, mL, mean (SD)	
Frontal	79.5 (8.2)
Parietal	52.0 (5.6)
Temporal	49.1 (5.3)
Occipital	22.8 (2.8)

Values are shown without imputation and therefore not always add up to 100%.
CES-D = Centre for Epidemiological Studies Depression Scale, IQR = interquartile range, N = number of participants, SD = standard deviation.

Table 1 Baseline characteristics of total study population (continued).

Characteristic	All participants (N=4622)
Subcortical structure volume, mL, mean (SD)	
Hippocampus	3.9 (0.4)
Amygdala	1.4 (0.2)
Caudate	3.3 (0.5)
Putamen	4.2 (0.5)
Thalamus	6.6 (0.7)
Pallidum	1.6 (0.2)
White matter microstructure*, mean (SD)	
Global fractional anisotropy	0.34 (0.02)
Global mean diffusivity, mm^2/s	$0.74 * 10^{-3}$ (0.03)
Cognitive function†	
Mini-Mental State Examination, median (IQR)	28.0 (27.0 to 29.0)
Word Fluency Test, mean (SD)	23.0 (5.9)
Letter-Digit Substitution Test, mean (SD)	30.6 (6.9)
Stroop Test: Naming, median (IQR)	16.4 (14.7 to 18.5)
Stroop Test: Reading, median (IQR)	22.4 (20.0 to 25.4)
Stroop Test: Interference, median (IQR)	44.3 (37.2 to 54.3)
Purdue Pegboard Test, mean (SD)	36.2 (5.2)
Word Learning Test: Immediate recall, mean (SD)	7.8 (2.1)
Word Learning Test: Delayed recall, mean (SD)	7.8 (2.9)
Word Learning Test: Recognition, median (IQR)	14.0 (13.0 to 15.0)
General cognitive factor, mean (SD)	0.0 (1.0)
Self-reported memory complaints‡, No. (%)	
More problems remembering	2082 (46.4)
Forgetting (daily) pursuits	1318 (29.4)
Word-finding problems	1182 (26.3)

Values are shown without imputation and therefore not always add up to 100%.
** FA and MD were measured in 4354 participants due to missing diffusion tensor imaging data. † Number of participants differed per cognitive test. ‡ Self-reported memory complaints were measured in 4486 participants.*
IQR = interquartile range, N = number of participants, SD = standard deviation.

Cerebral small vessel disease

No associations were found between white matter hyperintensity volume or presence of microbleeds and the risk of cancer (HR [95% CI] per SD increase in white matter hyperintensity volume = 0.98 [0.87 to 1.09], *P*=.67 and for presence of microbleeds = 1.00 [0.77 to 1.29], *P*=.98, **Table 2**). The largest HR for cerebral small vessel disease was observed for presence of lacunar infarcts and the risk of all cancers combined (HR [95% CI] = 1.39 [0.97 to 1.98], *P*=.07, **Table 2**). This effect estimate was more pronounced in sensitivity analyses when censoring the follow-up time after the first two years after the MRI scan (HR [95% CI] = 1.65 [0.95 to 2.86], *P*=.07, **Supplementary Table 2**).

We found no differences in associations for different cancer types (**Supplementary Table 4**), nor between men and women (**Supplementary Table 6**).

Table 2 Association between markers of cerebral small vessel disease and risk of cancer.

MRI measurement	Cancer (n/N = 353/4622)			
	Model I HR (95% CI)	*P*	Model II HR (95% CI)	*P*
White matter hyperintensity volume, mL*,†	0.99 (0.88 to 1.10)	.81	0.98 (0.87 to 1.09)	.67
Microbleeds	1.01 (0.78 to 1.31)	.96	1.00 (0.77 to 1.29)	.98
Lacunar infarcts	1.46 (1.02 to 2.07)	.04	1.39 (0.97 to 1.98)	.07

Model I: adjusted for sex and total intracranial volume. Model II: Model I plus adjusted for education, body mass index, hypertension, diabetes mellitus, smoking status, alcohol use, and CES-D sum score.
** Expressed per standard deviation increase. † Transformed with a natural logarithm.*
CES-D = Centre for Epidemiological Studies Depression Scale, CI = confidence interval, HR = hazard ratio, MRI = magnetic resonance imaging, n = number of participants with incident cancer, N = number of participants.

Brain tissue volumes

Overall, we found no associations between global and lobar brain tissue volumes and the risk of cancer. The majority of the effect estimates for brain tissue volumes were below one, with the most pronounced HR for total brain volume and the risk of cancer (HR [95% CI] per SD increase in total brain volume = 0.76 [0.55 to 1.04], *P*=.09, **Table 3**). We did not observe a non-linear pattern when categorising the volumes into quantiles (data not shown). No associations were found between cortical thickness and risk of cancer (HR [95% CI] per SD increase in cortical thickness = 0.94 [0.84 to 1.06], *P*=.33), and cortical surface area and risk of cancer (HR [95% CI] per SD increase in cortical surface area = 0.93 [0.72 to 1.20], *P*=.58). Regarding subcortical structures, the most pronounced effect estimate was found for hippocampal volume and the risk of cancer (HR [95% CI] = 0.87 [0.75 to 1.01], *P*=.07, **Table 3**).

Table 3 Association between brain tissue volumes and microstructural brain measurements and risk of cancer.

MRI measurement*	Cancer (n/N = 353/4622)			
	Model I HR (95% CI)	*P*	Model II HR (95% CI)	*P*
Global brain tissue volume, mL				
Total brain volume	0.74 (0.54 to 1.01)	.06	0.76 (0.55 to 1.04)	.09
Grey matter	0.89 (0.71 to 1.11)	.31	0.91 (0.73 to 1.14)	.41
Normal appearing white matter	0.86 (0.73 to 1.02)	.09	0.87 (0.73 to 1.03)	.11
Lobar brain tissue volume, mL				
Frontal	0.87 (0.70 to 1.08)	.06	0.90 (0.73 to 1.12)	.34
Parietal	0.86 (0.71 to 1.05)	.31	0.87 (0.72 to 1.07)	.19
Temporal	0.90 (0.74 to 1.10)	.09	0.92 (0.75 to 1.13)	.43
Occipital	0.99 (0.85 to 1.14)	.06	0.99 (0.85 to 1.14)	.85
Subcortical structure volume, mL				
Hippocampus	0.86 (0.74 to 1.00)	.05	0.87 (0.75 to 1.01)	.07
Amygdala	0.99 (0.86 to 1.15)	.94	1.00 (0.86 to 1.15)	.95
Caudate	1.04 (0.92 to 1.17)	.55	1.03 (0.92 to 1.16)	.61
Putamen	0.91 (0.79 to 1.03)	.15	0.90 (0.79 to 1.03)	.13
Thalamus	0.94 (0.80 to 1.12)	.51	0.95 (0.80 to 1.12)	.52
Pallidum	0.97 (0.85 to 1.10)	.63	0.97 (0.85 to 1.11)	.68
White matter microstructure†				
Global fractional anisotropy	0.98 (0.86 to 1.12)	.79	0.98 (0.86 to 1.12)	.75
Global mean diffusivity, 10^{-3} mm^2/s	1.02 (0.87 to 1.19)	.85	1.01 (0.86 to 1.19)	.89

Model I: adjusted for sex and total intracranial volume. For grey matter volume additionally adjustment for total white matter volume. For white matter microstructure additional adjustment for normal appearing white matter volume, white matter hyperintensity volume, and phase encoding direction. Model II: Model I plus adjusted for education, body mass index, hypertension, diabetes mellitus, smoking status, alcohol use, and CES-D sum score.

** Expressed per standard deviation increase. † Fractional anisotropy and mean diffusivity were measured in 4354 participants due to missing diffusion tensor imaging data.*

CES-D = Centre for Epidemiological Studies Depression Scale, CI = confidence interval, HR = hazard ratio, MRI = magnetic resonance imaging, n = number of participants with incident cancer, N = number of participants.

When limiting the follow-up to two years after the MRI scan, effect estimates were more pronounced for the association between volumes of total brain and hippocampus with the risk of cancer (**Supplementary Table 3**, HR [95% CI] per SD increase in total brain volume = 0.63 [0.35 to 1.12], *P*=.12 and per SD increase in hippocampal volume = 0.75 [0.58 to 0.98],

P=.04).

Regarding cancer type, we found that higher volumes of total brain, grey matter, and hippocampus were associated with a statistically significantly lower risk of lung cancer (**Figure 2**). In contrast, higher volumes of total brain and grey matter were associated with a higher risk of colorectal cancer. No differences were observed for the other cancer types and for metastatic cancer, but small numbers led to wide confidence intervals. Results for the remaining brain tissue volumes and risk of cancer stratified by cancer type are shown in **Supplementary Table 5**.

Lastly, we found no evidence for effect modification by sex (**Supplementary Table 7**).

White matter microstructure

Global measurements of white matter microstructure were not associated with the risk of cancer (HR [95% CI] per SD increase in global FA = 0.98 [0.86 to 1.12], *P*=.75 and in global MD = 1.01 [0.86 to 1.19], *P*=.89, **Table 3** and **Supplementary Table 3**).

Also no associations were found when stratifying by cancer type (**Supplementary Table 5**) and by sex (**Supplementary Table 7**).

Cognitive function

All effect estimates for the relation between individual cognitive tests and the risk of cancer were around 1.0, indicating that there are no associations between different cognitive test scores and the risk of cancer (**Supplementary Table 8**). Per SD increase in the general cognitive factor as measurement of global cognitive function the HR (95% CI) for cancer was 1.03 (0.89 to 1.20), *P*=.66. Also no associations were found between self-reported memory function and the risk of cancer.

DISCUSSION

In this population-based study, we aimed to obtain more insight into the impact of cancer on brain structure by investigating the presence of brain abnormalities in non-CNS cancer patients prior to the clinical manifestation of cancer. We found no meaningful associations between cerebral small vessel disease, brain tissue volumes, and white matter microstructure, and the risk of cancer. These findings suggest that persons who develop cancer do not have more brain abnormalities before cancer diagnosis than persons who remain free of cancer.

Our current findings obtained prior to cancer diagnosis deviate from previously observed

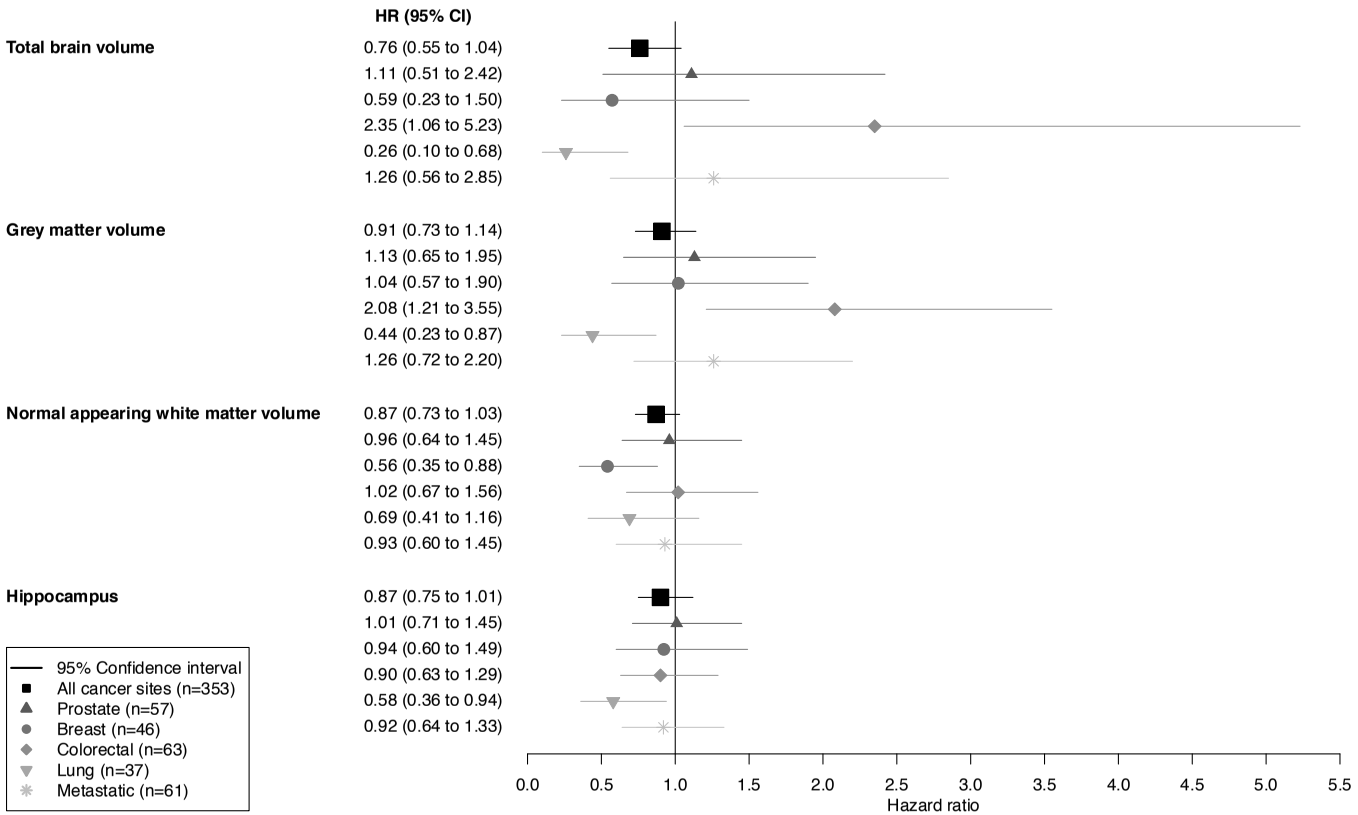

Figure 2 Adjusted hazard ratios for the association between global brain tissue volumes and hippocampus and risk of cancer at different organ sites and metastatic stage.

Hazard ratios are expressed per standard deviation increase in volume. Hazard ratios are adjusted for total intracranial volume, sex, education, body mass index, hypertension, diabetes mellitus, smoking status, alcohol use, and CES-D sum score. For grey matter volume additionally adjustment for total white matter volume. The boxes represent the effect size and the horizontal lines indicate the corresponding 95% confidence intervals. CI = confidence interval, HR = hazard ratio.

brain changes after diagnosis but before treatment such as lower grey matter volume and white matter microstructure (i.e., lower FA and higher axial diffusivity).[4,12,77] Although we did not find any statistically significant associations, we observed that almost all effect estimates for brain tissues volumes were below one, suggesting that we cannot completely rule out a subtle effect of cancer on the brain. In addition, effect estimates for the association between presence of lacunar infarcts, total brain volume, hippocampal volume, and the risk of cancer were more pronounced when the study follow-up was limited to two years after MRI scan. This may indicate that brain changes (i.e., more lacunar infarcts and smaller brain volumes) become more apparent closer to the date of cancer diagnosis. Given that we did not observe this pattern for any of the cognitive tests, this might suggest that brain changes might arise before they become clinically apparent, as seen in dementia.[78] This may also apply to cancer patients, with cognitive function first being preserved by compensation, followed by loss of compensatory activation, which results eventually in cognitive impairment.[1] Different underlying mechanisms by which non-CNS cancer may affect the brain have been proposed, including peripheral inflammation triggering neurotoxic cytokine response, oxidative stress, or vascular changes.[1,8,13-15,79] In addition, the associations were most pronounced for lung cancer, which is strongly associated with inflammation and oxidative stress.[80,81] Accordingly, we can conclude that if subclinical non-CNS cancer affects the brain, the effects are limited and may only result in subtle changes that are not evidently detected by measures of supratentorial brain tissue volumes, subcortical brain structure volumes, white matter pathology, and white matter microstructure, or effects are restricted to certain types of non-CNS cancer, such as lung cancer.

Our study has some limitations. First, measurement error in brain MRI volumes might have attenuated the association. For instance, it might have been possible that usage of a higher magnetic field strength or alternative imaging processing pipelines would have resulted in a more pronounced association between certain MRI measurements and the risk of cancer.[28,82] Second, although the statistical power in our main analysis was sufficient to detect a potential association (we were powered to detect a HR of 0.84 for the relation between total brain volume and risk of cancer [α=0.05, β=0.80]), the power might have been too limited to find statistically significant associations when limiting the follow-up time to two years and when focusing on different cancer types. Therefore, replication of this study in a larger sample with MRI scans performed more closely to the clinical manifestation of cancer is desirable.[5] In addition, it would be interesting to investigate the change in MRI measurements from before to after cancer diagnosis. Third, we had no information on fatigue and frailty, which may be confounding factors that would have further attenuated the effect estimates. Fourth, with the current analyses we were not able to study interrelationships between different brain

MRI measurements and therefore we might have missed more complex patterns of brain abnormalities related to the risk of cancer.

Strengths of this study include the unique design by which we could assess brain MRI before clinical manifestation of cancer. Hereby, we excluded the effects of psychological factors associated with a cancer diagnosis on the brain and the potential effects of selection bias.[29,30] Also, we have a larger sample size than that of other studies assessing brain MRI in cancer patients prior to treatment (number of patients ranging between 10 and 74, compared to 353 patients in our study), and we included different cancer types as outcome whereas previous studies primarily focused on breast cancer.

In conclusion, we found that persons who develop non-CNS cancer did not have more brain abnormalities before cancer diagnosis than persons who remained free of cancer. Our findings do not support that non-CNS cancer affects global brain structure measurements before clinical manifestation of cancer.

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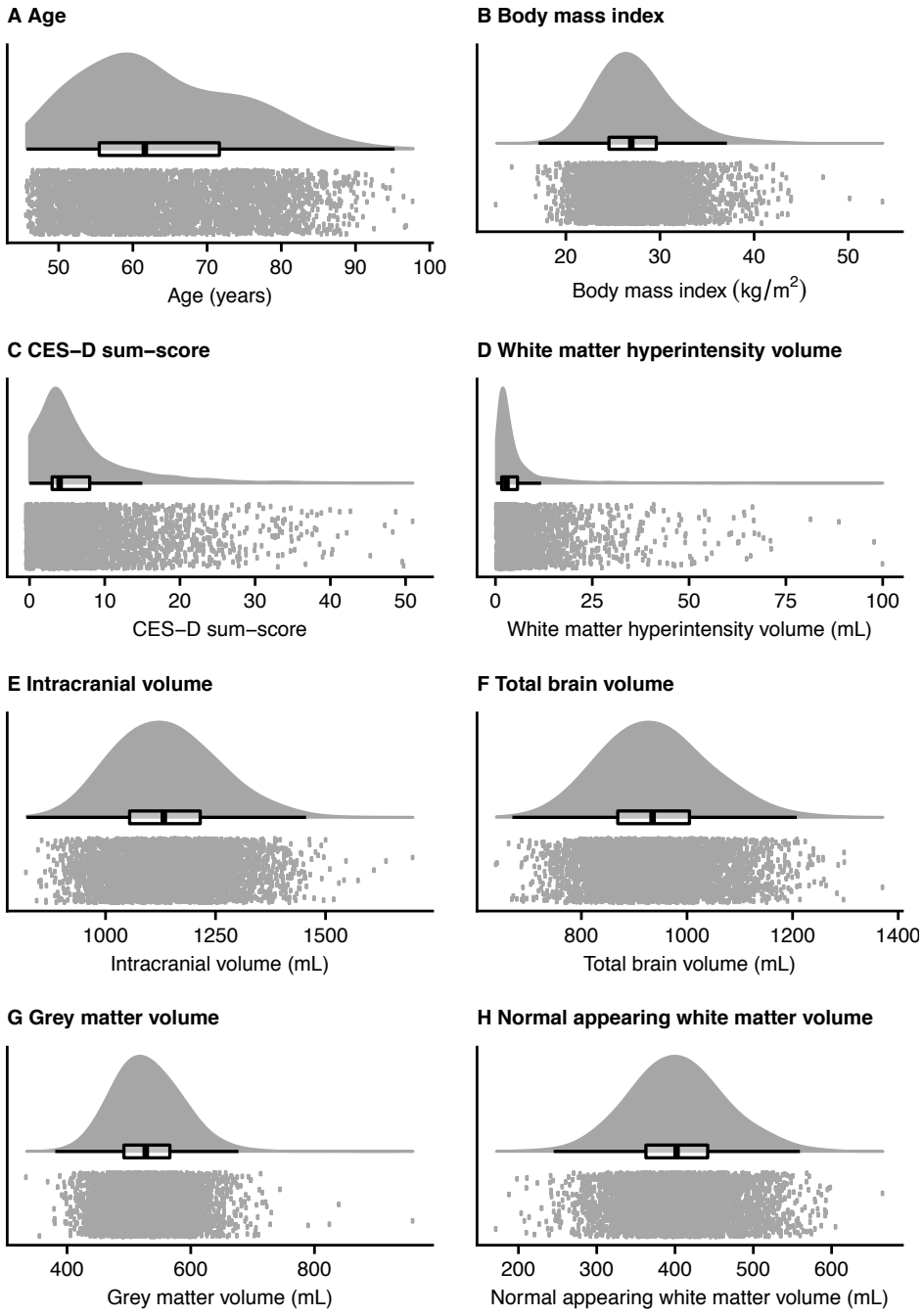
SUPPLEMENTARY MATERIAL



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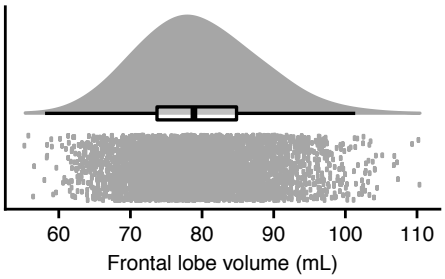

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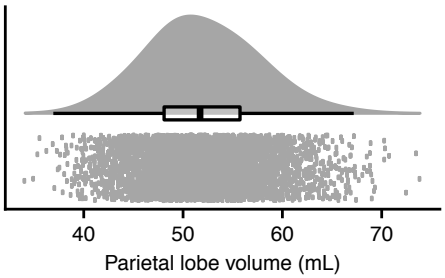

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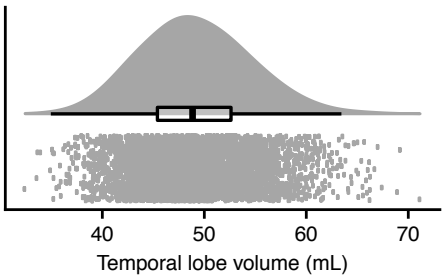

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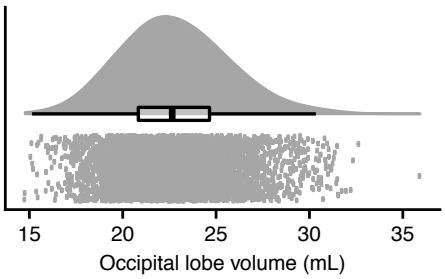

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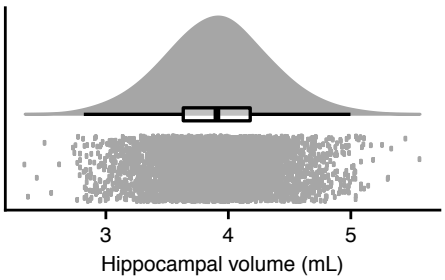
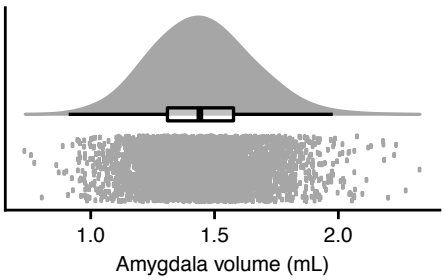


N Amygdala volume



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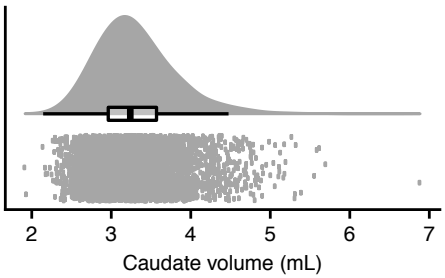

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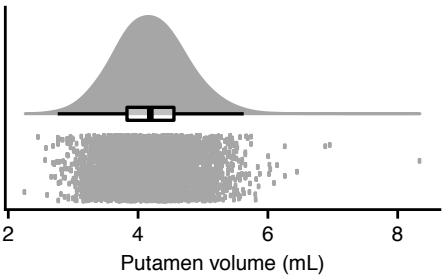
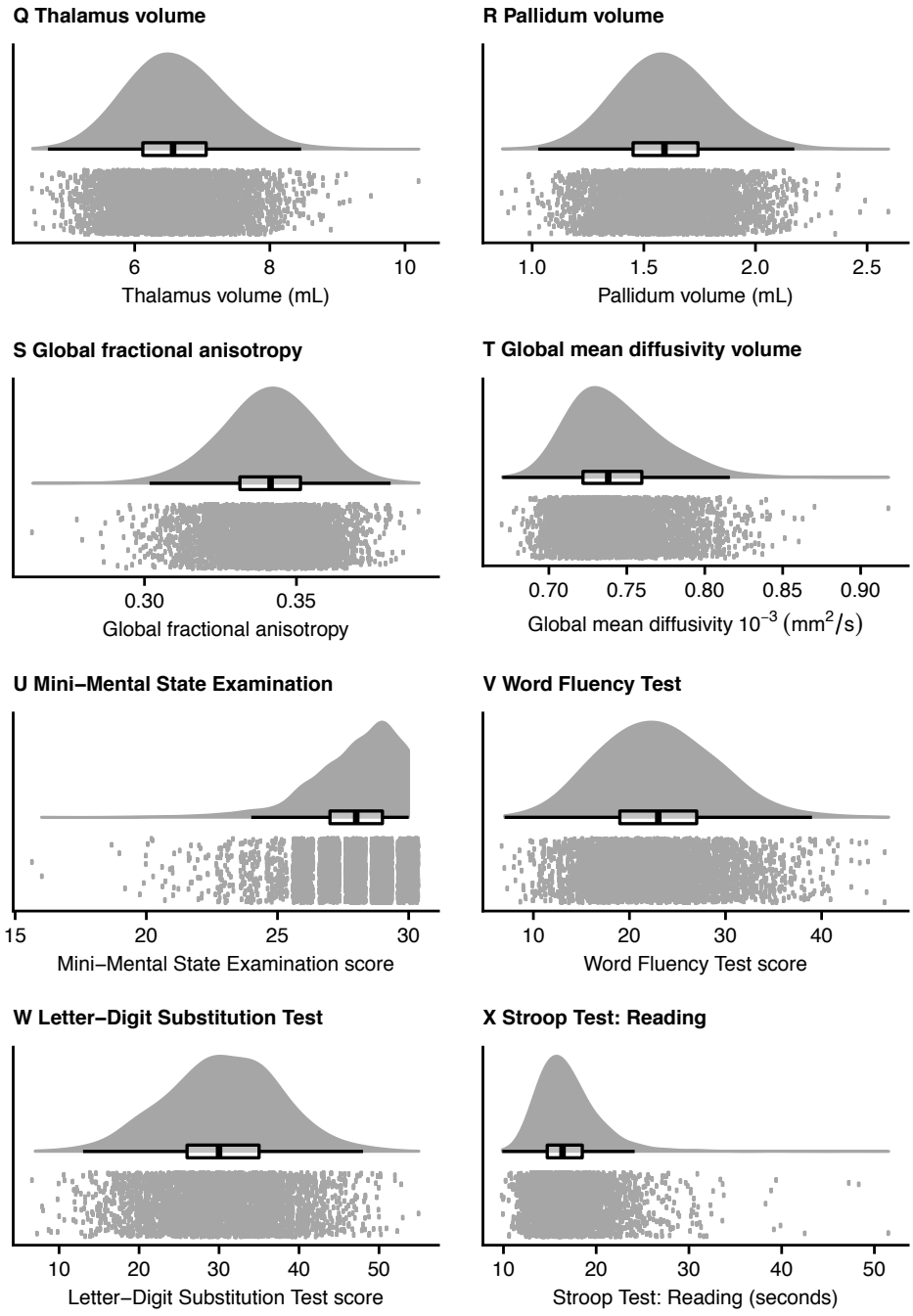
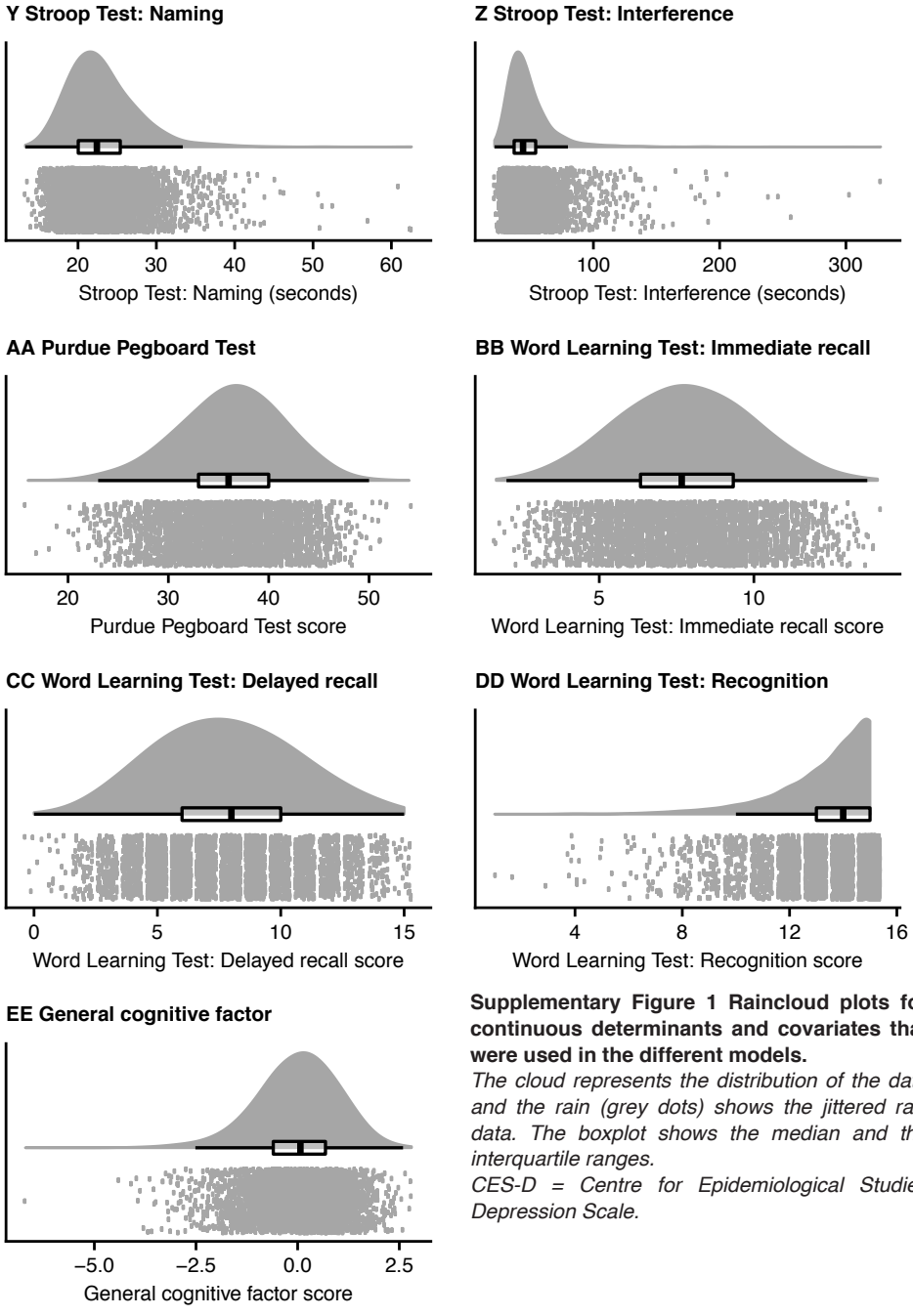



Q Thalamus volume
6
8
10
Thalamus volume (mL)
R Pallidum volume
1.0
1.5
2.0
2.5
Pallidum volume (mL)
S Global fractional anisotropy
0.30
0.35
Global fractional anisotropy
T Global mean diffusivity volume
0.70
0.75
0.80
0.85
0.90
Global mean diffusivity 10^{-3} (mm^2/s)
U Mini–Mental State Examination
15
20
25
30
Mini–Mental State Examination score
V Word Fluency Test
10
20
30
40
Word Fluency Test score
W Letter–Digit Substitution Test
10
20
30
40
50
Letter–Digit Substitution Test score
X Stroop Test: Reading
10
20
30
40
50
Stroop Test: Reading (seconds)



Supplementary Figure 1 Raincloud plots for continuous determinants and covariates that were used in the different models.

The cloud represents the distribution of the data and the rain (grey dots) shows the jittered raw data. The boxplot shows the median and the interquartile ranges.

CES-D = Centre for Epidemiological Studies Depression Scale.

Supplementary Table 1 Details of used sequences.

Sequence	Comment	Mode	Readout module	Time (min:sec)	TR/TE	TI (ms)	BW (kHz)	Flip angle (degrees)	Number of slices	Slice thickness (mm)	FOV (cm^2)	Matrix
PDw		2D	FSE	6:09	12 300/17.3		17.86	90 to 180	90	1.6	25	416 x 256
T1w		3D	GRE	6:24	13.8/2.8	400	12.5	20	96 (192)	1.6 (0.8)	25	416 x 256
FLAIR		2D	FSE	6:25	8000/120	2000	31.25	90 to 180	64	2.5	25	320 x 224
DTI	25 directions; b = 1000 mm^2/s, b_0 NEX = 3	2D	EPI	3:44	8000/74.6		250	90 to 180	39	3.5	21	64 x 96
T2*w		3D	GRE	5:55	45/31		14.71	13	96 (192)	1.6 (0.8)	25	320 x 224

*This Table has been obtained from 'Ikram et al. The Rotterdam Scan Study: design update 2016 and main findings, 2015: European Journal of Epidemiology'. BW = bandwidth, DTI = diffusion tensor imaging, EPI = echo-planar imaging, FLAIR = fluid-attenuated inversion recovery, FOV = field of view, GRE = gradient-recalled echo, FSE = fast spin echo, NEX = number of excitations, PDw = proton density-weighted, T1w = T1-weighted, T2*w = T2*- weighted, TE = echo time, TI = inversion time, TR = repetition time.*

Supplementary Table 2 Association between markers of cerebral small vessel disease and risk of cancer when limiting the follow-up time to two years after brain MRI.

MRI measurement	Cancer (n/N = 107/4622)	
	HR (95% CI)	*P*
White matter hyperintensity volume, mL*,†	0.94 (0.78 to 1.13)	.50
Microbleeds	0.85 (0.53 to 1.37)	.51
Lacunar infarcts	1.65 (0.95 to 2.86)	.07

Hazard ratios are adjusted for sex and total intracranial volume, education, body mass index, hypertension, diabetes mellitus, smoking status, alcohol use, and CES-D sum score.
** Expressed per standard deviation increase. † Transformed with a natural logarithm.*
CES-D = Centre for Epidemiological Studies Depression Scale, CI = confidence interval, HR = hazard ratio, MRI = magnetic resonance imaging, n = number of participants with incident cancer, N = number of participants.

Supplementary Table 3 Association between brain tissue volumes and microstructural brain measurements and risk of cancer when limiting the follow-up time to two years after brain MRI.

MRI measurement*	Cancer (n/N = 107/4622)	
	HR (95% CI)	*P*
Global brain tissue volume, mL		
Total brain volume	0.63 (0.35 to 1.12)	.12
Grey matter	0.76 (0.51 to 1.14)	.18
Normal appearing white matter	0.91 (0.66 to 1.24)	.54
Lobar brain tissue volume, mL		
Frontal	0.79 (0.53 to 1.17)	.23
Parietal	0.96 (0.66 to 1.38)	.81
Temporal	0.94 (0.65 to 1.35)	.73
Occipital	0.97 (0.74 to 1.26)	.82
Subcortical structure volume, mL		
Hippocampus	0.75 (0.58 to 0.98)	.04
Amygdala	0.90 (0.69 to 1.18)	.46
Caudate	0.95 (0.78 to 1.17)	.64
Putamen	0.90 (0.72 to 1.14)	.40
Thalamus	0.78 (0.57 to 1.07)	.13
Pallidum	0.84 (0.66 to 1.07)	.15
White matter microstructure†		
Global fractional anisotropy	1.06 (0.85 to 1.33)	.59
Global mean diffusivity, 10^{-3} mm^2/s	0.97 (0.74 to 1.27)	.80

Hazard ratios are adjusted for sex and total intracranial volume, education, body mass index, hypertension, diabetes mellitus, smoking status, alcohol use, and CES-D sum score. For grey matter volume additionally adjustment for total white matter volume. For white matter microstructure additional adjustment for normal appearing white matter volume, white matter hyperintensity volume, and phase encoding direction.
** Expressed per standard deviation increase. † Fractional anisotropy and mean diffusivity were measured in 4354 participants due to missing diffusion tensor imaging data.*
CES-D = Centre for Epidemiological Studies Depression Scale, CI = confidence interval, HR = hazard ratio, MRI = magnetic resonance imaging, n = number of participants with incident cancer, N = number of participants.

Supplementary Table 4 Association between markers of cerebral small vessel disease and risk of cancer stratified by cancer type.

MRI measurement	Prostate cancer (n=57)		Breast cancer (n=46)		Colorectal cancer (n=63)		Lung cancer (n=37)		Metastasized cancer (n=61)	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
White matter hyperintensity volume, mL[a,b]	1.09 (0.78 to 1.51)	.62	1.13 (0.83 to 1.53)	.45	0.93 (0.71 to 1.22)	.62	1.03 (0.80 to 1.32)	.81	1.02 (0.78 to 1.32)	.91
Microbleeds	0.73 (0.35 to 1.51)	.39	1.07 (0.49 to 2.33)	.87	0.88 (0.47 to 1.65)	.70	0.54 (0.22 to 1.34)	.19	0.69 (0.32 to 1.52)	.36
Lacunar infarcts	1.56 (0.69 to 3.53)	.28	1.55 (0.47 to 5.08)	.47	1.27 (0.54 to 3.02)	.59	2.07 (0.88 to 4.88)	.10	1.28 (0.49 to 3.35)	.61

Models are adjusted for sex, total intracranial volume, education, body mass index, hypertension, diabetes mellitus, smoking status, alcohol use, and CES-D sum score.

[a] Expressed per standard deviation increase. [b] Transformed with a natural logarithm.

CES-D = Center for Epidemiological Studies Depression Scale, CI = confidence interval, HR = hazard ratio, MRI = magnetic resonance imaging, n = number of participants with incident cancer.

Supplementary Table 5 Association between brain tissue volumes and microstructural brain measurements and risk of cancer stratified by cancer type.

MRI measurement[a]	Prostate cancer (n=57)		Breast cancer (n=46)		Colorectal cancer (n=63)		Lung cancer (n=37)		Metastasized cancer (n=61)	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Lobar brain tissue volume, mL										
Frontal	1.16 (0.68 to 1.98)	.59	1.38 (0.75 to 2.53)	.30	1.37 (0.83 to 2.28)	.22	0.45 (0.23 to 0.89)	.02	0.98 (0.58 to 1.66)	.94
Parietal	1.03 (0.62 to 1.69)	.92	1.72 (0.97 to 3.07)	.06	1.47 (0.91 to 2.36)	.11	0.41 (0.22 to 0.77)	.01	1.16 (0.71 to 1.90)	.55
Temporal	0.78 (0.48 to 1.26)	.31	1.41 (0.78 to 2.55)	.26	1.70 (1.06 to 2.72)	.03	0.55 (0.29 to 1.04)	.07	1.10 (0.68 to 1.80)	.69
Occipital	0.97 (0.69 to 1.36)	.84	1.14 (0.74 to 1.74)	.55	0.89 (0.64 to 1.26)	.52	0.80 (0.50 to 1.28)	.35	0.81 (0.58 to 1.15)	.25
Subcortical structure volume, mL										
Amygdala	0.94 (0.67 to 1.33)	.73	0.98 (0.63 to 1.53)	.94	1.04 (0.73 to 1.47)	.85	1.12 (0.70 to 1.81)	.64	1.04 (0.72 to 1.50)	.84
Caudate	1.25 (0.93 to 1.68)	.14	0.91 (0.64 to 1.30)	.60	0.86 (0.65 to 1.15)	.31	1.24 (0.89 to 1.73)	.20	1.05 (0.79 to 1.40)	.73
Putamen	1.08 (0.77 to 1.51)	.65	0.85 (0.57 to 1.26)	.42	0.98 (0.72 to 1.33)	.88	0.65 (0.42 to 1.02)	.06	0.83 (0.59 to 1.16)	.27
Thalamus	1.34 (0.91 to 1.96)	.13	0.77 (0.46 to 1.27)	.30	0.90 (0.60 to 1.35)	.61	0.46 (0.25 to 0.83)	.01	0.94 (0.62 to 1.44)	.78
Pallidum	1.13 (0.83 to 1.55)	.43	0.82 (0.54 to 1.23)	.33	1.00 (0.73 to 1.37)	.98	0.77 (0.50 to 1.18)	.23	0.98 (0.71 to 1.36)	.92
White matter microstructure[b]										
Global fractional anisotropy	0.91 (0.63 to 1.30)	.59	0.95 (0.66 to 1.35)	.77	0.96 (0.70 to 1.30)	.77	1.05 (0.70 to 1.58)	.81	1.20 (0.83 to 1.75)	.33
Global mean diffusivity, 10^{-3} mm^2/s	1.07 (0.69 to 1.66)	.77	1.11 (0.73 to 1.70)	.61	1.00 (0.69 to 1.45)	.98	0.97 (0.59 to 1.59)	.91	0.97 (0.62 to 1.52)	.88

Models are adjusted for sex, total intracranial volume, education, body mass index, hypertension, diabetes mellitus, smoking status, alcohol use, and CES-D sum score. For white matter microstructure additional adjustment for normal appearing white matter volume, white matter hyperintensity volume, and phase encoding direction.

[a] Expressed per standard deviation increase. [b] Fractional anisotropy and mean diffusivity were measured in less participants due to missing diffusion tensor imaging data. In these analyses, 40 participants were diagnosed with prostate cancer, 39 with breast cancer, 54 with colorectal cancer, and 29 with lung cancer. Of the participants with cancer, 37 had metastatic disease.

CES-D = Center for Epidemiological Studies Depression Scale, CI = confidence interval, HR = hazard ratio, MRI = magnetic resonance imaging, n = number of participants with incident cancer.

Supplementary Table 6 Association between markers of cerebral small vessel disease and risk of cancer stratified by sex.

MRI measurement	Women (n/N = 157/2574)		Men (n/N = 196/2048)	
	HR (95% CI)	*P*	HR (95% CI)	*P*
White matter hyperintensity volume, mL[a,b]	1.01 (0.85 to 1.21)	.87	0.96 (0.83 to 1.12)	.62
Microbleeds	1.12 (0.76 to 1.66)	.56	0.88 (0.62 to 1.25)	.49
Lacunar infarcts	1.60 (0.88 to 2.92)	.12	1.20 (0.77 to 1.87)	.42

Models are adjusted for total intracranial volume, education, body mass index, hypertension, diabetes mellitus, smoking status, alcohol use, and CES-D sum score.
[a] Expressed per standard deviation increase. [b] Transformed with a natural logarithm.
CES-D = Center for Epidemiological Studies Depression Scale, CI = confidence interval, HR = hazard ratio, MRI = magnetic resonance imaging, n = number of participants with incident cancer, N = number of participants.

Supplementary Table 7 Association between brain tissue volumes and microstructural brain measurements and risk of cancer stratified by sex.

MRI measurement[a]	Women (n/N = 157/2574)		Men (n/N = 196/2048)	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Global brain tissue volume, mL				
Total brain volume	0.73 (0.44 to 1.22)	.23	0.87 (0.57 to 1.33)	.52
Gray matter	0.83 (0.58 to 1.18)	.30	1.04 (0.77 to 1.38)	.81
Normal appearing white matter	0.91 (0.68 to 1.22)	.53	0.86 (0.69 to 1.08)	.19
Lobar brain tissue volume, mL				
Frontal	0.81 (0.58 to 1.13)	.22	1.04 (0.78 to 1.38)	.80
Parietal	1.00 (0.73 to 1.37)	.99	0.81 (0.62 to 1.05)	.11
Temporal	0.99 (0.72 to 1.38)	.97	0.91 (0.71 to 1.18)	.49
Occipital	0.97 (0.77 to 1.23)	.81	1.00 (0.84 to 1.20)	.97
Subcortical structure volume, mL				
Hippocampus	0.91 (0.72 to 1.16)	.45	0.91 (0.72 to 1.16)	.11
Amygdala	1.07 (0.84 to 1.36)	.58	1.07 (0.84 to 1.36)	.64
Caudate	0.94 (0.78 to 1.14)	.54	0.94 (0.78 to 1.14)	.15
Putamen	0.83 (0.67 to 1.02)	.08	0.83 (0.67 to 1.02)	.82
Thalamus	0.77 (0.58 to 1.01)	.06	0.77 (0.58 to 1.01)	.37
Pallidum	0.88 (0.71 to 1.10)	.26	0.88 (0.71 to 1.10)	.67
White matter microstructure[b]				
Global fractional anisotropy	0.88 (0.72 to 1.07)	.20	1.06 (0.89 to 1.27)	.53
Global mean diffusivity, 10^{-3} mm^2/s	1.15 (0.90 to 1.46)	.26	0.91 (0.73 to 1.14)	.42

Models are adjusted for total intracranial volume, education, body mass index, hypertension, diabetes mellitus, smoking status, alcohol use, and CES-D sum score. For gray matter volume additionally adjustment for total white matter volume. For white matter microstructure additional adjustment for normal appearing white matter volume, white matter hyperintensity volume, and phase encoding direction.
[a] Expressed per standard deviation increase. [b] Fractional anisotropy and mean diffusivity were measured in less participants due to missing diffusion tensor imaging data. In these analyses, 129 out of 2,426 women were diagnosed with cancer and 152 out of 1,928 men were diagnosed with cancer.
CES-D = Center for Epidemiological Studies Depression Scale, CI = confidence interval, HR = hazard ratio, MRI = magnetic resonance imaging, n = number of participants with incident cancer, N = number of participants.

Supplementary Table 8 Association between cognitive function and risk of cancer.

Cognitive test	Cancer		
	n/N	HR (95% CI)	*P*
Mini-Mental State Examination	353/4614	1.00 (0.94 to 1.07)	.95
Word Fluency Test	347/4486	1.01 (0.99 to 1.03)	.47
Letter-Digit Substitution Test	347/4496	1.01 (0.99 to 1.03)	.34
Stroop Test*			
Naming	334/4343	1.02 (0.99 to 1.05)	.28
Reading	333/4342	1.00 (0.97 to 1.02)	.84
Interference	333/4336	1.00 (1.00 to 1.01)	.58
Purdue Pegboard Test	326/4326	0.99 (0.97 to 1.02)	.56
Word Learning Test			
Immediate recall	327/4278	1.01 (0.96 to 1.07)	.64
Delayed recall	327/4277	1.00 (0.96 to 1.04)	.85
Recognition	331/4318	0.97 (0.92 to 1.02)	.25
General cognitive factor	298/3927	1.03 (0.89 to 1.20)	.66
Self-reported memory complaints			
More problems remembering	342/4486	1.18 (0.95 to 1.47)	.14
Forgetting (daily) pursuits	342/4486	0.99 (0.77 to 1.26)	.91
Word-finding problems	342/4486	1.17 (0.92 to 1.48)	.21

Hazard ratios are adjusted for sex, education, body mass index, hypertension, diabetes mellitus, smoking status, alcohol use, and CES-D sum score.
** Better performance corresponds to lower scores.*
CES-D = Centre for Epidemiological Studies Depression Scale, CI = confidence interval, HR = hazard ratio, n = number of participants with incident cancer, N = number of participants.

Chapter 7

Change in cognitive function before and after non-central nervous system cancer diagnosis

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ABSTRACT

Background Studies showing that non-central nervous system cancer patients can develop cognitive impairment, have primarily focused on patients with specific cancer types and intensive treatments. These results may have been unduly influenced by patient selection. Such selection may be limited by studying cancer patients in a population-based setting. To better understand the course of cognitive function in the general population of cancer patients, we assessed cognitive trajectories of patients before and after cancer diagnosis in such population-based setting and compared these with cognitive trajectories of cancer-free controls.

Methods We evaluated 718 of the 2211 participants from the population-based Rotterdam Study who had been diagnosed with cancer between 1989 and 2014 and who had undergone at least one cognitive assessment before and after diagnosis. Cognition was measured every three to six years using a neuropsychological battery. Linear mixed models were used to compare cognitive trajectories of patients before and after diagnosis with those of age-matched cancer-free controls (sampled in a ratio of 1:3).

Results The median age at cancer diagnosis was 70.3 years and 47.1% were women. Most patients (68.1%) had received local treatment only. Cognitive trajectories of patients before and after cancer diagnosis were largely similar to those of controls. After diagnosis, the largest difference was found on a memory test (patients declined with 0.14 units per year on the Word Learning Test: Delayed recall [95% confidence interval = -0.35 to 0.07] and controls with 0.09 units [95% confidence interval = -0.18 to -0.00], *P* for difference = .59).

Conclusions At a population-level, cognitive function in cancer patients declines similarly to that in cancer-free controls. This finding provides some reassurance to patients who have received local treatment. Larger numbers are needed to evaluate cognitive change in patients with specific cancer types and treatments.

INTRODUCTION

During and following cancer treatment, non-central nervous system (CNS) cancer patients frequently report cognitive problems that adversely affect their quality of life and daily functioning.[1-4] Multiple clinical studies have shown that cancer treatment, and in particular chemotherapy, can negatively affect cognitive function.[5-9] In addition, it has been found that in some patients cognitive impairment occurs before start of cancer treatment, suggesting that cancer itself may also impact cognitive function.[10-16]

Although these studies have contributed greatly to our current understanding of cognitive impairment in cancer patients, they have generally focused on patients with specific types of cancer – in particular breast cancer – or patients treated with intensive, systemic cancer treatments. The clinical setting of most of these studies might also have attracted more participants with cognitive problems. This potential selection of patients may have unduly influenced the prevalence and severity of cognitive problems in cancer patients.[1] As yet, there is therefore insufficient understanding of the course of cognitive function in the general, unselected population of cancer patients with different cancer types and treatments.

We have previously investigated the course of cognitive function before cancer diagnosis in such an unselected population of cancer patients by studying participants from the population-based Rotterdam Study.[17] In this cohort study participants undergo cognitive testing every three to six years, and some of these participants are subsequently diagnosed with cancer. In this population-based setting, we found no evidence that cognitive function declines differently in individuals who will be diagnosed with cancer than in those who will remain cancer-free, indicating that the impact of cancer itself on the brain is limited before clinical manifestation of the disease.

The current study expands our previous investigation and studies the cognitive trajectories of cancer patients before and after cancer diagnosis in the Rotterdam Study, allowing us to include cancer patients with different cancer types and cancer treatments. Our primary aim was to assess if the rate of change in cognitive function among cancer patients before and after cancer diagnosis is different from the rate of change in cognitive function in cancer-free controls over a similar time period.

METHODS

Setting

This study is embedded within the Rotterdam Study, a prospective population-based cohort designed to study the occurrence and determinants of age-related diseases in the general population. The design has been described in detail previously.[18] Briefly, in 1989 all inhabitants aged 55 years and over of the district Ommoord in Rotterdam, the Netherlands, were invited to participate. This initial cohort comprised 7983 participants (response of 78%) and was extended with a second subcohort in 2000 with 3011 participants (response of 67%) who had become 55 years of age or moved into the study district. In 2006, the cohort was further extended with 3932 participants (response of 65%) aged 45 years and over.

Participants were interviewed at home by a trained research assistant, followed by two visits to the research centre for different examinations including laboratory assessments, imaging, and physical examinations. Follow-up examinations took place every three to six years.

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus Medical Centre and by the Ministry of Health, Welfare and Sport of the Netherlands. Written informed consent was obtained from all participants.

Study population

Of the total of 14 926 participants, we excluded those with a history of cancer (n=567), history of dementia (n=521) or who were insufficiently screened for history of dementia (n=601), history of stroke (n=341), or history of Parkinson's disease (n=77). Next, we excluded those without informed consent to access medical records for follow-up (n=165), leaving 12 654 eligible participants (**Figure 1**).

Cases

Out of the 12 654 eligible participants, 2403 were diagnosed with cancer during a median (interquartile range [IQR]) follow-up of 10.0 years (7.0 to 5.2, up to January 1st, 2015). Of these participants, we excluded those with primary CNS cancer (n=15) and those diagnosed with dementia (n=66), stroke (n=102), or Parkinson's disease (n=9) after study entry but before cancer diagnosis. Participants who were diagnosed with dementia, stroke, Parkinson's disease, or secondary CNS cancer after non-CNS cancer diagnosis were censored at date of diagnosis to exclude cognitive test results obtained after such diagnoses. Lastly, we excluded participants without any cognitive assessment (n=45), those without at least one

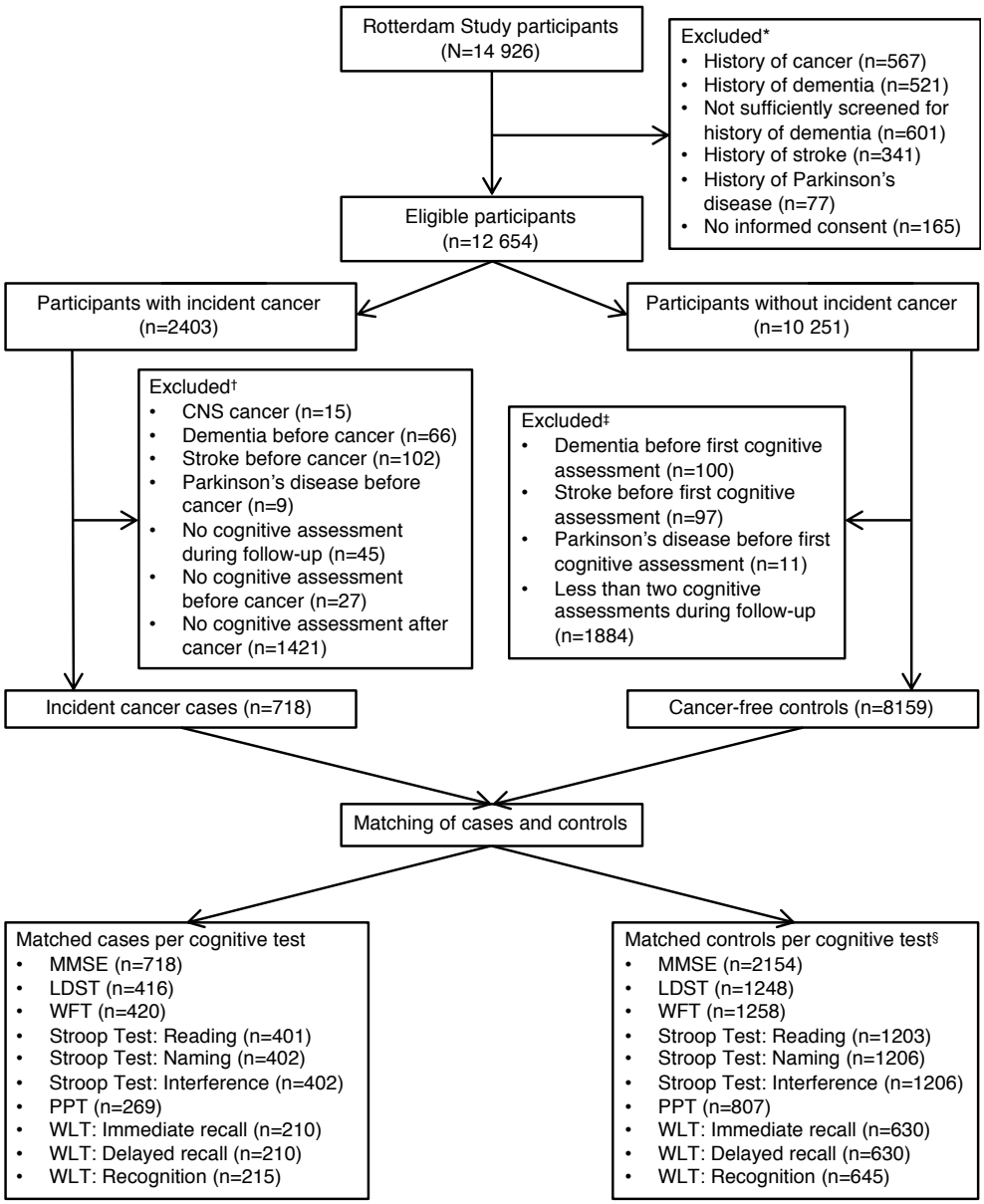


Figure 1 Flowchart of the study population.

** History of diseases at study entry. † CNS cancer, dementia, stroke, or Parkinson's disease diagnosis after study entry but before non-CNS cancer diagnosis. ‡ Dementia, stroke, or Parkinson's disease after study entry but before first cognitive assessment. § Number of matched controls depended on the cognitive test.*

CNS = central nervous system, LDST = Letter-Digit Substitution Test, MMSE = Mini-Mental State Examination, PPT = Purdue Pegboard Test, WFT = Word Fluency Test, WLT = Word Learning Test.

cognitive assessment before cancer diagnosis (n=27), or those without at least one cognitive assessment after diagnosis (n=1421), resulting in 718 cases for analysis who had at least two cognitive assessments.

Cancer-free controls

Out of the 12 654 eligible participants, 10 251 remained free of cancer during follow-up. We censored these participants at date of diagnosis of dementia, stroke, or Parkinson's disease. Participants were excluded if their diagnosis of dementia (n=100), stroke (n=97), or Parkinson's disease (n=11) was before their first cognitive assessment. We subsequently excluded participants without at least two cognitive test results during follow-up (n=1884), leaving 8159 participants as eligible controls.

Matching procedure

Each case was individually matched to three randomly selected cancer-free controls for the age at cancer diagnosis of the case (index age). To avoid overmatching, we only matched for age.[19] An eligible control had undergone at least one cognitive assessment before and one assessment after the index age. Matching started with the oldest case and was done without replacement. We separately performed matching for each individual cognitive test.

Ascertainment of cancer

Diagnoses of cancer were based on medical records of general practitioners (including hospital discharge letters) and through linkage with Dutch Hospital Data, Netherlands Cancer Registry, and histology and cytopathology registries in the region. Incident cancer was defined as any primary malignant tumour, excluding non-melanoma skin cancer. Diagnoses were coded independently by two physicians according to the International Classification of Diseases, tenth revision (ICD-10). In case of discrepancy, consensus was sought through consultation with a physician specialised in internal medicine. Date of diagnosis was based on date of biopsy (solid tumours) and laboratory assessment (haematological tumours), or – if unavailable – date of hospital admission or discharge letter. Only pathology-confirmed cancers were included in the analysis. We collected information about cancer treatment, which was categorised into no or local treatment (yes or no), hormonal therapy (yes or no), or chemotherapy (yes or no). Follow-up was completed up to January 1st, 2015.

Cognitive function assessment

Between 1989 and 2014, participants underwent cognitive screening using the Mini-Mental State Examination (MMSE)[20] during home interviews by trained interviewers. From 1997

onwards, participants underwent in addition cognitive assessments at the research centre using a neuropsychological battery that included the Letter-Digit Substitution Test (LDST),[21,22] Word Fluency Test (WFT),[23] and Stroop Test (Reading, Naming, and Inference subtask).[24,25] In 1999, the Purdue Pegboard Test (PPT) was added to the test battery.[26] The test battery was further expanded in 2002 with the 15-Word Learning Test (WLT, Immediate recall, Delayed recall, and Recognition).[27,28] Because tests were implemented into the study protocol at different moments, the number of participants differs per cognitive test (**Supplementary Table 1**).

Measurement of covariates

During the home interview, participants provided information on educational level, smoking habits, and alcohol use. Educational level was classified into primary education, lower (lower or intermediate general education, or lower vocational education), intermediate (intermediate vocational education or higher general education), or higher (higher vocational education or university). Smoking was categorised as never, current, or former. Alcohol use was based on total consumption in grams per day and was categorised as 0, ≤10, 10-≤20, or >20 grams.[29] Symptoms of depression were evaluated with the Centre for Epidemiologic Studies Depression scale (CES-D), which was converted to a sum-score.[30] Height and weight were measured at the research centre and the body mass index (BMI, kg/m^2) was computed.

Statistical analysis

Differences in characteristics between cases and cancer-free controls were investigated using the independent samples *t* test (normally distributed continuous variables), the Wilcoxon signed-rank test (non-normally distributed continuous variables), and the chi-square test (for categorical variables). Next, we used the same statistical tests to explore differences in characteristics between included cases and cancer patients who were excluded because they had no cognitive assessment after diagnosis.

We determined and visualised cognitive trajectories of cases before and after cancer diagnosis and cognitive trajectories of controls over a similar time period using a two-level linear mixed model with a random intercept and slope for each cognitive test. If models did not converge with both random intercepts and time slopes, only a random intercept was used. Skewed cognitive test results (i.e., MMSE, Stroop tests, and WLT: Recognition) were transformed with the natural logarithm to reach an approximate normal distribution and were back-transformed for visualisation. Time to cancer diagnosis was used as underlying time scale (i.e., Time = 0 at cancer diagnosis, i.e., index age for controls, Time = -5 for five years before cancer diagnosis, and Time = 5 for five years after cancer diagnosis). To capture

possible non-linearity, time was represented as a natural cubic B-spline with one interior knot at time of index age (Time = 0) via the ns(Time, 2, knots = 0) function using the 'splines' package from R software Version 3.3.2. An interaction term between time and case-control status (Cancer = 1 for cases and Cancer = 0 for controls) allowed a difference in change over time between cases and controls. These models were only adjusted for the age at first cognitive test (continuous). Visualisations of the trajectories were shown for the median age at first cognitive test of cases and controls combined.

Next, we determined the change in cognitive test score per year using two-level linear mixed models, because usage of splines limits the interpretation of the corresponding coefficients. In these models, we evaluated different slopes of the trajectory before and after index age, because we hypothesised that cognitive function might decline faster after cancer diagnosis than before diagnosis. In order to do so, we included a second time variable (Time2), which is obtained by subtracting the time at which the slope is allowed to change (i.e., Time = 0) from the original time variable. Time2 is set at 0 if it has a negative value, i.e., before cancer diagnosis. This linear fit is the best linear approximation to the true functional relationship. Skewed cognitive test results were also transformed with the natural logarithm for these analyses, but when transformation did not change the statistical significance, we reported the results based on untransformed values for interpretation purposes. We modelled the j^{th} cognitive test result of participant i as:

$$\text{Cognitive test result}_{ij} = (\beta_{00} + u_{0i}) + (\beta_1 + u_{1i})\text{Time}_{ij} + (\beta_2 + u_{2i})\text{Time2}_{ij} + \beta_3\text{Cancer}_i + \beta_4\{\text{Time}_{ij}*\text{Cancer}_i\} + \beta_5\{\text{Time2}_{ij}*\text{Cancer}_i\} + \beta_6\text{Age}_i + \beta_7\text{Sex}_i + \beta_8\text{Education}_i + \beta_9\text{Smoking}_i + \beta_{10}\text{Alcohol}_i + \beta_{11}\text{CESD}_i + \beta_{12}\text{BMI}_i + \varepsilon_{ij}$$

In this formula, cognitive test result$_{ij}$ is the score of the cognitive assessment. Time$_{ij}$ represents the time to cancer diagnosis. The β parameters are fixed effects, while the u parameters are random errors, allowing variation of the intercept and slope of time between subjects. The residual term ε was modelled with the autocorrelation structure of the variance-covariance matrix, and the general positive-definite matrix was used for the random part.

The average change in cognitive test result per year for controls before index age was equal to β_1, whereas the change for cases before cancer diagnosis was equal to $(\beta_1 + \beta_4)$, i.e., β_4 indicates whether the change differs between cases and controls. After the index age, the average change in cognitive test result per year was $(\beta_1 + \beta_2)$ for controls and $(\beta_1 + \beta_2 + \beta_4 + \beta_5)$ for cases, with $(\beta_4 + \beta_5)$ indicating the difference in change between cases and controls.

Models were adjusted for age at first test and in addition for sex (women or men), educational level (primary, lower, intermediate, or higher), smoking status (never, current, or former), alcohol use (0, ≤10, 10-≤20, or >20 grams/day), CES-D sum-score (continuous), and

BMI (continuous). Values of covariates measured closest to the index age were used.

Because the slope of the estimated trajectories might have been influenced by test results of participants who had been assessed multiple years before or after the index age, we subsequently examined the difference in cognitive test scores between cases and controls using one-level linear regression models. This analysis was limited to the last cognitive assessment before the index age and the first cognitive assessment after the index age. We modelled the difference in cognitive test result of participant i as:

Cognitive test result after index age$_i$ - Cognitive test result before index age$_i$ = β_0 + β_1Cancer$_i$ + β_2Cognitive test result before index age$_i$ + β_3Time$_i$ + β_4Age$_i$ + β_5Sex$_i$ + β_6Education$_i$ + β_7Smoking$_i$ + β_8Alcohol$_i$ + β_9CESD$_i$ + β_{10}BMI$_i$

The outcome was defined as the cognitive test score after index age minus the cognitive test score before index age. Analyses were additionally adjusted for the cognitive test score before index age (continuous) and follow-up time between the assessment before and after index age (continuous).

Lastly, we performed analyses by cancer type (prostate, breast, colorectal, and other cancers) and cancer treatment (no or local treatment, hormonal therapy, and chemotherapy) in cases only to examine the effects of cancer-related variables on cognitive decline after cancer diagnosis. These analyses were adjusted for index age (continuous) instead of age at first cognitive assessment.

Missing values of covariates (maximum of 2.1%) were replaced with mean (continuous, except CES-D sum-score), median (CES-D sum-score) or mode (categorical) values of the observed data (cases and controls combined).

Statistical analyses were performed using the ‘nlme’ and ‘splines’ packages from R software Version 3.3.2.[31]

RESULTS

Characteristics of included cases and controls are presented in **Table 1A**. Cases were slightly older at the first cognitive assessment than controls. Also, they were more often men and former smokers. The median (IQR) age at cancer diagnosis was 70.3 years (65.1 to 76.2). Most frequently diagnosed cancer types were prostate (27.6%), female breast (20.6%), and colorectal (15.5%). Most cases had received local cancer treatment (68.1%).

Table 1A Characteristics of cases and matched cancer-free controls.

Characteristic	Study population		*P*†	Excluded cases (n=1421)*	*P*‡
	Cases (n=718)	Controls (n=4859)§			
Age at first cognitive assessment, years, median (IQR)	62.8 (59.3 to 69.0)	62.1 (58.5 to 68.0)	.002	65.3 (60.5 to 72.3)	<.001
Women, No. (%)	338 (47.1)	2917 (60.0)	<.001	687 (48.3)	.46
Educational level, No. (%)			.07		<.001
Primary	92 (12.8)	567 (11.7)		265 (18.6)	
Lower	281 (39.1)	2049 (42.2)		591 (41.6)	
Intermediate	239 (33.3)	1423 (29.3)		385 (27.1)	
Higher	106 (14.8)	820 (16.9)		163 (11.5)	
Body mass index, kg/m^2, mean (SD)	26.7 (4.9)	26.9 (5.2)	.12	27.1 (4.1)	.69
Smoking status, No. (%)			.007		<.001
Never	164 (22.8)	1389 (28.6)		295 (20.8)	
Former	427 (59.5)	2640 (54.3)		746 (52.5)	
Current	127 (17.7)	830 (17.1)		374 (26.3)	
Daily grams of alcohol consumption, No. (%)			.008		<.001
0	121 (16.9)	647 (13.3)		339 (23.9)	
≤10	338 (47.1)	2887 (59.4)		557 (39.2)	
10-≤20	108 (15.0)	757 (15.6)		182 (12.8)	
>20	101 (14.1)	568 (11.7)		169 (11.9)	
CES-D sum-score, median (IQR)	3 (0 to 8)	3 (1 to 7)	.24	3 (0 to 8)	.99

** Cases were excluded because they had no cognitive measurement after cancer diagnosis. Missing data for these cases were not imputed and therefore numbers do not always add up to 100%. † P-value for difference in characteristics between included cases and cancer-free controls. ‡ P-value for difference in characteristics between included and excluded cases. § Controls were matched to cancer patients per individual cognitive test. Therefore, some controls were used for different cognitive tests, whereas other controls were only matched to cancer patients for one cognitive test, and some were not used at all (n=3300). The controls in this table represent all unique controls used for the different cognitive test analyses. Of the 4859 controls, 41% were matched for one test, 24% for two tests, 16% for three tests, and 19% for four or more tests.*

CES-D = Centre for Epidemiologic Studies Depression Scale, IQR = interquartile range, SD = standard deviation.

Table 1B Cancer-related characteristics of included and excluded cases.

Characteristic	Included cases (n=718)	Excluded cases (n=1421)*	*P*†
Age at cancer diagnosis, years, median (IQR)	70.3 (65.1 to 76.2)	75.7 (69.5 to 81.4)	<.001
Cancer type, No. (%)			<.001
Head and neck	31 (4.3)	46 (3.2)	
Oesophagus and gastric	16 (2.2)	107 (7.5)	
Colorectal	111 (15.5)	233 (16.4)	
Hepato-pancreato-biliary	2 (0.3)	90 (6.3)	
Lung and mesothelioma	26 (3.6)	261 (18.4)	
Female breast	148 (20.6)	151 (10.6)	
Female genital organs	36 (5.0)	59 (4.2)	
Male genital organs	199 (27.7)‡	152 (10.7)§	
Urinary tract	50 (7.0)	103 (7.2)	
Haematological	50 (7.0)	127 (8.9)	
Other	48 (6.7)	41 (2.9)	
Unknown primary origin	1 (0.1)	51 (3.6)	
Cancer treatment‖, No. (%)			<.001
No treatment	61 (8.5)	60 (4.2)	
Local treatment	489 (68.1)	982 (69.1)	
Hormonal therapy	87 (12.1)	108 (7.6)	
Chemotherapy	81 (11.3)	271 (19.1)	

** Cases were excluded because they had no cognitive measurement after cancer diagnosis. † P-value for difference in characteristics between included and excluded cases. ‡ 198 out of 199 were prostate cancers. § 151 out of 152 were prostate cancers. ‖ Any line of cancer treatment for included cases, first line cancer treatment for excluded cases. If cases received more than one treatment, cases were categorised as receiving either chemotherapy or hormonal therapy.*
IQR = interquartile range.

Cancer patients who were excluded because they had only cognitive assessments before, but not after diagnosis, were older at the first cognitive assessment and at cancer diagnosis than included cases (**Table 1B**). In addition, they had more often a primary educational level, were more frequently current smokers, and less frequently alcohol users. Most frequent cancer types among excluded cancer patients were lung and mesothelioma (18.4%), colorectal (16.4%), female breast (10.6%), and prostate (10.6%). Reasons for missing cognitive assessments after diagnosis are presented in **Table 2**. The majority of the excluded cancer patients (46.9%) had died within five years after their last visit.

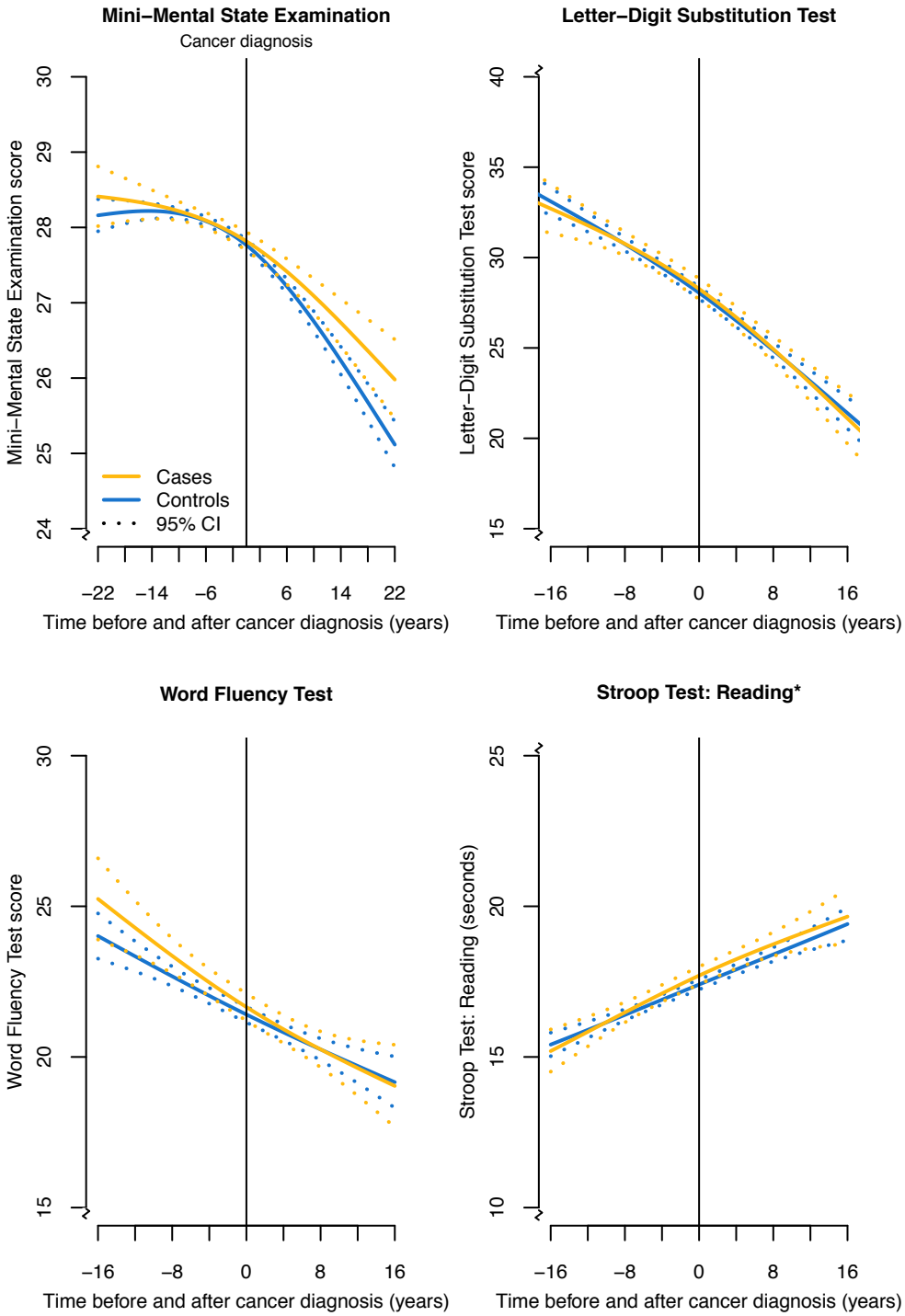
Table 2 Reasons for absent cognitive assessment after cancer diagnosis.

Reason for absent cognitive assessment	Cancer patients without cognitive assessment after cancer diagnosis (n=1421)
Dementia <5 years after last visit	15 (1.1)
Age at dementia diagnosis, years	81.0 (75.2 to 85.6)
Stroke <5 years after last visit	41 (2.9)
Age at stroke diagnosis, years	77.4 (72.4 to 85.6)
Parkinson's disease <5 years after last visit	4 (0.3)
Age at Parkinson's disease diagnosis, years	75.5 (71.1 to 77.1)
End of follow-up (2015) <5 years after last visit	120 (8.4)
Deceased <5 years after last visit	666 (46.9)
Age at death, years	76.3 (69.8 to 81.8)
Other reasons	575 (40.5)
Refused	305 (53.0)
Physical or mental problems	143 (24.9)
Deceased >5 years after last visit	32 (5.6)
Other	22 (3.8)
Unknown	73 (12.7)

Categorical data are presented as count (percent), continuous data are presented as median (interquartile range).

Figure 2 shows the cognitive trajectories of cases and controls. Cases declined with a similar rate as controls on all cognitive tests before cancer diagnosis (**Figure 2** and **Table 3**). Also after diagnosis, trajectories were largely similar between cases and controls after diagnosis. The largest difference was found on the WLT: Delayed recall (cases declined with 0.14 units per year [95% confidence interval [CI] = -0.35 to 0.07] whereas controls declined with 0.09 units [95% CI = -0.18 to -0.00], *P* for difference = .59, **Table 3**). The total number of cognitive assessments and the time between assessments are presented in **Supplementary Table 1**.

When including only one cognitive assessment before and one assessment after index age to minimise possible effects of leverage points, we found that the difference in WLT: Immediate and Delayed recall scores was larger in cases than in controls (cases declined with an additional 0.27 units [95% CI = -0.53 to -0.01] on the WLT: Immediate recall and with an additional 0.34 units [95% CI = -0.68 to 0.00] on the WLT: Delayed recall, **Supplementary Table 2**).


Mini–Mental State Examination
Cancer diagnosis
Mini–Mental State Examination score
30
29
28
27
26
25
24
Cases
Controls
95% CI
–22
–14
–6
6
14
22
Time before and after cancer diagnosis (years)
Letter–Digit Substitution Test
Letter–Digit Substitution Test score
40
35
30
25
20
15
–16
–8
0
8
16
Time before and after cancer diagnosis (years)
Word Fluency Test
Word Fluency Test score
30
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Time before and after cancer diagnosis (years)
Stroop Test: Reading*
Stroop Test: Reading (seconds)
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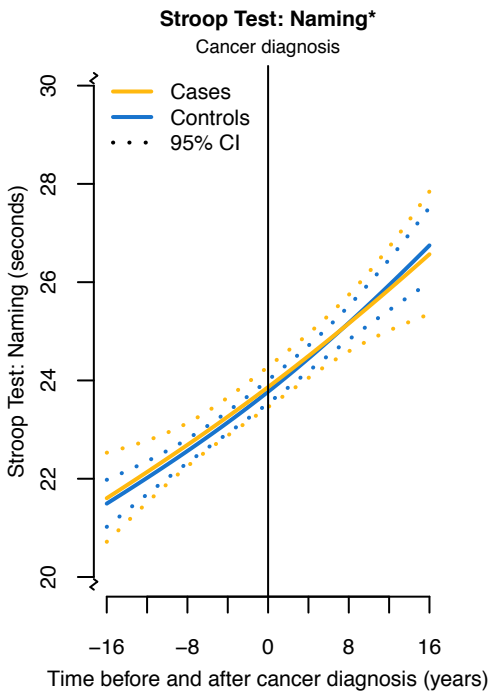
Time before and after cancer diagnosis (years)


Stroop Test: Naming*
Cancer diagnosis
Cases
Controls
95% CI
Stroop Test: Naming (seconds)
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24
26
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−16
−8
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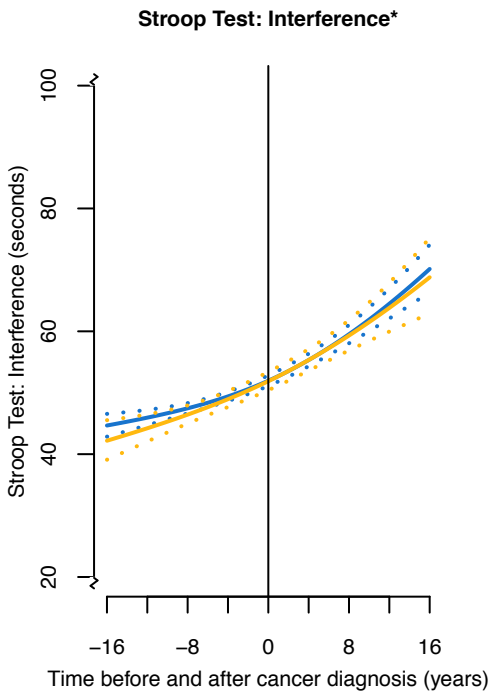
8
16
Time before and after cancer diagnosis (years)


Stroop Test: Interference*
Stroop Test: Interference (seconds)
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40
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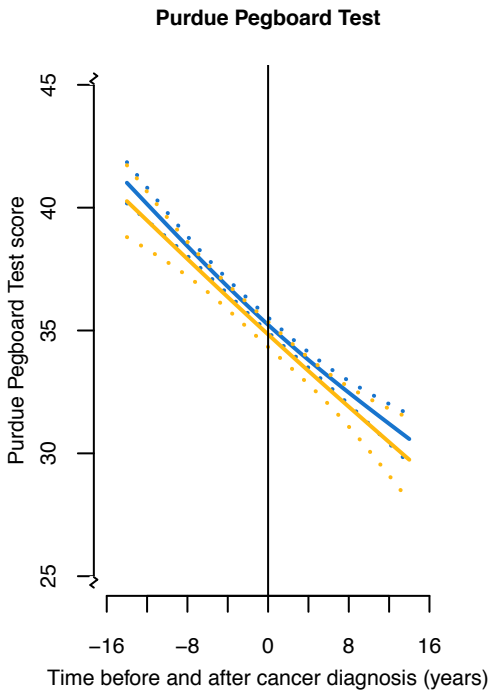
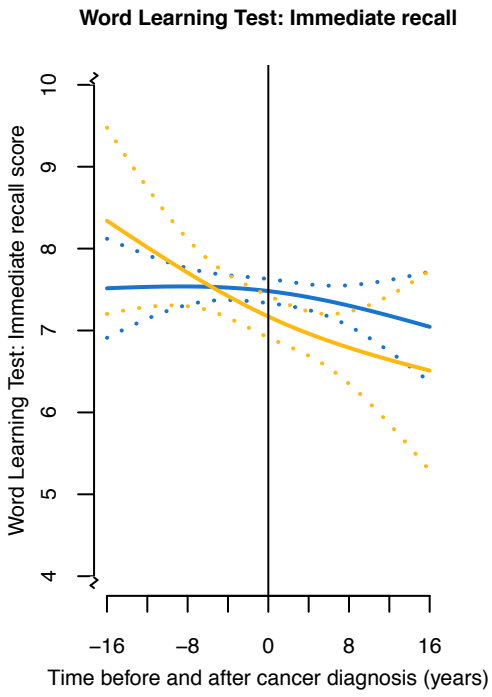
80
100
−16
−8
0
8
16
Time before and after cancer diagnosis (years)


Purdue Pegboard Test
Purdue Pegboard Test score
25
30
35
40
45
−16
−8
0
8
16
Time before and after cancer diagnosis (years)


Word Learning Test: Immediate recall
Word Learning Test: Immediate recall score
4
5
6
7
8
9
10
−16
−8
0
8
16
Time before and after cancer diagnosis (years)

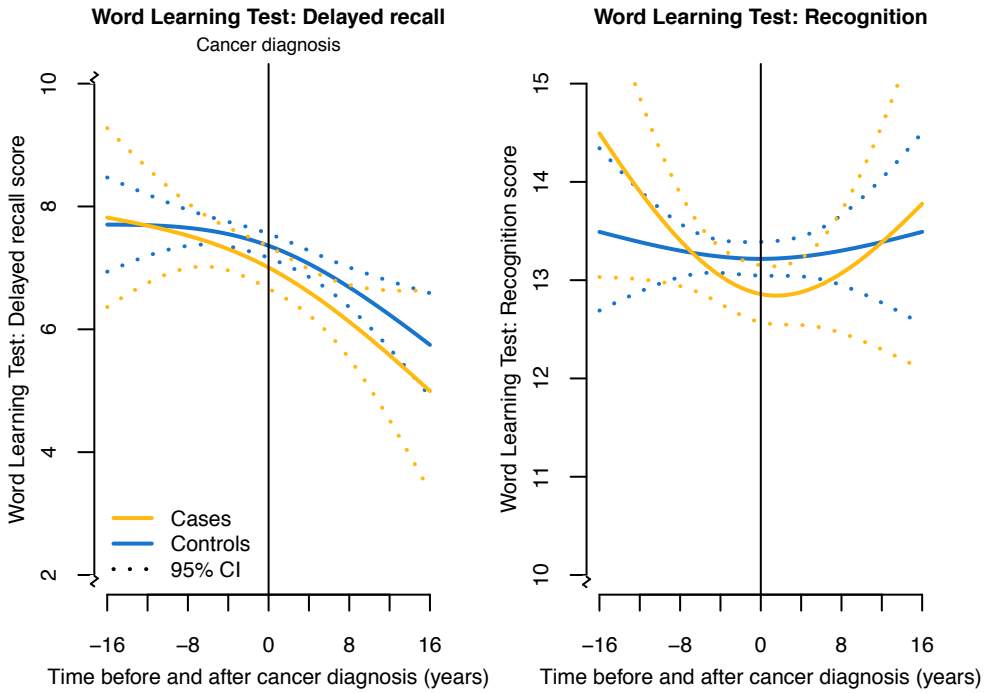


Figure 2 Trajectories of cognitive decline for cases (yellow) before and after cancer diagnosis and for cancer-free controls (blue) over a similar time period.
** Higher score indicates worse performance.*
CI = confidence interval.

Subgroup analyses by cancer type showed that cognitive function in patients with breast, prostate, and colorectal cancer declined with a similar rate after cancer diagnosis as cognitive function in patients with other cancer types (**Supplementary Table 3**). We found that cases who received chemotherapy declined slightly faster on Stroop Test reading subtask and PPT than patients who had received no or local cancer treatment, but effect sizes were small (**Supplementary Table 4)**.

DISCUSSION

In this population-based study we found that the mean rate of cognitive decline seen in cancer patients from before to after cancer diagnosis is no faster than that observed in cancer-free controls over a similar time period. When focusing on only one cognitive assessment before and after diagnosis, cognitive test scores related to memory function of cancer patients

Table 3 Estimated change in cognitive function in cases and cancer-free controls before and after cancer diagnosis.

			Before cancer diagnosis			After cancer diagnosis		
Cognitive test	N Cases	N Controls	Change/year (95% CI) cases	Change/year (95% CI) controls	*P*	Change/year (95% CI) cases	Change/year (95% CI) controlsII	*P*
MMSE	718	2154	-0.03 (-0.05 to -0.01)	-0.03 (-0.04 to -0.02)	.75	-0.08 (-0.12 to -0.03)	-0.10 (-0.13 to -0.08)	.11
LDST	416	1248	-0.29 (-0.38 to -0.19)	-0.32 (-0.36 to -0.28)	.36	-0.44 (-0.63 to -0.25)	-0.40 (-0.49 to -0.32)	.31
WFT*	420	1258	-0.22 (-0.32 to -0.13)	-0.16 (-0.21 to -0.12)	.17	-0.17 (-0.37 to 0.03)	-0.14 (-0.23 to -0.05)	.64
Stroop Test: Reading†	401	1203	0.16 (0.09 to 0.22)	0.13 (0.10 to 0.16)	.41	0.14 (0.01 to 0.28)	0.13 (0.07 to 0.19)	.86
Stroop Test: Naming†	402	1206	0.16 (0.07 to 0.24)	0.15 (0.11 to 0.18)	.80	0.21 (0.01 to 0.41)	0.23 (0.14 to 0.32)	.71
Stroop Test: Interference†	402	1206	0.82 (0.32 to 1.33)	0.55 (0.33 to 0.77)	.24	1.22 (0.22 to 2.22)	1.32 (0.87 to 1.77)	.35
PPT	269	807	-0.38 (-0.51 to -0.24)	-0.39 (-0.45 to -0.33)	.85	-0.38 (-0.65 to -0.11)	-0.36 (-0.48 to -0.24)	.76
WLT: Immediate recall	210	630	-0.06 (-0.14 to 0.01)	-0.00 (-0.04 to 0.03)	.11	-0.06 (-0.21 to 0.10)	-0.03 (-0.09 to 0.04)	.70
WLT: Delayed recall	210	630	-0.03 (-0.13 to 0.07)	-0.03 (-0.07 to 0.02)	.89	-0.14 (-0.35 to 0.07)	-0.09 (-0.18 to -0.00)	.59
WLT: Recognition	215	645	-0.05 (-0.13 to 0.03)	0.00 (-0.03 to 0.04)	.17	0.02 (-0.15 to 0.19)	0.01 (-0.06 to 0.08)	.35

** One out of the 420 cases was matched to just one control. † Positive values indicate worse performance.*
CI = confidence interval, LDST = Letter-Digit Substitution Test, MMSE = Mini-Mental State Examination, N = number, PPT = Purdue Pegboard Test, WFT = Word Fluency Test, WLT = Word Learning Test.

appeared to decline faster, but this difference was only marginally significant. Our analyses within cancer patients showed that patients treated with chemotherapy declined faster on two out of ten cognitive tests, but numbers were small and additional data is needed for definite statements about systemic treatment.

Previous clinical studies that have investigated cognitive effects of specific cancer types or systemic cancer treatments have shown that cancer patients have more often impaired cognitive function than cancer-free controls, also before start of cancer treatment.[1-16] These studies were often limited to specific cancer types, leaving doubts about generalisability to other cancer types. Animal studies have confirmed findings from clinical studies, showing that for example both treatment-naive rodents with cancer and rodents treated with chemotherapy can have cognitive impairment.[32-36] Based on these clinical and preclinical findings, different causes and mechanisms underlying cancer-related cognitive impairment have been proposed, including psychological factors that accompany a cancer diagnosis, cancer itself, and cancer treatment.[3,37]

Our current findings at a population-level show similar trajectories of cognitive function between cancer patients and cancer-free controls. When limiting the number of cognitive assessments – and thus the study period – we found only a slight tendency for a steeper decline on the memory tests WLT: Immediate and Delayed recall in cancer patients after diagnosis. These findings indicate that in general, cognitive function in cancer patients does not decline faster than in cancer-free controls.

An important explanation for the discrepancy between our findings and those of previous clinical studies lies in the difference between study populations. Firstly, we have included a heterogeneous population of cancer patients with different cancer types and treatments. Most cancer patients (68.1%) did receive local treatment only. Secondly, we had to exclude almost two thirds of cancer patients in the Rotterdam Study, because they had no cognitive assessment after cancer diagnosis. Compared to these excluded patients, included cancer patients were younger, had more favourable cancer types, and underwent less aggressive treatments. Also, because cognitive assessments in this population-based study take place every three to six years, and patients had to visit the research centre to undergo the variety of neuropsychological tests, we may have selected the most 'healthy' cancer patients. In clinical studies, patients are assessed shortly after diagnosis. Therefore, patients who die within a few years after diagnosis may still have been included in a clinical study. Despite this selection of healthier cancer patients, we nevertheless feel that our study provides key insights into the course of cognitive decline for a large group of cancer patients. Such insights into cognitive decline in later life are especially important given the growing number of long-term cancer survivors.

A few limitations of our study need to be addressed. Although we included a large number of cancer patients, they represent a very heterogeneous group in terms of cancer types and treatments. Therefore, the statistical power to detect small to moderate differences in cognitive function over time within different subgroups of cancer patients was likely low (for instance, only 19 out of 148 breast cancer patients were treated with chemotherapy). Another limitation is that we were unable to identify any risk factors that might be associated with accelerated cognitive decline in cancer patients as risk factors for cognitive decline such as smoking and alcohol can also affect the risk of cancer. To be able to determine the effect of smoking on cognitive decline in cancer patients, it is necessary to take into account the mediating effect of smoking through cancer. As yet, the application of mediation analysis in longitudinal data is limited. Another possibility is to apply the well-established prediction models for cognitive decline, since we assume that these risk factors will not affect cognitive function in cancer patients differently than that in cancer-free controls. However, application of such models requires a larger population of cancer patients.

Despite these limitations, our study design and setting also have many strengths. Firstly, the baseline assessment in the current study was a cognitive assessment before cancer diagnosis, which is necessary to control the effects of psychological factors that accompany a new cancer diagnosis. Secondly, instead of assessing cognitive function at a single time point after cancer diagnosis or treatment, we assessed cognitive function over time. Thirdly, we included a large number of cancer patients with different types of cancer and different treatments, enabling us to generalise our findings to a larger population of cancer patients.

In conclusion, we found that at a population-level, cognitive function in cancer patients from before to after diagnosis declines similarly to that in cancer-free controls. This finding provides some reassurance to cancer patients with favourable cancer types and those who have received local treatment. Even larger numbers of patients with cognitive assessments would be needed to evaluate cognitive changes in patients with specific cancer types and following certain treatments and to identify subgroups of cancer patients who are at high risk for developing cognitive impairment.

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Supplementary Table 1 Overview of number of cognitive assessments before and after cancer diagnosis.

Cognitive test	N Cases	N Controls	Before cancer diagnosis				After cancer diagnosis			
			Cases		Controls		Cases		Controls	
			N assess-ments	Years between assessment and diagnosis*	N assess-ments	Years between assessment and index age*,†	N assess-ments	Years between diagnosis and assessment*	N assess-ments	Years between index age and assessment*,†
Mini-Mental State Examination	718	2154	1597	4.9 (8.7 to 2.4)	4791	5.3 (9.5 to 2.6)	1154	3.8 (1.8 to 7.3)	3365	3.7 (1.6 to 6.9)
Letter-Digit Substitution Test	416	1248	619	4.0 (6.2 to 2.0)	1898	4.2 (6.6 to 2.2)	549	3.2 (1.7 to 6.0)	1592	3.2 (1.5 to 5.5)
Word Fluency Test‡	420	1258	619	4.0 (6.4 to 2.0)	1893	4.1 (6.7 to 2.2)	550	3.2 (1.7 to 6.3)	1609	3.1 (1.5 to 5.7)
Stroop Test: Reading	401	1203	596	4.0 (6.4 to 2.0)	1817	4.2 (6.5 to 2.3)	523	3.2 (1.8 to 6.4)	1543	3.1 (1.5 to 5.9)
Stroop Test: Naming	402	1206	597	4.0 (6.4 to 2.0)	1819	4.2 (6.7 to 2.1)	524	3.2 (1.8 to 6.3)	1543	3.2 (1.5 to 5.6)
Stroop Test: Interference	402	1206	597	4.0 (6.4 to 2.0)	1819	4.3 (6.7 to 2.2)	522	3.2 (1.7 to 6.2)	1523	3.2 (1.5 to 5.6)
Purdue Pegboard Test	269	807	342	4.0 (5.7 to 2.2)	1050	4.1 (5.8 to 2.3)	310	2.7 (1.5 to 5.0)	931	2.9 (1.4 to 4.9)
Word Learning Test: Immediate recall	210	630	241	3.8 (4.9 to 2.0)	722	3.8 (5.4 to 2.1)	238	2.7 (1.7 to 4.8)	700	2.9 (1.4 to 4.8)
Word Learning Test: Delayed recall	210	630	241	3.8 (4.9 to 2.0)	718	3.7 (5.4 to 2.0)	238	2.7 (1.7 to 4.8)	719	2.9 (1.3 to 4.9)
Word Learning Test: Recognition	215	645	247	3.7 (4.9 to 2.0)	734	3.7 (5.4 to 2.1)	242	2.7 (1.7 to 4.7)	725	2.9 (1.3 to 4.7)

** Presented as median (interquartile range). † Corresponding to the age at which the matched case was diagnoses with cancer. ‡ One out of the 420 cases was matched to just one control.*
N = number.

Supplementary Table 2 Difference between cases and cancer-free controls in the change in cognitive test scores between before and after cancer diagnosis based on one cognitive test before cancer diagnosis and one cognitive test after cancer diagnosis using linear regression models.

Cognitive test	N Cases	N Controls	β Cancer* (95% CI)
Mini-Mental State Examination	718	2154	0.07 (-0.08 to 0.23)
Letter-Digit Substitution Test	416	1248	-0.32 (-0.77 to 0.12)
Word Fluency Test	420	1258	-0.10 (-0.53 to 0.34)
Stroop Test: Reading†	401	1203	0.33 (-0.02 to 0.68)
Stroop Test: Naming†	402	1206	-0.40 (-0.98 to 0.18)
Stroop Test: Interference†	402	1206	-0.37 (-3.19 to 2.45)
Purdue Pegboard Test	269	807	-0.28 (-0.74 to 0.18)
Word Learning Test: Immediate recall	210	630	-0.27 (-0.53 to -0.01)
Word Learning Test: Delayed recall	210	630	-0.34 (-0.68 to 0.00)
Word Learning Test: Recognition	215	645	-0.24 (-0.51 to 0.04)

Results based on the model Cognitive test result after index age$_i$ - Cognitive test result before index age$_i$ = $\beta_0 + \beta_1 Cancer_i + \beta_2 Cognitive\ test\ result\ before\ index\ age_i + \beta_3 Time_i + \beta_4 Age_i + \beta_5 Sex_i + \beta_6 Education_i + \beta_7 Smoking_i + \beta_8 Alcohol_i + \beta_9 CESD_i + \beta_{10} BMI_i$ for participant i.
** β_1, † Positive values indicate worse test scores.*
BMI = body mass index, CES-D = Centre for Epidemiologic Studies Depression Scale, CI = confidence interval, N = number.

Supplementary Table 3 Estimated change in cognitive function in cases with different cancer types following cancer diagnosis.

Cognitive test	Other cancer types		Prostate cancer			Breast cancer			Colorectal cancer		
	N	Change/year (95% CI)*	N	Change/year (95% CI)†	*P*‡	N	Change/year (95% CI)†	*P*‡	N	Change/year (95% CI)†	*P*‡
Mini-Mental State Examination	261	-0.07 (-0.12 to -0.01)	198	-0.08 (-0.18 to 0.02)	.70	148	-0.08 (-0.18 to 0.02)	.77	111	-0.10 (-0.20 to 0.01)	.49
Letter-Digit Substitution Test	145	-0.52 (-0.78 to -0.27)	119	-0.20 (-0.63 to 0.22)	.06	83	-0.29 (-0.74 to 0.17)	.22	69	-0.23 (-0.68 to 0.21)	.12
Word Fluency Test	147	-0.17 (-0.44 to 0.09)	117	-0.12 (-0.56 to 0.33)	.75	84	-0.17 (-0.64 to 0.31)	.98	72	-0.08 (-0.54 to 0.39)	.62
Stroop Test: Reading§	139	0.13 (-0.07 to 0.33)	112	0.18 (-0.15 to 0.51)	.74	81	0.12 (-0.23 to 0.47)	.94	69	0.02 (-0.33 to 0.37)	.43
Stroop Test: Naming§	139	0.19 (-0.11 to 0.48)	112	0.41 (-0.11 to 1.92)	.31	82	0.24 (-0.30 to 0.79)	.81	69	0.17 (-0.36 to 0.69)	.93
Stroop Test: Interference§	139	1.50 (0.22 to 2.78)	112	1.79 (-0.30 to 3.87)	.73	82	-0.54 (-2.76 to 1.68)	.03	69	1.13 (-1.07 to 3.32)	.68
Purdue Pegboard Test	95	-0.28 (-0.61 to 0.06)	69	-0.30 (-0.88 to 0.28)	.92	57	-0.59 (-1.20 to 0.03)	.24	48	-0.21 (-0.81 to 0.39)	.80
Word Learning Test: Immediate recall	76	-0.02 (-0.25 to 0.20)	49	-0.14 (-0.53 to 0.26)	.49	46	-0.30 (-0.70 to 0.10)	.10	39	0.10 (-0.29 to 0.48)	.45
Word Learning Test: Delayed recall	76	-0.13 (-0.42 to 0.16)	49	-0.19 (-0.70 to 0.32)	.78	46	-0.33 (-0.83 to 0.18)	.35	39	0.03 (-0.48 to 0.53)	.46
Word Learning Test: Recognition	78	0.04 (-0.19 to 0.28)	51	0.04 (-0.38 to 0.46)	.99	47	-0.14 (-0.58 to 0.30)	.33	39	0.05 (-0.37 to 0.46)	.97

Results based on the model: Cognitive test result$_{ij}$ = $(\beta_{00} + u_{0i}) + (\beta_1 + u_{1i})Time_{ij} + (\beta_2 + u_{2i})Time2_{ij} + \beta_3 Cancer\ type_i + \beta_4(Time_{ij} * Cancer\ type_i) + \beta_5(Time2_{ij} * Cancer\ type_i) + \beta_6 Age_i + \beta_7 Sex_i + \beta_8 Education_i + \beta_9 Smoking_i + \beta_{10} Alcohol_i + \beta_{11} CESD_i + \beta_{12} BMI_i + \varepsilon_{ij}$ for participant i and repeated measure j, assuming autocorrelation structure of the variance-covariance matrix of residuals and the general positive-definite matrix of the random part.

** $\beta_1 + \beta_2$, † $\beta_1 + \beta_2 + \beta_4 + \beta_5$, ‡ Test of H_0: $\beta_5 = 0$ that compares each cancer type with other cancer types (reference). § Positive values indicate worse performance.*

BMI = body mass index, CES-D = Centre for Epidemiologic Studies Depression Scale, CI = confidence interval, N = number of cases with cancer at that specific site.

Supplementary Table 4 Estimated change in cognitive function in cases with different types of cancer treatment after cancer diagnosis.

Cognitive test	Local or no treatment		Treatment with hormonal therapy			Treatment with chemotherapy		
	N	Change/year (95% CI)*	N	Change/year (95% CI)†	*P*‡	N	Change/year (95% CI)†	*P*‡
Mini-Mental State Examination	550	-0.07 (-0.11 to -0.04)	87	-0.10 (-0.18 to -0.01)	.59	81	0.01 (-0.09 to 0.11)	.09
Letter-Digit Substitution Test	304	-0.43 (-0.59 to -0.28)	58	-0.32 (-0.67 to 0.03)	.47	54	-0.57 (-0.94 to -0.20)	.44
Word Fluency Test	309	-0.20 (-0.37 to -0.03)	57	-0.14 (-0.48 to 0.20)	.67	54	0.10 (-0.28 to 0.48)	.08
Stroop Test: Reading§	294	0.11 (-0.02 to 0.23)	55	0.23 (-0.02 to 0.48)	.27	52	0.40 (0.11 to 0.69)	.03
Stroop Test: Naming§	294	0.14 (-0.04 to 0.33)	55	0.48 (0.05 to 0.92)	.09	53	0.33 (-0.16 to 0.82)	.42
Stroop Test: Interference§	294	1.17 (0.34 to 2.00)	55	0.83 (-1.02 to 2.68)	.69	53	1.00 (-1.01 to 3.02)	.86
Purdue Pegboard Test	191	-0.31 (-0.53 to -0.10)	37	-0.47 (-0.93 to -0.01)	.45	41	-0.87 (-1.37 to -0.38)	.01
Word Learning Test: Immediate recall	146	-0.07 (-0.22 to 0.08)	32	-0.11 (-0.36 to 0.14)	.66	32	-0.07 (-0.38 to 0.23)	.95
Word Learning Test: Delayed recall	146	-0.14 (-0.33 to 0.05)	32	-0.28 (-0.58 to 0.02)	.23	32	-0.23 (-0.59 to 0.14)	.57
Word Learning Test: Recognition	151	0.07 (-0.09 to 0.22)	32	-0.17 (-0.46 to 0.12)	.06	32	-0.00 (-0.33 to 0.32)	.63

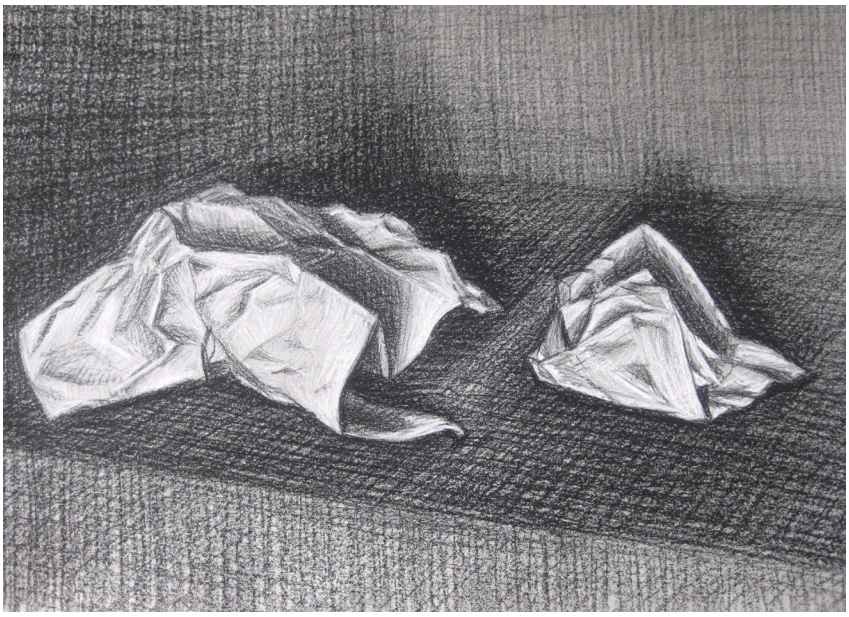
*Results based on the model: Cognitive test result$_{ij}$ = ($\beta_{00} + u_{0i}$) + ($\beta_1 + u_{1i}$)Time$_{ij}$ + ($\beta_2 + u_{2i}$)Time2$_{ij}$ + β_3Cancer treatment$_i$ + β_4(Time2$_{ij}$*Cancer treatment$_i$) + β_5Age$_i$ + β_6Sex$_i$ + β_7Education$_i$ + β_8Smoking$_i$ + β_9Alcohol$_i$ + β_{10}CESD$_i$ + β_{11}BMI$_i$ + ε_{ij} for participant i and repeated measure j, assuming autocorrelation structure of the variance-covariance matrix of residuals and the general positive-definite matrix of the random part.*

** $\beta_1 + \beta_2$, † $\beta_1 + \beta_2 + \beta_4$, ‡ Test of H_0: $\beta_4 = 0$. § Positive values indicate worse performance.*

BMI = body mass index, CES-D = Centre for Epidemiologic Studies Depression Scale, CI = confidence interval, N = number of cases with cancer with that specific treatment.

Part III

Cancer and dementia



Chapter 8

Alzheimer's disease as a multistage process

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ABSTRACT

Background In cancer research, multistage models are used to assess the multistep process that leads to the onset of cancer. In view of biological and clinical similarities between cancer and dementia, we used these models to study Alzheimer's disease (AD).

Methods From the population-based Rotterdam Study, we included 9362 participants free from any type of dementia, of whom 1124 developed AD during up to 26.1 years of follow-up. Under a multistage model, we regressed the logarithm of AD incidence rate against the logarithm of five-year age categories. The slope in this model reflects the number of steps (n–1) required for disease onset before the final step leading to disease manifestation.

Results A linear relationship between log incidence rate and log age was observed, with a slope of 12.8 (95% CI 9.0 to 16.6), equivalent to 14 steps. We observed fewer steps for those at high genetically determined risk: 12 steps for *APOE*-ε4 carriers, and ten steps for those at highest genetic risk based on *APOE* and a genetic risk score.

Conclusions The pathogenesis of AD complies with a multistage disease-model, requiring 14 steps before disease manifestation. Genetically predisposed individuals require fewer steps indicating that they already inherited multiple of these steps. Unravelling these steps in AD pathogenesis could benefit the development of intervention strategies.

INTRODUCTION

Over the past decades, major advances have been made in the understanding of the role of amyloid and cerebrovascular pathology in the onset and progression of Alzheimer's disease (AD).[1] However, the underlying number of pathological changes and the subsequent final trigger leading to clinical disease manifestation, remain largely unclear. AD has a strong genetic component with a heritability of 60 to 80%, with additional AD-susceptibility genes that are still being identified.[2,3] These findings suggest that an individual's genetic architecture is key in determining if and when disease emerges.[2-4] Notwithstanding the importance of environmental and lifestyle factors, it remains difficult to quantify to what extent this genetic predisposition is deterministic for AD onset.

Originated in cancer research, multistage models have been used to gain more insight in the number of steps before disease manifestation. These models are able to estimate the number of steps ('mutations') required for a healthy cell to become malignant.[5] After undergoing several of these rate-limiting steps, the last mutation will ultimately lead to clinical manifestation of the disease. These models have yielded consistent findings across a variety of cancers, supporting the notion that the occurrence of cancer is the end result of seven, successful mutations.[5]

Cancer and neurodegenerative disease, including AD as its most common form, may be seen as two opposite ends in cell proliferation. Yet they share biological and clinical characteristics, including dysregulations in key DNA repair and inflammation processes, an increasing incidence with advancing age, and rapid disease progression after diagnosis.[6,7] Moreover, they share a complex inheritance pattern with genetic pleiotropy.[8] For instance, a recent genome-wide association study (GWAS) found a positive genetic correlation between AD and cancer genes, further supporting the genetic overlap between these two diseases.[8]

Given the commonalities between neurodegenerative diseases and cancer, the multistage model has recently been successfully applied to model the incidence rate of amyotrophic lateral sclerosis, a rare neurodegenerative disease, as a six-step process.[9] So far, this multistage modelling has not been used for AD. We therefore applied a multistage model within a large, population-based study to test the hypothesis that AD is a multistage process. We determined the number of steps required for disease onset and hypothesised that if AD complies with a multistage process, the number of steps will be smaller in genetically predisposed individuals as these individuals may already inherited one of these key steps.

METHODS

Study design

This study was conducted within the Rotterdam Study, a prospective population-based cohort designed to study the occurrence and determinants of age-related diseases in the general population. Details regarding the objectives and design have been reported previously.[10] Briefly, in 1989 inhabitants aged 55 years and older from a well-defined suburb in the city of Rotterdam, the Netherlands were invited to participate. The initial cohort comprised 7983 individuals. In 2000, 3011 individuals who had become 55 years of age or moved into the study district since the start of the study if aged at least 55 years, were added to the cohort. In 2006, a further extension of the cohort was initiated in which 3932 individuals were included who were aged at least 45 years. In total, the Rotterdam Study comprises 14 926 individuals aged 45 years and older. The overall response rate for all three recruitment waves was 72%.

To model AD as a multistage process, we excluded participants with a history of any type of dementia at baseline (N=531) and those who were insufficiently screened for dementia (N=637). We further excluded participants who did not provide informed consent to access medical records or hospital discharge letters (N=159). Lastly, participants without information on their *APOE* genotype (N=964) or AD-associated genetic variants to calculate the genetic risk score (N=1565) were excluded, leaving 11 070 participants for analyses (**Figure 1**).

APOE genotyping and calculation of a weighted genetic risk score

DNA was extracted from blood samples drawn by venepuncture at baseline. *APOE* genotype was determined using polymerase chain reaction on coded DNA samples in the initial cohort and with a bi-allelic TaqMan assay (rs7412 and rs429358) in the two extensions (RS-II and RS-III). The majority of samples (81.1%) were further genotyped with the Illumina 610K and 660K chips and imputed to the Haplotype Reference Consortium reference panel (version 1.0) with Minimac 3. We included 23 genetic variants that showed genome wide significant evidence of association with AD to calculate a weighted genetic risk score (**Supplementary Table 1** for an overview of the included variants).[9,11-25] This score was calculated as the sum of the products of single nucleotide polymorphism dosages of the 23 genetic variants (excluding *APOE*) and their respective reported effect estimates. All 23 variants selected for the calculation of the genetic risk score were well imputed (imputation score R2 >0.3, median 0.99).

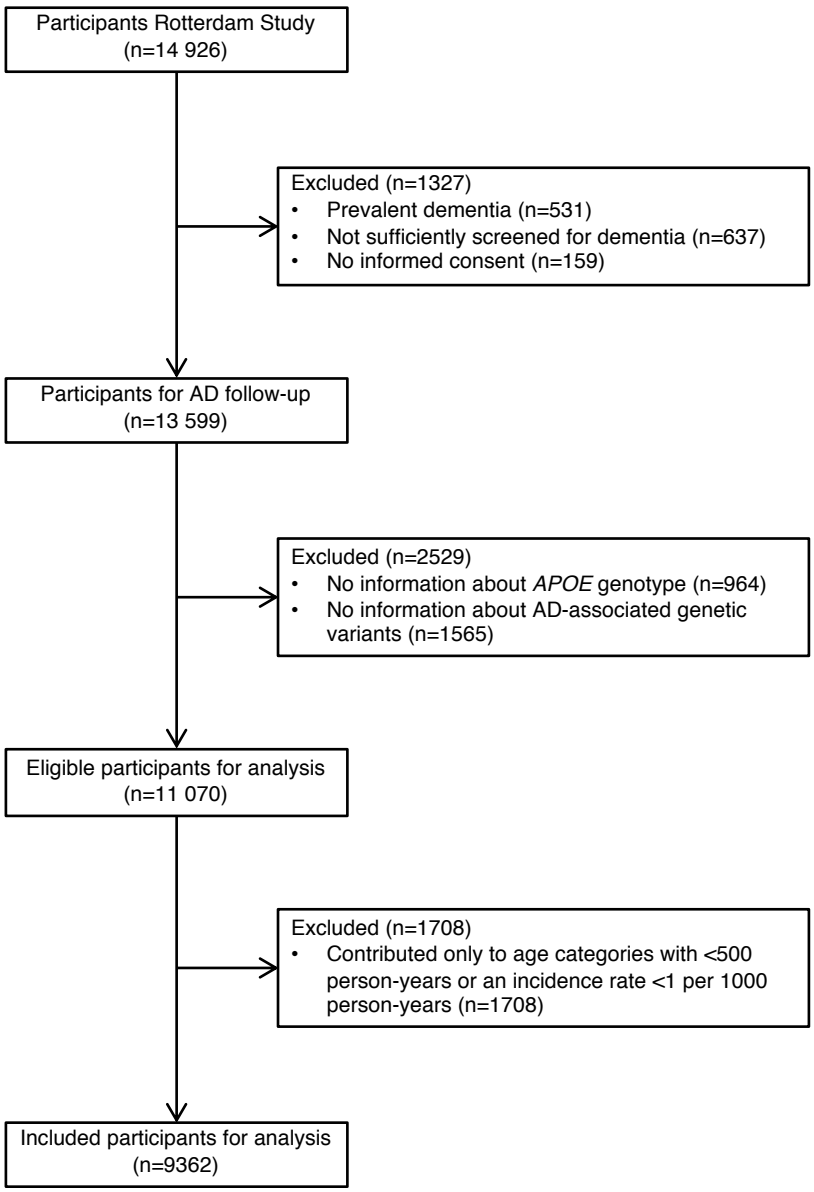

Figure 1 Flowchart of study population.
AD = Alzheimer's disease, APOE = Apolipoprotein E.

Ascertainment methods of dementia

Baseline and follow-up ascertainment methods for dementia have previously been described in detail.[26] Participants were screened for dementia at baseline and subsequent centre visits with the Mini-Mental State Examination and the Geriatric Mental Schedule organic level. Those with a Mini-Mental State Examination score <26 or Geriatric Mental Schedule score >0 underwent further investigation and informant interview, including the Cambridge Examination for Mental Disorders of the Elderly. All participants also underwent routine cognitive assessment. In addition, the entire cohort was continuously under surveillance for dementia through electronic linkage of the study database with medical records from general practitioners and the regional institute for outpatient mental health care. Available information on cognitive testing and clinical neuroimaging was used when required for diagnosis of dementia subtype. A consensus panel led by a consultant neurologist established the final diagnosis according to standard criteria for AD (NINCDS–ADRDA). Participants were censored at date of any type of dementia diagnosis, death, loss to follow-up, or January 1st, 2016, whichever came first. Follow-up was virtually complete (96.3% of potential person-years).[27]

The multistage model

Multistage models originate from cancer epidemiology, where they were first employed to study the age distribution of several cancer types.[5,28-30] Within this framework it is assumed that cancer manifests clinically after a certain threshold number has been reached composed of n mutations within one cell. This threshold for disease occurrence in that cell has a certain probability distribution over time (t), e.g., for an individual the n^{th} mutation occurs at age 50 years, whereas for another individual this n^{th} mutation may occur at age 80 years. Of the required mutations, (n–1) mutations have independently taken place at a certain point during the lifespan. For each of these mutations, a certain probability per time unit (e.g., year) exists that a mutation will occur (λ). When a cell is primed, such that it has undergone all of these necessary preceding mutations, the final mutation (n^{th} mutation) leads to clinical manifestation of disease. Subsequently, this final n^{th} mutation has to occur after all of these steps and can for example not occur in between preceding steps. So, the probability density function of time-point t, when the n^{th} change takes place is:

$$f(t) \sim \lambda_1\lambda_2...\lambda_{n-1}\lambda_n t^{n-1}$$

It was noted in cancer epidemiology that the age-specific incidence rate of cancer ('i') roughly coincided with the probability that at least one cell of all independent cells acquired the necessary number of seven mutations by that specific age. This means that for most

types of cancer six preceding rate-limiting steps (n–1) are necessary during the lifespan, with a seventh and final mutation (n^{th} mutation), leading to disease manifestation.[31] It can subsequently be shown that if the disease under study fits a multistep process, the number of these steps (n) can be estimated with the following formula:

$$\log(i) = (n\text{-}1)\log(t) + c$$

in which c is a constant number containing $\log(\lambda_1\lambda_2...\lambda_{n-1}\lambda_n)$. The common ground of these rate-limiting definitions is that the speed of a reaction step will have a significant effect on the speed of the overall chain of events to which the step belongs.[32] A reaction step is thus subsequently considered a rate-limiting step, when the rate of that particular step is identical to the overall rate of the entire reaction.

Statistical analysis

We applied a multistage model to determine the slope and the number of steps for the development and clinical onset of AD. In line with previous studies, the incidence rate of AD was calculated per five years age categories.[5,9] Each participant contributed person-years to specific age categories, until the age at AD diagnosis or censoring. To minimise the effects of outliers on the slope of the model, we excluded age categories with less than 500 person-years or with an incidence rate below 1 per 1000 person-years given that estimated incidence rates often become instable in the extremes of the age distribution.[30] This additional criterion resulted in an exclusion 213 530.6 person-years, which corresponded to the exclusion of 1708 of the 11 070 participants with age at AD or censoring below the first included age category. This left 9362 participants available for the final analyses (**Figure 1**). The incidence rate of AD and the five-years age categories (log age) were natural log-transformed. Linearity was tested based on the adjusted R-squared obtained from a linear regression model with log age and incidence rate of AD as outcome. Linear models were unadjusted.

Additionally, we stratified according to *APOE* ε4 carrier status and on tertiles of a weighted genetic risk score in mutually exclusive categories of genetic risk and by combining both in order to be able to stratify those individuals with the lowest and those with the highest AD genetic risk.

Data were handled and analysed with SPSS Statistics version 24.0.0.1 (IBM Corp., Armonk, NY) and R, CRAN version 3.4.3.

RESULTS

During a follow-up of up to 26.1 years, 1124 out of 9362 participants were diagnosed with AD during a median (interquartile range) follow-up 10.3 years (5.1 to 15.3). **Table 1** shows the baseline characteristics of the study population. In this sample, 58.2% of the participants were women. Of the included participants, 2624 were *APOE* ε4 carriers (28.0%).

Table 1 Baseline characteristics of total study population.

Characteristic	Study population (N=9362)
Age, years, median (IQR)	65.0 (60.1 to 72.7)
Women, No. (%)	5453 (58.2)
APOE ε4 carrier status, No. (%)	
Carrier	2624 (28.0)
Non-carrier	6738 (72.0)
Weighted genetic risk score, No. (%)	
First tertile	3146 (33.6)
Second tertile	3120 (33.3)
Third tertile	3096 (33.1)
Educational level, No. (%)	
Primary	1689 (18.0)
Lower	3941 (42.1)
Intermediate	2512 (26.8)
Higher	1101 (11.8)
Body mass index, mean (SD), kg/m^2	26.8 (3.9)
Systolic blood pressure, mm Hg, mean (SD)	140 (22)
Diastolic blood pressure, mm Hg, mean (SD)	76 (12)
Total cholesterol, mmol/L, mean (SD)	6.3 (1.2)
Diabetes mellitus, No. (%)	1026 (11.0)
Smoking status, No. (%)	
Never	3021 (32.2)
Former	4276 (45.7)
Current	1938 (20.7)
Alcohol use, No. (%)	6763 (72.2)

Values are shown without imputation and therefore not always add up to 100%.
APOE = apolipoprotein E, IQR = interquartile range, SD = standard deviation.

Multistep model

The adjusted R-squared for the relation between log AD incidence rate and log age was 0.93, indicating a linear correlation, which is in line with the multistage model. The estimate of the slope (number of steps minus 1) for AD was 12.8 (95% confidence interval [CI] 9.0 to 16.6), indicating that 14 steps are needed for the development of AD (**Figure 2**, **Table 2**).

Considering genetic risk

When considering only the *APOE*-related risk of developing AD, we found that *APOE* ε4 genotype non-carriers needed more steps to develop AD than *APOE* ε4 carriers (16 steps for non-carriers, 12 for carriers). In an exploratory analysis, we also examined the number of steps among participants homo- or heterozygous for *APOE* ε4 separately. Participants homozygous for the *APOE* ε4 allele required ten steps, while participants heterozygous for *APOE* with ε3 and ε4 or ε2 and ε4 required 16 steps to develop AD. Similarly, we found for participants in the low-risk tertile of the genetic risk score that more steps were required to develop AD than for those in the high-risk tertile (16 steps versus 13 steps). When stratifying on both *APOE* ε4 carriership and the genetic risk score, we found that for every increase in tertile of the genetic risk score, *APOE* ε4 carriers needed less steps to develop AD than *APOE* ε4 non-carriers. This translated into ten steps for *APOE* ε4 carriers in the high-risk tertile, compared to 16 steps for non-carriers for *APOE* in the low-risk tertile (**Table 2**).

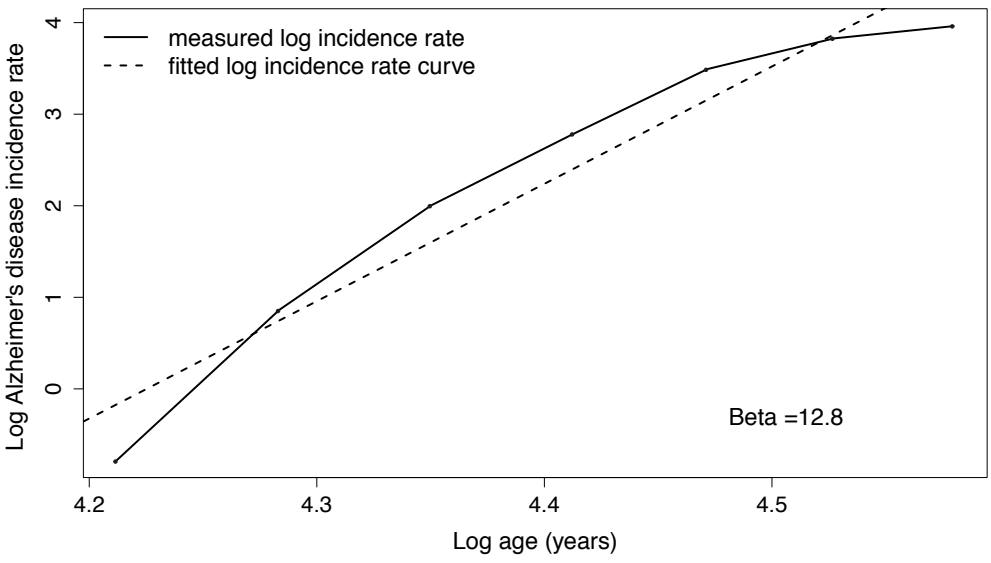

Figure 2 Plotted log incidence rate of Alzheimer's disease (y-axis) against log age (x-axis).
The dashed line shows the most optimal linear correlation.

Table 2 Overview of estimates for slopes across groups with different genetic risks.

Study population	n/N	n-1 (95% CI)	R-squared*
Total study population	1124/9362	12.8 (9.01 to 16.6)	0.925
APOE ε4			
Carrier	481/2624	10.6 (6.0 to 15.2)	0.849
Homozygote	70/213	8.9 (5.7 to 12.1)	0.923
Heterozygote	411/2411	14.9 (8.1 to 21.7)	0.878
Non-carrier	643/6738	15.0 (11.3 to 18.8)	0.946
Weighted genetic risk score - tertiles			
First	296/3146	15.0 (9.4 to 20.7)	0.885
Second	376/3120	12.8 (9.9 to 15.7)	0.956
Third	452/3096	11.7 (7.4 to 16.1)	0.886
Weighted genetic risk score - first tertile			
APOE ε4 carrier	124/843	8.5 (1.9 to 15.0)	0.886
APOE ε4 non-carrier	172/2303	15.3 (11.8 to 18.8)	0.954
Weighted genetic risk score - second tertile			
APOE ε4 carrier	161/930	10.3 (6.8 to 13.8)	0.905
APOE ε4 non-carrier	215/2190	15.5 (12.0 to 19.0)	0.955
Weighted genetic risk score - third tertile			
APOE ε4 carrier	196/851	8.9 (3.5 to 14.4)	0.738
APOE ε4 non-carrier	256/2245	14.4 (9.8 to 19.0)	0.915

** Obtained from linear regression model* $\log(\text{Alzheimer's disease incidence}_i) = \beta_0 + \beta_1 * \log(\text{age}_i)$
APOE = apolipoprotein E, n = number of incident Alzheimer's disease events, N = total number of participants, n-1 = estimate for slope (i.e., number of steps minus 1).

DISCUSSION

In this population-based study using long-term follow-up of AD, we found evidence that the development of AD follows a multistage process with 14 steps. This indicates that 14 steps are required for the clinical occurrence of AD in the general population. The number of steps was modified by the level of genetic predisposition, translating into six less steps for those individuals at highest genetic risk for AD than the number of steps for those at the lowest genetic risk.

The multistage models have been extensively used in cancer research to provide more

insight in their underlying pathogenesis.[28,33-36] Several studies have shown that seven steps were required to develop cancer, which may reflect somatic mutations, genomic rearrangements, or changes in tissue interactions and environment. Neurodegenerative diseases show several similarities with cancer such as dysregulation of DNA repair mechanisms. Yet, the multistage model has only been applied to amyotrophic lateral sclerosis which appears to follow a multistage process with six rate-limiting steps. In this study, we show that AD can also be modelled as a multistage condition consisting of 14 steps, stressing the genetic complexity and the variety of potential biological pathways involved in the development of this disease.

We found that the number of steps for AD differed between individuals with different degrees of genetic predisposition. *APOE* ε4 carriers require a smaller number of steps to develop AD than *APOE* ε4 non-carriers. In addition, these effects became even more pronounced when additionally considering 23 AD-associated genetic variants. Compared to those at highest genetic risk (i.e., *APOE* ε4 carrier and within the third tertile of the weighted genetic score), individuals at lowest genetic risk (i.e., *APOE* ε4 non-carrier and within the first tertile of weighted genetic score) needed six more rate-limiting steps to develop AD. These findings are in line with previous observations in cancer research showing different thresholds before the disease becomes clinically apparent for inherited and sporadic cancer events. For instance, individuals with familial adenomatous polyposis are at increased risk of colon cancer due to one mutated copy of the *APC* gene. It has been shown that these individuals need one step less in the overall pathological process to develop clinical colon cancer than individuals without this mutated gene.[33] Also, children with inherited retinoblastoma required only one hit to develop this disease, whilst sporadic retinoblastoma cases became clinically apparent after two hits.[37] Our findings may suggest that individuals with genetic predisposition begin several stages further down the chain of the required pathological threshold before AD becomes clinically apparent.

Although our findings suggest that 14 steps are needed for AD to emerge clinically, the underlying biological pathways and changes reflected by these steps still need to be identified. To date, eight different biological pathways involved in the pathogenesis of AD have been identified using genetic variants in AD.[38] The *APOE* ε4 allele is the most significant genetic risk factor due to its high prevalence and strong relation to AD. It is involved in four of these pathways, including cholesterol transport, haematopoietic cell lineage, clathrin/AP2 adaptor complex, and protein folding pathways. Our finding that *APOE* ε4 non-carriers need four more steps before AD clinically manifests compared to *APOE* ε4 carriers taps into this observation, and could indicate that changes in the abovementioned four pathways are indeed necessary to acquire before AD manifests clinically. This could mean that these pathways are already changed or dysregulated at birth in *APOE* ε4 carriers, indicating that these individuals

subsequently have a lower resilience to the development of dementia. This could in turn lead to a lower required number of subsequent steps before disease manifestation. Indeed, up to 18% of the *APOE* ε4 carriers in this study developed AD during follow-up, yet the lifetime risk of AD among these individuals is even higher with almost half of all them developing AD in their remaining lifetime. For carriers homozygous for *APOE* ε4 in the high-risk tertile, this risk is even higher, and the disease moreover manifests earlier, with a 29-year difference in age at onset for AD, compared to homozygous *APOE* carriers at the low-risk tertile of the genetic risk score.[2]

The search of finding successful AD therapies is among the most challenging and expensive healthcare issue to date. So far, many disease-modifying agents reduce the production of amyloid-beta (Aβ), or target only one other specific part of the disease process.[39] Our present study shows that as many as 14 steps are required before AD becomes clinically apparent. This high number of required steps may signal the need to develop multi-domain approaches to target various underlying disease-processes simultaneously in order to halt or deter neurodegeneration.

Several limitations of this study need to be discussed. Firstly, although the use of multistage models produces a number as simple, and concrete result, its exact biological meaning is complex and remains hard to interpret. For instance, multistage models reflect the notion and the trajectory of a single cell or cell lineage to become malignant in several rate limiting steps in cancer research. However, the biological unit and meaning of these independent steps is more variable in the case of AD, as indeed it is for other neurodegenerative diseases such as amyotrophic lateral sclerosis. This could for instance reflect an essential pathophysiological change in a single neurovascular unit, but could also relate to a key genetic mutation in a single cell or cell lineage. Secondly, the underlying multistage model assumes that disease development is predominantly genetically determined. This means that a certain number of steps, all with a similar exposure time, have to occur before the specific disease manifests clinically. In most instances, this means that the exposure under study must be present at birth or during an individual's early life, such as their genes, ethnicity, sex, or environmental factors present from birth onwards. This leaves little room for the incorporation of environmental factors that start later in life, such as smoking. While AD has a strong genetic component,[2] the importance of lifestyle and environmental factors is also substantial.[26,40] These factors remain however in part unaddressed in the current multistage models. Some studies in cancer epidemiology have tried to model these effects in more complex multistage models, but the results of these models turned out to be difficult to interpret and are currently poorly validated.[33] Since this is the first application of the multistage modelling in AD, we relied on a simpler, yet widely used multistage model. Future research is encouraged to incorporate

(time-varying) extensions with environmental and lifestyle factors. Thirdly, results derived from exploratory analyses amongst participants either homo- or heterozygous for *APOE* ε4 should be interpreted with caution as these analyses are based on relatively small sample sizes. Fourthly, due to various reasons including for instance selection bias, the presented frequencies of homo- and heterozygous carriers for the *APOE* ε4 allele in this population-based cohort study (2.8% homozygous, 25.8% heterozygous), may differ from those in the unselected general population.[41] Nevertheless, the frequencies in this study fell within the reported ranges from several other, large population-based cohort studies (**Supplementary Table 2**). Finally, estimates of multistage models are vulnerable for several artificial influences on the observed incidence patterns, such as community-wide disease screening programs or misclassification of diagnoses at high ages due to restrained diagnostic work-ups.[42] For some diseases, this subsequently could influence the estimation of the slope and thus the number of steps needed for disease onset. We nevertheless minimised these effects by using a cohort study with standardised and consistent AD ascertainment over time with virtually complete follow-up (>95% of potential person-years).

In conclusion, we found that AD complies with a multistage model characterised by 14 steps that include essential facets of biological change which are required before AD becomes clinically apparent. Moreover, we observed that individuals with a higher genetic susceptibility require less of these additional steps before disease manifests clinically. Future research is warranted to validate the number of steps, to study the effects of environmental and lifestyle factors, and to further investigate the processes underlying these rate-limiting steps. These findings could further increase the understanding of the pathogenesis of AD, which in turn could benefit the development of prevention and treatment strategies.

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Supplementary Table 1 Genetic variants included in the genetic risk score.

Chr	Rs-id	ALT-HRC	Assigned-gene	Locus discovered in	Effect estimate	Maf	Weight ALT-HRC	R^2-RS-I	R^2-RS-II	R^2-RS-III
19	rs4147929	G	ABCA7	Hollingworth et al., Naj et al.	Lambert et al. (2013)	0.19	-0.135	0.916	0.917	0.991
2	rs6733839	T	BIN1	Seshadri et al.	Lambert et al. (2013)	0.409	0.188	0.960	0.911	0.962
20	rs7274581	C	CASS4	Lambert et al. (2013)	Lambert et al. (2013)	0.083	-0.139	0.990	0.989	0.990
6	rs10948363	G	CD2AP	Hollingworth et al., Naj et al.	Lambert et al. (2013)	0.266	0.098	0.998	0.998	0.998
11	rs10838725	C	CELF1	Lambert et al. (2013)	Lambert et al. (2013)	0.316	0.075	0.998	0.998	0.998
8	rs9331896	T	CLU	Harold et al., Lambert et al. (2009)	Lambert et al. (2013)	0.379	0.146	0.902	0.974	0.901
1	rs6656401	G	CR1	Lambert et al. (2009)	Lambert et al. (2013)	0.197	-0.157	0.953	0.948	0.950
10	rs7920721	G	ECHDC3	Desikan et al.	Desikan et al.	0.387	-0.067	1.000	1.000	1.000
7	rs11771145	A	EPHA1	Hollingworth et al., Naj et al.	Lambert et al. (2013)	0.338	-0.102	0.998	0.998	0.999
14	rs17125944	C	FERMT2	Lambert et al. (2013)	Lambert et al. (2013)	0.092	0.122	1.000	1.000	1.000
6	rs111418223	A	HLA-DRB1/5	Lambert et al. (2013)	Lambert et al. (2013)	0.276	-0.108	0.314	0.312	0.314
4	rs13113697	G	HS3ST1	Desikan et al.	Desikan et al.	0.283	-0.067	0.999	0.998	0.999
2	rs35349669	T	INPP5D	Lambert et al. (2013)	Lambert et al. (2013)	0.488	0.066	0.975	0.973	0.976
17	rs118172952	G	KANSL1	Jun et al.	Lambert et al. (2013)	0.873	-0.151	0.710	0.700	0.708
5	rs190982	A	MEF2C	Lambert et al. (2013)	Lambert et al. (2013)	0.408	0.080	0.979	0.934	0.978

Ordered by assigned gene name. Minor allele Frequency (Maf) of Rotterdam Study (RS)-I is shown and is representative of the MAF in RS-II and RS-III. R^2 = imputation quality. RS-I = initial Rotterdam Study cohort, RS-II = first extension Rotterdam Study, RS-III = second extension. Rotterdam Study cohorts were imputed separately. Gene names are ncbi gene names assigned to the loci in the corresponding references. References are shown on the next page.

Supplementary Table 1 Genetic variants included in the genetic risk score (continued).

Chr	Rs-id	ALT-HRC	Assigned-gene	Locus discovered in	Effect estimate	Maf	Weight ALT-HRC	R^2-RS-I	R^2-RS-II	R^2-RS-III
11	rs983392	G	MS4A6A	Hollingworth et al., Naj et al.	Lambert et al. (2013)	0.403	-0.108	0.989	0.990	0.991
7	rs2718058	G	NME8	Lambert et al. (2013)	Lambert et al. (2013)	0.373	-0.070	1.000	1.000	1.000
11	rs10792832	G	PICALM	Harold et al.	Lambert et al. (2013)	0.358	0.130	0.999	0.999	0.999
8	rs28834970	C	PTK2B	Lambert et al. (2013)	Lambert et al. (2013)	0.366	0.096	0.993	0.990	0.994
14	rs10498633	T	SLC24A4-RIN3	Lambert et al. (2013)	Lambert et al. (2013)	0.217	-0.104	0.999	0.999	1.000
11	rs11218343	C	SORL1	Lambert et al. (2013)	Lambert et al. (2013)	0.039	-0.270	0.998	0.995	0.998
6	rs75932628	T	TREM2	Guerreiro et al., Jonsson et al.	Ruiz et al.	0.0016	0.889	0.762	0.726	0.668
7	rs1476679	T	ZCWPW1	Lambert et al. (2013)	Lambert et al. (2013)	0.287	0.078	0.995	0.996	0.995

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Supplementary Table 2 Frequency of *APOE* alleles in study population.

APOE genotype	Study population (N=9362)
ε2/ε2	63 (0.7)
ε2/ε3	1208 (12.9)
ε2/ε4	250 (2.7)
ε3/ε3	5467 (58.4)
ε3/ε4	2161 (23.1)
ε4/ε4	213 (2.8)

Data are presented as number (percentage) of participants.
APOE = apolipoprotein E.

Chapter 9

Mild cognitive impairment and dementia show contrasting associations with cancer

van der Willik KD, Ruiter R, Wolters FJ, Ikram MK, Stricker BHCh, Hauptmann M, Compter A, Schagen SB, Ikram MA

ABSTRACT

Background To investigate and to compare the relation between dementia and cancer with the association between mild cognitive impairment (MCI) and cancer.

Methods A total of 13 207 persons from the Rotterdam Study were followed between 1989 and 2013 for the onset of dementia and cancer (sample 1). Between 2002 and 2005, a subset of 5181 persons underwent extensive cognitive testing for MCI and were subsequently followed up for cancer until 2013 (sample 2). We used Cox proportional hazards models to determine the association between dementia and cancer, and MCI and cancer.

Results In sample 1, 1404 patients were diagnosed with dementia, and 2316 developed cancer (63 among dementia cases). Dementia was associated with a decreased risk of cancer (hazard ratio [HR] 0.53, 95% confidence interval [CI] = 0.41 to 0.68). In sample 2, 513 persons were diagnosed with MCI and 670 persons developed cancer (81 among MCI cases). In contrast to individuals with dementia, those with MCI tended to have an increased risk of cancer (HR 1.25, 95% CI = 0.99 to 1.58).

Conclusions We found that persons with MCI tended to have an increased risk of cancer, whereas patients with dementia had a decreased risk of cancer. These findings call into question a biological explanation for the inverse link between dementia and cancer, thereby suggesting the presence of methodological bias.

INTRODUCTION

Dementia, including Alzheimer's disease (AD), and cancer are global health priorities. Interestingly, several studies have consistently shown an inverse link between the two diseases. Patients with dementia have a decreased risk of cancer,[1-7] while persons with a history of cancer are affected less often from subsequent dementia.[1,2,4,5,8]

Different biological mechanisms underlying this inverse association have been proposed, including pathways of cell proliferation and cell survival.[9,10] In addition, epigenetic processes including DNA methylation have been considered contributing to this inverse association. Yet, patients with dementia are less likely to be screened for other diseases and have a limited life expectancy, both potentially contributing to a decreased subsequent incidence of cancer. Therefore, methodological bias, such as surveillance and survival bias, possibly explaining the inverse link between dementia and cancer has so far not been satisfactory ruled out.

If indeed a biological mechanism underlies the association between dementia and cancer, this would likely extend across the different preclinical stages of cognitive impairment. Mild cognitive impairment (MCI) is often considered the transitional stage between normal cognition and dementia, although not all cases of MCI ultimately lead to dementia.[11,12] As such, it is considered an early clinical manifestation of the same pathological processes that underlie dementia and AD. Accordingly, we hypothesised that if the inverse link between dementia and cancer is truly biologically determined, this should be also reflected in the association between MCI and cancer.

We therefore investigated and compared the association between dementia and cancer with the association between MCI and cancer.

METHODS

Setting

This study is embedded in the Rotterdam Study, a population-based prospective cohort that started in 1989 in the Netherlands. The initial cohort (RS-I) consisted of 7983 participants (78% of invitees) aged 55 years or older residing in the district Ommoord in Rotterdam. The second cohort (RS-II) started in 2000 and was composed of 3011 participants (67% of invitees) in the same district who had turned 55 years or moved into this area. The third cohort (RS-III) was started in 2006, in which 3392 participants (65% of invitees) were included. The design of the

Rotterdam study has been described in detail previously.[13]

The Rotterdam Study was approved by the Medical Ethics Committee of Erasmus Medical Centre and by the board of The Netherlands Ministry of Health, Welfare, and Sports. A written informed consent was obtained from all participants.

Study population

For the current study, two partly overlapping samples from the Rotterdam Study were defined. First, in sample 1, we investigated the association between dementia and risk of cancer, using dementia as a time-varying exposure. This analysis used the complete sample and follow-up of the Rotterdam Study. Of 14 926 study participants, we excluded patients with prevalent dementia (n=527), participants who were not sufficiently screened for dementia (n=743), and participants with prevalent cancer (n=449), leaving a total of 13 207 persons (**Figure 1A**).

Second, in sample 2, we investigated the association between MCI and risk of cancer, using MCI at a single assessment, since assessment of incident MCI is more difficult than incident dementia in a population-based setting due to limited information about the date of onset. This sample originated from the fourth follow-up round of RS-I, the second round of RS-II, and the first round of RS-III. In total 9065 participants were assessed for MCI, of whom we excluded patients with prevalent dementia (n=124), persons not sufficiently screened for dementia (n=283), not sufficiently screened for MCI (n=326), or aged below sixty years (n=2599). In addition, participants with prevalent cancer (n=214) or incident cancer before MCI assessment (n=338) were excluded, resulting in 5181 participants for the MCI analysis (**Figure 1B**). To enhance comparability between the analyses for dementia and MCI, we additionally performed a comparative analysis between dementia and cancer in sample 2 by using a single assessment of prevalent dementia (n=124). Persons with MCI (n=513) were excluded for this analysis (**Figure 1B**).

Ascertainment of incident dementia

Participants were screened for dementia at baseline and subsequent centre visits with the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level.[14] Those with a MMSE score <26 or GMS score >0 underwent further investigation and informant interview, including the Cambridge Examination for Mental Disorders of the Elderly. During each centre visit, all participants also underwent routine cognitive assessment, including a verbal fluency test (Word Fluency Test [WFT], animal categories), Letter-Digit Substitution Task (LDST), Stroop Test, Purdue Pegboard Test, and 15-Word Learning Test (15-WLT). In addition, the entire cohort was continuously under surveillance for dementia through electronic linkage of the study database with medical records from general practitioners and the regional institute for outpatient

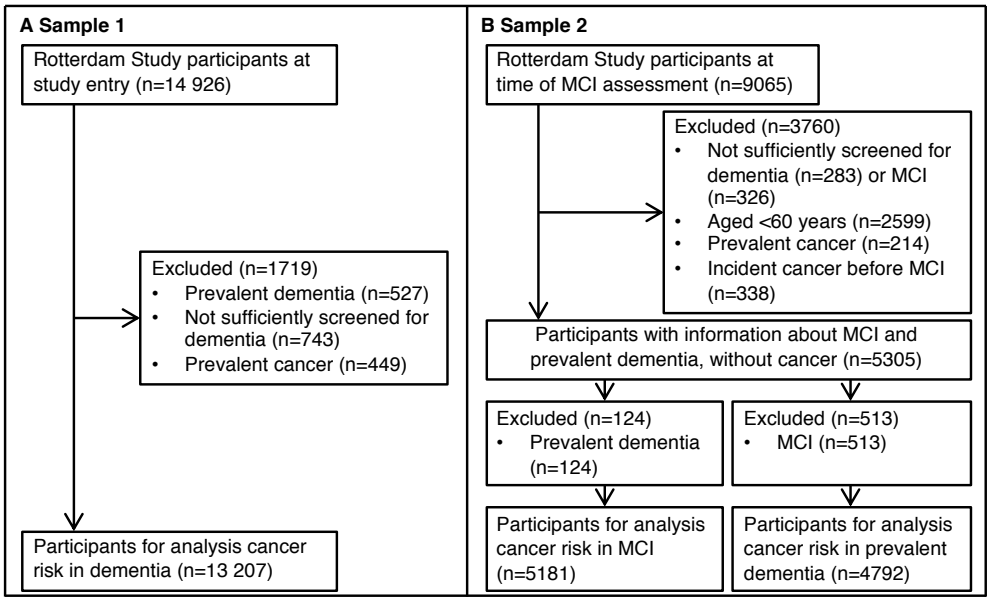

Figure 1 Flowchart of participants in sample 1 and 2.
The association between incident dementia and cancer was studied in sample 1. For sample 1, all participants of the Rotterdam Study were included at study entry, that is, the first rounds of the first (RS-I), second (RS-II), and third cohort (RS-III). In total, sample 1 consisted of 13 207 participants. Sample 2 originated from the fourth follow-up round of RS-I, the second round of RS-II, and the first round of RS-III. In this sample, the association between MCI and cancer was investigated after excluding participants with prevalent dementia, since absence of dementia is part of the definition of MCI. In addition, a comparative analysis was performed in sample 2 investigating the risk of cancer in patients with prevalent dementia. For this comparative analysis, persons with MCI were excluded.
MCI = mild cognitive impairment.

mental health care. Available information on clinical neuroimaging was used when required for diagnosis of dementia subtype. A consensus panel led by a consultant neurologist established the final diagnosis according to standard criteria for dementia (DSM-III-R, Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised), AD (NINCDS-ADRDA, National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association), and vascular dementia (NINDS-AIREN, National Institute of Neurological Disorders and Stroke and the Association Internationale pour la Recherché et l'Enseignement en Neurosciences). Follow-up until January 1st, 2013 was virtually complete (92.4% of potential person-years).

Assessment of MCI

Extensive cognitive testing for MCI assessment was implemented in the Rotterdam Study between 2002 and 2005, which encompasses the fourth examination round of RS-I, the

second examination round of RS-II, and the first examination round of RS-III. MCI was defined as the presence of self-perceived cognitive complaints (defined as at least one of six questions on memory and daily functioning) and cognitive impairment as assessed with neuropsychological tests in the absence of dementia, in persons aged at least 60 years.[15] The neuropsychological tests measured memory function (15-WLT: Immediate and Delayed recall), information-processing speed (LDST, Stroop: Reading and Naming subtask), and executive functioning (LDST, Stroop: Interference subtask, and WFT).

MCI was classified as amnestic (impaired scores on memory function irrespective of other domains) and non-amnestic MCI (normal memory function but impaired score on information-processing speed or executive function).

Assessment of incident cancer

The primary outcome of interest was incidence of cancer. Two research physicians independently assessed the diagnosis of cancer based on medical records obtained through general practitioners and hospital discharge letters. Additional information was collected through linkage with the Dutch Hospital Data, Netherlands Cancer Registry, and Dutch pathology database (PALGA). Only cases confirmed by pathology were used. Cancer was classified according to the International Classification of Diseases tenth edition. In case of discrepancy, consensus was sought through consultation with a cancer epidemiologist. Follow-up of cancer registration was completed up to January 1st, 2013. Non-melanoma skin cancers (NMSC) were not included in the definition of cancer for the analysis.

Other assessments

Baseline was study entry for sample 1 and time of MCI assessment for sample 2. Educational level (primary: primary education, lower: lower general education, intermediate general education, or lower vocational education, intermediate: intermediate vocational education or higher general education, or higher: higher vocational education or university), smoking status (current, former, or never), alcohol use (yes or no), and psycholeptic drug use (yes or no) were assessed at baseline by interview. Body mass index (BMI, kg/m^2) was computed from measurements of height and weight.

Statistical analysis

Cox proportional hazards models were used to study the association between incident dementia and cancer in sample 1. Dementia was used as time-varying variable. In sample 2, we used Cox proportional hazards models investigating the relation between MCI and cancer. All analyses were adjusted for age (continuous), sex, BMI (continuous), educational

level, smoking status, alcohol use, and psycholeptic drug use. Ethnicity was not used as a confounder since nearly all participants (98%) were of European descent. Follow-up time started from inclusion in the Rotterdam Study until the date of incident cancer, death, loss to follow-up, or January 1st, 2013, whichever came first. Censoring non-exposed participants at date of death allowed us to compute cause-specific hazard ratios (HRs). To minimise the potential impact of pre-existing subclinical malignancy on cognition (i.e., reverse causation), we repeated analyses after excluding the first two and five years of follow-up. This was performed in sample 1 by excluding the first two and five years following study entry for persons free of dementia, and the first two and five years after dementia diagnosis for dementia patients.[16,17] In sample 2, the first two and five years after baseline were excluded for both persons with and without MCI. Additionally, we explored effect modification by stratifying for age, sex, and smoking status. In sensitivity analyses, we repeated the analyses using age instead of follow-up time as time scale. The proportional hazards assumption was checked by visual inspection of the Schoenfeld residuals.

To enhance comparability between the dementia and MCI analyses, we performed a Cox proportional hazard analysis in sample 2 to study the risk of cancer in patients with prevalent dementia. In this sample, we additionally censored follow-up time at date of NMSC, stroke, or dementia diagnosis, limiting the effect of possible over- or underdiagnoses of cancer after these conditions.

Finally, direct comparison of the risk of cancer between dementia and MCI was performed by testing whether the HRs of cancer for dementia in sample 1 and 2 differed from the HR of cancer for MCI in sample 2 using a *t* test.

Missing covariates were imputed using the mean of five imputations based on the investigated covariates and outcome. All analyses were performed using IBM SPSS Statistics Version 21.0 and the 'survival' package in RStudio Version 3.3.2.

RESULTS

Table 1 shows the characteristics of the study population. Persons who developed dementia during follow-up were at baseline older, were more often women, had a lower BMI, had more often a primary or lower educational level, and less often an intermediate or higher educational level than participants who were not diagnosed with dementia during follow-up. Additionally, persons who developed dementia were less frequently smokers and alcohol users, and used less often psycholeptic drugs. Participants with MCI were older, were more often men, had a

Table 1 Characteristics of study population for dementia and MCI.

	Sample 1		Sample 2	
Characteristic	No dementia (N=11 803)	Dementia (N=1404)	No MCI (N=4668)	MCI (N=513)
Age, years, median (IQR)	61.8 (57.6 to 70.2)	73.2 (67.2 to 79.0)	69.5 (64.1 to 75.8)	72.0 (65.9 to 78.6)
Women, No. (%)	6676 (56.6)	996 (70.9)	2732 (58.5)	265 (51.7)
Body mass index, kg/m^2, mean (SD)	26.9 (4.0)	26.5 (3.6)	27.7 (4.1)	27.8 (4.0)
Educational level, No. (%)				
Primary	1869 (15.8)	430 (30.6)	488 (10.5)	97 (18.9)
Lower	4743 (40.2)	603 (42.9)	2087 (44.7)	214 (41.7)
Intermediate	3318 (28.1)	289 (20.6)	1392 (29.8)	143 (27.9)
Higher	1873 (15.9)	82 (5.8)	701 (15.0)	59 (11.5)
Smoking status, No. (%)				
Never	3689 (31.3)	615 (44.8)	1415 (30.3)	143 (27.9)
Former	5395 (45.7)	556 (39.6)	2644 (56.6)	293 (57.1)
Current	2719 (23.0)	233 (16.6)	609 (13.0)	77 (15.0)
No alcohol use, No. (%)	1823 (15.4)	304 (21.7)	628 (13.5)	98 (19.1)
No psycholeptic drug use, No. (%)	10 410 (88.2)	1142 (81.3)	4065 (87.1)	416 (81.1)

IQR = interquartile range, MCI = mild cognitive impairment, N = number of persons, SD = standard deviation.

lower educational level, were less often alcohol users, and used less frequently psycholeptic drugs than participants without MCI.

Dementia and the risk of cancer

In sample 1, 1404 (10.6%) participants were diagnosed with dementia and 2316 (17.5%) participants developed cancer, of whom 63 (4.5%) after a diagnosis of dementia. Those who developed dementia had a median follow-up time of 13.2 years (interquartile range [IQR] 8.8 to 19.0 years), whereas the median follow-up time for participants who were not diagnosed with dementia was 8.4 years (IQR 5.3 to 13.0 years). The most frequently observed cancer sites were colorectal (15.7%), prostate (15.5%), breast (13.9%), and lung (11.9%).

Dementia was associated with a decreased risk of cancer (HR 0.53 [95% confidence interval [CI] = 0.41 to 0.68], **Figure 2**). The risk estimates were similar for AD and vascular dementia. The risk was still reduced after excluding the first two and five years of follow-up time (the respective HRs were 0.44 [95% CI = 0.30 to 0.65] and 0.48 [95% CI = 0.26 to 0.90]). Dementia-related cancer risks did not significantly differ by age, sex, and smoking.

Reduced risks were observed when using age as time scale in the Cox model; for instance, the HR for cancer among dementia patients was 0.61 (95% CI = 0.47 to 0.78).

MCI and the risk of cancer

In sample 2, 513 (9.9%) participants had MCI. Six hundred and seventy (12.9%) participants developed cancer, of whom 81 (12.0%) had MCI. The median follow-up time for persons with MCI was 7.6 years (IQR 4.6 to 8.9 years), and 7.9 years (IQR 5.5 to 9.1 years) for those without MCI. A similar distribution of cancer sites was observed as in sample 1.

Participants with MCI had a borderline statistically significantly increased risk of cancer (HR 1.25 [95% CI = 0.99 to 1.58], **Figure 3**). This increased risk was particularly pronounced for amnestic MCI (HR 1.42 [95% CI = 1.02 to 1.98]). This risk increase was consistent when excluding the first two and five years of follow-up time (the respective HRs were 1.25 [95% CI = 0.95 to 1.66] and 1.73 [95% CI = 1.19 to 2.51]). Risk estimates for younger participants tended to be stronger than for older individuals, but a formal interaction term did not reach statistical significance (*P*=.09). Results were comparable when using age as time scale.

The risk of cancer in patients with prevalent dementia in sample 2 was comparable to the cancer risk after incident dementia in sample 1 (HR 0.47 [95% CI = 0.21 to 1.06], **Table 2**). Risk estimates did not change materially after censoring for NMSC, stroke, or dementia (**Table 2**).

The abovementioned HR of cancer for MCI (1.25) was significantly different from the HR of cancer for dementia in sample 1 (0.53, *P*=.001) and sample 2 (0.47, *P*=.02), that is, persons with MCI had a significantly higher HR for cancer than patients with dementia.

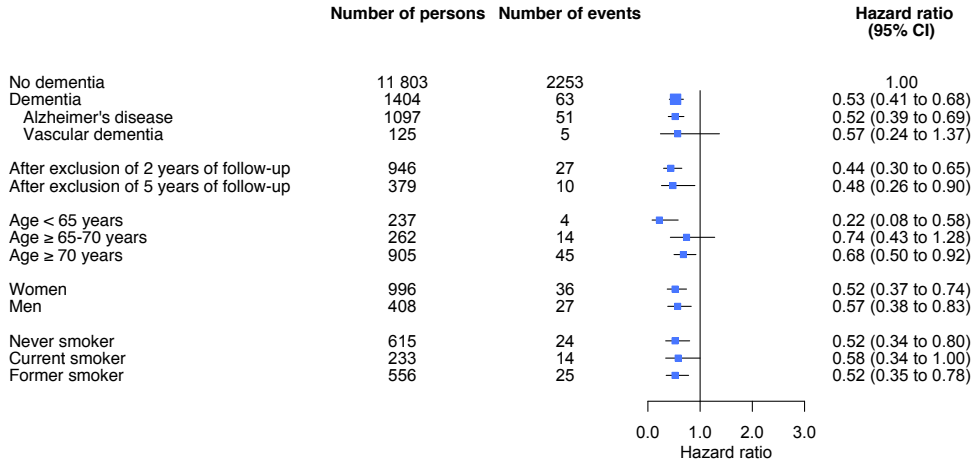

Figure 2 Forest plot dementia and risk of cancer.

Hazard ratios with 95% confidence intervals for the risk of cancer among patients with dementia. Hazard ratios are adjusted for age, sex, body mass index, educational level, smoking status, alcohol use, and psycholeptic drug use. Characteristics are measured at time of study entry.
CI = confidence interval.

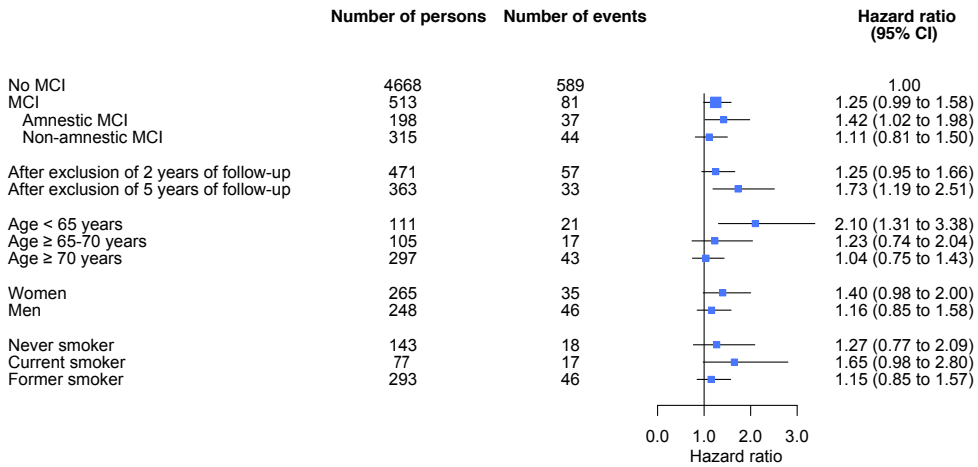

Figure 3 Forest plot MCI and risk of cancer.

Hazard ratios with 95% confidence intervals for the risk of cancer among persons with MCI. Hazard ratios are adjusted for age, sex, body mass index, educational level, smoking status, alcohol use, and psycholeptic drug use. Characteristics are measured at the time of MCI assessment.
CI = confidence interval, MCI = mild cognitive impairment.

Table 2 Risk of cancer in persons with MCI or prevalent dementia at time of MCI assessment.

		Solid and haematological cancer		Solid cancer	
	Number of persons	Number of events	HR (95% CI)	Number of events	HR (95% CI)
MCI and risk of cancer					
No MCI	4668	589	1.00	540	1.00
MCI	513	81	1.25 (0.99 to 1.58)	76	1.29 (1.02 to 1.65)
Censored for NMSC	483	74	1.28 (1.00 to 1.63)	69	1.30 (1.01 to 1.68)
Censored for stroke	454	73	1.36 (1.07 to 1.74)	69	1.42 (1.10 to 1.83)
Censored for dementia	513	73	1.22 (0.95 to 1.56)	68	1.24 (0.96 to 1.60)
Censored for stroke and dementia	454	65	1.32 (1.01 to 1.71)	61	1.36 (1.04 to 1.77)
Prevalent dementia and risk of cancer					
No prevalent dementia	4668	589	1.00	540	1.00
Prevalent dementia	124	6	0.47 (0.21 to 1.06)	5	0.43 (0.18 to 1.05)
Censored for NMSC	121	6	0.49 (0.22 to 1.09)	5	0.45 (0.18 to 1.09)
Censored for stroke	104	6	0.60 (0.27 to 1.35)	5	0.56 (0.23 to 1.35)

Hazard ratios are adjusted for age, sex, body mass index, educational level, smoking status, alcohol use, and psycholeptic drug use.
CI = confidence interval, HR = hazard ratio, MCI = mild cognitive impairment, NMSC = non-melanoma skin cancer.

DISCUSSION

In this population-based cohort study, we found opposite effects of MCI and dementia with respect to subsequent risk of cancer. While we confirmed that persons with dementia had a decreased risk of cancer, those with MCI did not have a decreased risk and even tended to have an increased risk of cancer.

Strengths of our study are its prospective, population-based design, the number of cancer diagnoses, and the standardised ascertainment of the determinants and outcome. Also, by focusing on MCI we were able to reduce the effect of a possible surveillance bias and the decreased life expectancy in dementia patients. In addition, we excluded the first two and five years of follow-up time in order to limit reverse causality.

Our study has some limitations. First, the cognitive tests for assessing MCI were implemented between 2002 and 2005, precluding MCI assessment at baseline for RS-I and RS-II. To increase the comparability between the samples used for analyses of dementia and MCI, we investigated the risk of cancer in patients with prevalent dementia in the same sample as the MCI-analysis and we found similar results to the overall population. Second, we did not have baseline information about potential confounders such as depressive and anxiety disorders, which could have resulted in an overestimation of the observed associations between dementia and cancer, and MCI and cancer. Third, the Rotterdam Study includes mostly white, middle class persons, possibly limiting the generalisability of our findings to other ethnic and socioeconomic groups. Finally, patients with AD represented the largest group of people with dementia. Therefore, we were not able to reliably study the association with cancer for other dementia types.

We found that dementia is associated with a decreased risk of cancer, which is in line with findings from previous studies for various cancer types including breast, prostate, colon, and NMSC.[1-8,18] Various biological mechanisms have been proposed, and the most frequently postulated mechanisms are to do with a genetic predisposition for either promoting or suppressing metabolic survival or apoptotic cellular pathways.[10] For instance, the tumour suppressor protein p53 induces apoptosis in the face of DNA damage, which protects against cancer, while in dementia, it could induce neuronal death.[19] Methodological explanations – like surveillance and survival bias – could also have accounted for the observed inverse relation, but these have not been sufficiently ruled out. As MCI is often considered an early manifestation of the same pathological processes as dementia and AD, we investigated the risk of cancer among persons with MCI. We argued that if the inverse link between dementia and cancer is rooted in biology, this decreased risk would be reflected in persons with MCI as well. One previous study has looked into history of cancer among persons with and without MCI and has shown that 31% of the persons with MCI were previously diagnosed with cancer.[8] However, no longitudinal analysis was performed in this group of persons and the risk of cancer after MCI or dementia diagnosis was not investigated.

In contrast to the decreased risk of cancer observed in our patients with dementia, we found that MCI was associated with an increased risk of cancer, which was borderline significant. Importantly, the difference between the risk of cancer after dementia and MCI was statistically significant. Before interpreting our results further, a word of caution is warranted. A basic premise of our study is that MCI and dementia share the same pathological underpinnings.[11] We do emphasise though that only half of MCI patients convert to dementia over a five year period with the other half remaining stable or even reverting back to normal, suggesting that the underlying pathology between MCI and dementia does not entirely overlap.[11,12] Nevertheless,

two observations in our study support our basic premise in interpreting our findings. First, we found stronger effects for amnestic MCI than non-amnestic MCI. Indeed, amnestic MCI is more closely linked to AD pathology than non-amnestic MCI.[15] Second, censoring for dementia did not materially change the risk of cancer after MCI – if anything, the risk slightly attenuated. This suggests that those persons with MCI that went on to develop dementia (i.e., those that were censored) actually had an even higher risk of cancer than those with MCI that did not develop dementia.

The inverse link between dementia and cancer is often linked to genes involved in pathways with opposite effects in dementia and cancer. Our findings, however, point towards biological mechanisms with similar effects in both diseases. Several processes including angiogenesis, inflammation, and oxidative stress have proven to be important for tumorigenesis and there is increasing evidence that these processes also have a prominent role in the pathophysiology of AD.[20,21] For instance, different inflammatory biomarkers are elevated in both MCI and dementia, suggesting a chronic inflammatory state.[22] Inflammatory cells can promote tumour cell growth, facilitate genomic instability, and influence tumour cell migration, and many chronic inflammatory conditions are associated with cancer.[23] Further, tumour cells can produce various cytokines and chemokines, such as tumour necrosis factor-alpha, interleukins, and interferons, to attract leukocytes and enhance inflammation. This shows that dementia and cancer could be parallel processes as a result of inflammation. In addition, several proteins are involved in the pathogenesis of both dementia and cancer. For instance, AD is characterised by the accumulation of plaques containing amyloid-beta (Aβ) peptide within the brain. It has been shown that plasma levels of Aβ40 and Aβ42 are increased in cancer patients.[24] Also, Aβ precursor protein can promote cell proliferation and is increased in different types of cancer, suggesting a potential role for Aβ in cancer.[25] Finally, there has been increasing evidence that patients with cancer have lower cognitive function and differences in brain structure prior to cancer treatment compared to persons without cancer, indicating continuity with dementia rather than an inverse association.[26,27] Investigation of the risk of MCI in cancer patients would therefore be very interesting, although appropriate methods should be used to deal with same potential biases as in the current study.

In conclusion, this is the first study to show that persons with MCI do not have a decreased risk of cancer as observed in patients with dementia, and even tended to have an increased risk. This suggests that the previously reported inverse link between dementia and cancer is based on methodological limitations. Future studies should further verify our observations and seek to elucidate the underlying shared – instead of opposite – mechanisms between dementia and cancer. Clinically, our findings imply that for persons presenting with dementia, treating physicians should be aware of their increased risk of cancer.

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Chapter 10

Higher plasma amyloid-β levels are associated with a higher risk of cancer

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ABSTRACT

Background Various studies have shown an inverse relation between Alzheimer's disease (AD) and cancer, but findings are likely to be biased by surveillance and survival bias. Plasma amyloid-β (Aβ) is defined as a preclinical feature of AD, with lower levels of Aβ42 being associated with a higher risk of AD. To get more insight into the biological link between AD and cancer, we investigated plasma Aβ levels in relation to the risk of cancer.

Methods Between 2002 and 2005, we measured plasma Aβ40 and Aβ42 levels in 3949 participants from the population-based Rotterdam Study. These participants were followed for the onset of cancer, all-cause dementia, death, loss to follow-up, or January 1st, 2014, whichever came first. We used Cox proportional hazards models to investigate the association between plasma Aβ40 and Aβ42 levels, and the risk of cancer. Analyses were stratified by cancer site.

Results During a median (interquartile range) follow-up of 9.0 years (6.9 to 10.1), 560 participants were diagnosed with cancer. Higher levels of $\log_2$ plasma Aβ40 and Aβ42 were associated with a higher risk of cancer (hazard ratio [95% confidence interval] per standard deviation increase for Aβ40 = 1.12 [1.02 to 1.23] and Aβ42 = 1.12 [1.03 to 1.23]). These effect estimates were most pronounced for haematological cancers, urinary tract cancers, and cancers of unknown primary origin.

Conclusions We found that higher levels of both plasma Aβ40 and Aβ42 were associated with a higher risk of cancer. This suggests a potential biological link between AD and cancer. The pathophysiological role of Aβ in cancer and its causality warrant further investigation.

INTRODUCTION

Alzheimer's disease (AD) and cancer are common diseases in the elderly population that pose a high burden of morbidity and mortality on societies.[1,2] Various observational studies have suggested that patients with AD have a lower risk of non-central nervous system (CNS) cancer and vice versa,[3-7] which was not driven by a specific cancer type. However, methodological issues including surveillance and survival bias may drive the association towards an inverse direction.[8] In fact, recent evidence points towards the possibility of a positive link,[9,10] which is supported by overlapping risk factors for AD and cancer, such as age and smoking, and by overlapping pathways, including genome instability and inflammation.[11] Against this background, we recently showed that persons with mild cognitive impairment (MCI), a preclinical stage of AD, tended to have a higher risk of cancer (hazard ratio [HR] = 1.25 [95% confidence interval [CI] = 0.99 to 1.58]). This risk was statistically significantly higher than the decreased risk of cancer of patients with AD (HR = 0.52 [95% CI = 0.39 to 0.69]).[10]

Investigating the preclinical stage of AD and linking it to cancer could further unravel the association between these diseases. Accumulation of plaques containing amyloid-β (Aβ) in the brain is a defining feature of AD pathology.[12] Aβ is currently the earliest detectable pathological change in the preclinical stage of AD[13] and can be measured non-invasively in plasma. Although endothelial cells of blood vessels and platelets are the main source of circulating Aβ,[14] Aβ is also produced by neuronal cells and is subsequently transported across the blood-brain barrier.[15] During the earliest stages of AD, neuronal Aβ production is first increased, resulting in higher plasma Aβ levels. Plasma Aβ40 and Aβ42 levels decrease during the incipient clinical phase of the disease as a result of Aβ deposition in the brain.[16,17] A substantial amount of Aβ accumulates before the manifestation of clinical symptoms – thus also before MCI – but the exact onset of deposition is as yet unknown.[13] In turn, lower plasma Aβ42 levels are associated with a higher risk of dementia,[18-21] although not all persons with Aβ accumulation will eventually have clinically manifested dementia.[22] Previous work on Aβ in the oncology field has shown that cancer patients, in particular those with hepatic cancer, have higher plasma Aβ40 and Aβ42 levels than controls.[23] Yet, to understand the role of Aβ in the context of the relation between AD and cancer, it is pivotal to study plasma Aβ before cancer diagnosis.

We hypothesised that if there is a biological link between AD and cancer, this should probably extend through all preclinical stages of AD. The life expectancy of persons in the preclinical stage of AD is longer than that of patients with clinically manifested AD, thereby

limiting the effects of survival bias. We have previously shown in the prospective population-based Rotterdam Study that higher levels of plasma Aβ42 are associated with a lower risk of AD.[21] Given that early-stage AD is characterised by higher plasma Aβ levels, whereas Aβ levels decrease during disease progression, any association between plasma Aβ and cancer may support a biological association between AD and cancer. A positive association might suggest that the very early stage of AD is related to cancer, whereas a negative association might reflect a link between a later preclinical stage of AD and cancer. We therefore determined the association between plasma Aβ40 and Aβ42 levels and the risk of cancer in the Rotterdam Study.

METHODS

Study population

This study is embedded within the Rotterdam Study, a prospective population-based cohort designed to study the occurrence and determinants of diseases in the elderly population. The details of this cohort have been described in detail previously.[24] Briefly, in 1990, after the pilot phase in 1989, all inhabitants aged 55 years and over from the Ommoord area, a suburb of in Rotterdam, the Netherlands, were invited to participate. This initial cohort (RS-I) consisted of 7983 participants and was subsequently extended with a second subcohort (RS-II) in 2000 with 3011 participants who had reached the age of 55 years or moved into the study area. In 2006, the cohort was further expanded (RS-III) with 3932 participants aged 45 years and over. In total, the Rotterdam Study comprises 14 926 participants (overall response rate 72%).

The Rotterdam Study complies with the Declaration of Helsinki and has been approved by the Medical Ethics Committee of Erasmus Medical Centre and by the board of The Netherlands Ministry of Health, Welfare, and Sports. A written informed consent was obtained from all participants.

The population for the current study was defined by availability of -80^{0}C stored plasma samples obtained from participants during the fourth visit of RS-I between January 2002 and July 2004, and the second visit of RS-II between July 2002 and December 2005. From this selection of 5094 participants with plasma samples available, we excluded participants who did not provide informed consent to access medical records and hospital discharge letters during follow-up (n=6), those with a history of cancer at blood sample draw (n=408), participants with a history of all-cause dementia (n=25) or insufficient data to determine their cognitive status (n=1), and participants with missing (n=471) or invalid test results for plasma Aβ40 and Aβ42

levels (n=234). Missing and invalid test results were missing at random. As a result, the final sample included 3949 participants for analyses.

Assessment of plasma Aβ40 and Aβ42

Blood samples of participants were collected during the research centre visit. Blood was sampled in EDTA-coated tubes and centrifuged, of which subsequently plasma was aliquoted and frozen at -80^{0}C according to standard procedures. Measurements of plasma Aβ levels were performed at Quanterix (Lexington, MA, USA) on a Simoa HD-1 analyser platform using the Simoa Human Neurology 3-Plex A assay.[25] Measurements were done in two separate batches and samples were tested in duplicate. Two quality control samples were run on each plate for each analyte. When duplicate measurements were missing (e.g. blood sample was not good) or inconsistent, or if the concentration coefficient of variation exceeded 20%, participant's samples were not valid and were therefore not included.

Assessment of cancer

Registration of prevalent and incident cancer diagnoses was based on medical records of general practitioners (including hospital discharge letters) and through linkage with the Netherlands Cancer Registry, Dutch Hospital Data, and histology and cytopathology registries in the region (PALGA). Each diagnosis of cancer was coded independently by two physicians and classified according to the International Classification of Diseases, 10th revision. In case of discrepancy between the two physicians, consensus was sought through consultation with a physician specialised in internal medicine. Cancer diagnosis was defined as any primary malignant tumour that was confirmed by pathology, excluding non-melanoma skin cancer. Date of diagnosis was based on date of biopsy for solid tumours, laboratory assessment for haematological tumours, or – if information on these dates was unavailable – date of hospital admission or hospital discharge letter. Follow-up of cancer registration was completed up to January 1st, 2014. Only non-CNS cancers were included in the analysis.

Other assessments

Participants provided information on educational level, smoking habits, and alcohol use during the home interview. Educational level was classified into primary, lower (lower or intermediate general education or lower vocational education), intermediate (intermediate vocational education or higher general education), or higher (higher vocational education or university). Smoking habits were classified into never, current, or former smoker. Alcohol use was categorised as any use or no use.

Height and weight were measured at the research centre from which the body mass

index (kg/m^2) was computed. Hypertension was defined as a systolic blood pressure ≥140 mm Hg, a diastolic blood pressure ≥90 mm Hg, or use of antihypertensive medication.[26] Hypercholesterolaemia was defined as a serum total cholesterol >6.5 mmol/L or use of lipid-lowering medication. Diabetes mellitus was defined as a fasting serum glucose level ≥7.1 mmol/L, a random serum glucose level ≥11.1 mmol/L, or use of antidiabetic medication.[27] Symptoms of depression were assessed using the Centre for Epidemiologic Studies Depression scale (CES-D) and were converted into a sum-score. Apolipoprotein E (*APOE)* genotype was determined using polymerase chain reaction on coded DNA samples in the subcohort RS-I and with a bi-allelic TaqMan assay in the subcohort RS-II.[28,29] *APOE* ε4 carrier status was defined as carrier of at least one *APOE* ε4 allele. Granulocyte, lymphocyte, and platelet counts were quantified using the COULTER® Ac·T diff2™ Haematology Analyser (Beckman Coulter, San Diego, California, USA). Granulocyte-to-lymphocyte ratio (GLR) was calculated as the ratio of granulocyte to lymphocyte count and platelet-to-lymphocyte ratio (PLR) as the ratio of platelet to lymphocyte count. Systemic immune-inflammation index (SII) was calculated as platelet count times GLR. Serum creatinine was measured with an enzymatic assay method and was subsequently standardised to isotope–dilution mass spectrometry–traceable measurements.[30]

Incidence of all-cause dementia was evaluated by screening of participants at the research centre visit using the Mini-Mental State Examination and the Geriatric Mental Schedule organic level. Participants with a Mini-Mental State Examination score below 26 or Geriatric Mental Schedule score of at least one underwent further investigation, including the Cambridge Examination for Mental Disorders of the Elderly. In addition, the cohort was electronically linked with medical records from general practitioners and the regional institute for outpatient mental health care to ensure continuous surveillance for all-cause dementia.[31]

Statistical analysis

We used Cox proportional hazards models to obtain HRs and 95% CIs to investigate the association between plasma Aβ40 and Aβ42 levels with risk of non-CNS cancer. Aβ levels were $\log_2$ transformed to reach a normal distribution and were subsequently standardised. The standard deviation (SD) increase in Aβ level on the $\log_2$ scale can then be multiplied with two to obtain the corresponding SD increase in Aβ level on the original scale. As yet, neither reference values for plasma Aβ nor thresholds for preclinical AD are available. We therefore investigated the association with continuous Aβ and Aβ levels divided into quartiles, using two nested models: Model I was adjusted for age at blood sample draw, sex, and assay batch number; Model II was Model I plus additional adjustment for covariates related to both AD and cancer, including education, body mass index, hypertension, hypercholesterolaemia, diabetes

mellitus, smoking status, alcohol use, depression (as measured with CES-D sum-score), and inflammation (using the SII). Because of collinearity, we only adjusted for SII and not for GLR and PLR. Follow-up time was used as time scale. Follow-up time started at date of blood sample draw (i.e., at the fourth visit of participants of RS-I and at the second visit of participants of RS-II) until date of incident cancer, all-cause dementia, death, loss to follow-up, or January 1st, 2014, whichever came first. Participants were censored at date of CNS cancer diagnosis, because we hypothesised that the potential mechanisms underlying any association between Aβ and cancer would differ between non-CNS and CNS cancers, given that CNS cancer can cause direct damage to the brain.[32] We repeated analyses using age as time scale instead of follow-up to verify that the choice of the time scale did not affect the results. We checked the proportional hazards assumption by visual inspection of the Schoenfeld residuals.

To further evaluate the robustness of our findings, we performed two sensitivity analyses: (i) adjusting for creatinine to minimise the effect of impaired kidney function, because plasma Aβ is partly cleared by the kidney; and (ii) excluding the first two and five years of follow-up time to examine reverse causation (i.e., the possible effect of subclinical cancer on plasma Aβ). In this latter analysis, only those participants with a follow-up time of more than, respectively, two and five years were included.

We studied effect modification for median age, sex, education, smoking status, *APOE* ε4 carrier status, and inflammatory ratios by stratification and by adding multiplicative interaction terms to the model. Lastly, we stratified analyses by primary cancer site.

We used multiple imputation for missing covariates (maximum of 3.2%), with five imputed datasets based on the covariates and outcome. We used Rubin's method for pooled HRs and 95% CIs.[33] Statistical analyses were performed using the R package 'survival' in RStudio Version 3.3.2.[34]

RESULTS

Characteristics of the study population are presented in **Table 1**. The median (interquartile range [IQR]) age at blood sample draw was 70.4 years (65.8 to 76.4) and 57.7% were women. During a median (IQR) follow-up of 9.0 years (6.9 to 10.1), 560 out of 3949 participants were diagnosed with cancer. In the same follow-up period, 303 participants developed all-cause dementia (of whom 247 with AD) and 712 participants died. Most frequently diagnosed cancer sites were breast (30.4% among women), male genital organs (29.1% among men), colorectal (16.3%), and lung (14.5%).

Table 1 Characteristics of the study population.

Characteristic	Included participants (N=3949)	Excluded participants (N=705)*
Age, years, median (IQR)	70.4 (65.8 to 6.4)	75.6 (69.7 to 80.9)
Women, No. (%)	2277 (57.7)	442 (62.7)
Educational level, No. (%)		
Primary	421 (10.7)	118 (16.7)
Lower	1711 (43.3)	318 (45.1)
Intermediate	1200 (30.4)	194 (27.5)
Higher	557 (14.1)	69 (9.8)
Body mass index, kg/m^2, mean (SD)	27.6 (4.1)	27.6 (4.4)
Hypertension, No. (%)	3091 (78.3)	595 (84.4)
Hypercholesterolaemia, No. (%)	1580 (40.0)	263 (37.3)
Diabetes mellitus, No. (%)	421 (10.7)	100 (14.2)
Smoking, No. (%)		
Never	1159 (29.3)	221 (31.3)
Former	2222 (56.3)	394 (55.9)
Current	491 (12.4)	70 (9.9)
Alcohol use, No. (%)	3319 (84.0)	565 (80.1)
CES-D sum-score, median (IQR)	3 (1 to 8)	5 (1 to 10)
APOE ε4 carrier, No. (%)	1044 (26.4)	186 (26.4)
Inflammatory ratios, median (IQR)		
Granulocyte-to-lymphocyte ratio	1.8 (1.4 to 2.4)	2.0 (1.5 to 2.5)
Platelet-to-lymphocyte ratio	120 (96 to 153)	128 (100 to 161)
Systemic immune-inflammation index	455 (338 to 621)	497 (355 to 682)
Plasma amyloid-β40, pg/mL, median (IQR)	258 (229 to 291)	
Plasma amyloid-β42, pg/mL, median (IQR)	10.3 pg/mL (8.9 to 11.9)	

Characteristics were measured during the visit of the blood sample draw (i.e., at the fourth visit of participants of RS-I and at the second visit of participants of RS-II). Missing values are not imputed and therefore numbers do not always sum up to 100%.
** Excluded participants in this table only include those participants who were excluded due to missing test results (n=471) or invalid test results (n=234).*
APOE = apolipoprotein E, CES-D = Centre for Epidemiologic Studies Depression scale, IQR = interquartile range, SD = standard deviation.

Higher levels of $\log_2$ plasma Aβ40 and Aβ42 were associated with a higher risk of cancer (HR [95% CI] per SD increase in Aβ40 = 1.12 [1.02 to 1.23] and Aβ42 = 1.12 [1.03 to 1.23], **Table 2**). Given that the unadjusted HR for the risk of cancer per one year increase in age is 1.01 (95% CI = 1.00 to 1.02), the HR per SD increase in Aβ corresponds with a risk increase

Table 2 The association between standardised log_2 transformed plasma amyloid-β40 and amyloid-β42 levels with risk of cancer.

Plasma assessment (pg/mL)*	Cancer		
	n/N	Model I HR (95% CI)	Model II HR (95% CI)
Amyloid-β40			
Per SD increase	560/3949	1.13 (1.03 to 1.24)	1.12 (1.02 to 1.23)
Quartiles (range)			
1st quartile (-8.05 to -0.61)	126/988	1.00	1.00
2nd quartile (-0.61 to -0.01)	138/987	1.10 (0.86 to 1.40)	1.11 (0.87 to 1.42)
3rd quartile (-0.01 to 0.59)	136/987	1.12 (0.87 to 1.43)	1.13 (0.88 to 1.44)
4th quartile (0.59 to 4.24)	160/987	1.47 (1.14 to 1.88)	1.43 (1.11 to 1.83)
P for trend		.004	.005
Amyloid-β42			
Per SD increase	560/3949	1.12 (1.03 to 1.23)	1.12 (1.03 to 1.23)
Quartiles (range)			
1st quartile (-11.4 to -0.53)	124/988	1.00	1.00
2nd quartile (-0.53 to 0.03)	136/987	1.11 (0.87 to 1.42)	1.13 (0.88 to 1.44)
3rd quartile (0.03 to 0.59)	140/987	1.14 (0.89 to 1.45)	1.16 (0.91 to 1.47)
4th quartile (0.59 to 8.46)	160/987	1.38 (1.09 to 1.75)	1.38 (1.09 to 1.76)
P for trend		.009	.009

Hazard ratios in Model I are adjusted for age at blood draw, sex, and assay batch number. Hazard ratios in Model II are adjusted for covariates in model I plus adjustment for education, body mass index, hypertension, hypercholesterolaemia, diabetes mellitus, smoking, alcohol use, CES-D sum-score, and systemic immune-inflammation index.

** Plasma assessments are log_2 transformed and standardised.*

CES-D = Centre for Epidemiologic Studies Depression scale, CI = confidence interval, HR = hazard ratio, n = number of participants with incident cancer, N = total number for participants, SD = standard deviation.

in cancer of at least 10.2 years of age (the unrounded HR for age is 1.0117 and for Aβ40 is 1.1193, thus .1193/.0117 = 10.2). Participants with plasma Aβ40 and Aβ42 levels in the highest quartile had a higher risk of cancer than those with levels in the lowest quartile (HR [95% CI] for Aβ40 = 1.43 [1.11 to 1.83] and Aβ42 = 1.38 [1.09 to 1.76]). Additional adjustment for creatinine did not meaningfully change the estimated HRs (**Table 3**). Also, exclusion of the first two and five years of follow-up time did not affect the risk estimates (**Table 3**).

Stratified analyses showed that the association between plasma Aβ and cancer was more profound in older participants, men, former smokers, participants with an intermediate educational level, and *APOE* ε4 carriers (**Figure 1**). Regarding inflammatory status,

Table 3 Sensitivity analyses for the association between plasma amyloid-β40 and amyloid-β42 levels with risk of cancer.

Plasma assessment (pg/mL)*	Cancer	
	n/N	HR (95% CI)
Additional adjustment for creatinine level (µmol/L)†		
Amyloid-β40	229/1514	1.13 (0.96 to 1.33)
Amyloid-β42	229/1514	1.10 (0.94 to 1.28)
Excluding first two years of follow-up		
Amyloid-β40	438/3672	1.08 (0.97 to 1.20)
Amyloid-β42	438/3672	1.07 (0.97 to 1.18)
Excluding first five years of follow-up		
Amyloid-β40	242/3271	1.12 (0.96 to 1.29)
Amyloid-β42	242/3271	1.15 (1.01 to 1.32)

Hazard ratios are adjusted for age at blood draw, sex, assay batch number, education, body mass index, hypertension, hypercholesterolaemia, diabetes mellitus, smoking, alcohol use, CES-D sum-score, and systemic immune-inflammation index.
** Plasma assessments are log_2 transformed and standardised. † Creatinine levels were measured in a random sample of 1514 out of 3949 participants.*
CES-D = Centre for Epidemiologic Studies Depression scale, CI = confidence interval, HR = hazard ratio, n = number of participants with incident cancer, N = total number for participants.

participants with higher GLR had a higher risk of cancer than those with a lower GLR, which was not observed for PLR and SII (**Supplementary Figure 1**). All interactions were tested on the multiplicative scale and did not reach statistical significance.

Analyses per cancer site showed that the association was most pronounced between plasma Aβ40 and Aβ42 and haematological cancer (HR [95% CI] per SD increase in log_2 Aβ40 = 1.56 [1.12 to 2.17] and Aβ42 = 1.30 [0.94 to 1.79], **Figure 2**). The association was also stronger for cancer of unknown primary origin and cancer in the urinary tract, oesophagus and stomach (only for Aβ40), and head and neck (only for Aβ42), albeit not statistically significantly. In a post-hoc analysis we found a strong association with pancreatic cancer, although the power was limited by the number of participants with pancreatic cancer (n=13, HR [95% CI] per SD increase in log_2 Aβ40 = 1.52 [0.83 to 2.79] and Aβ42 = 1.51 [0.98 to 2.31]).

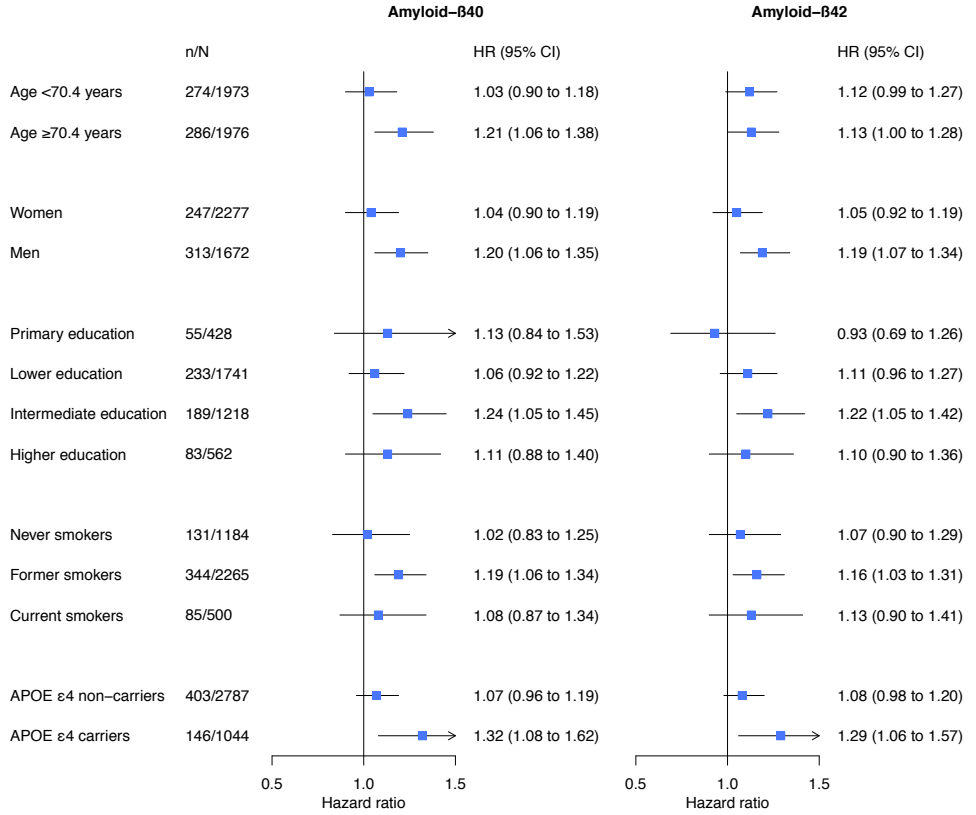

Figure 1 Forest plot of association between $\log_2$ transformed and standardised plasma amyloid-β40 and amyloid-β42 levels with risk of cancer, stratified by median age at blood draw, sex, education, smoking status, and *APOE* ε4 carrier status.

Hazard ratios are adjusted for age at blood draw, sex, assay batch number, education, body mass index, hypertension, hypercholesterolaemia, diabetes mellitus, smoking, alcohol use, CES-D sum-score, and systemic immune-inflammation index. APOE ε4 carrier status was missing for 118 participants. Missing values of education and smoking were imputed and therefore the total number of participants per smoking category is higher than the number of participants presented in Table 1.

APOE = apolipoprotein E, CES-D = Centre for Epidemiologic Studies Depression scale, CI = confidence interval, HR = hazard ratio, n = number of participants with incident cancer, N = total number for participants.

DISCUSSION

In this population-based cohort study, we found that higher plasma levels of both Aβ40 and Aβ42 were associated with a higher risk of cancer. This indicates that Aβ could be involved in the pathophysiology of cancer and may further support a potential biological link between AD and cancer. The direction of this link, that is, inverse or positive, and the causal effect of Aβ on

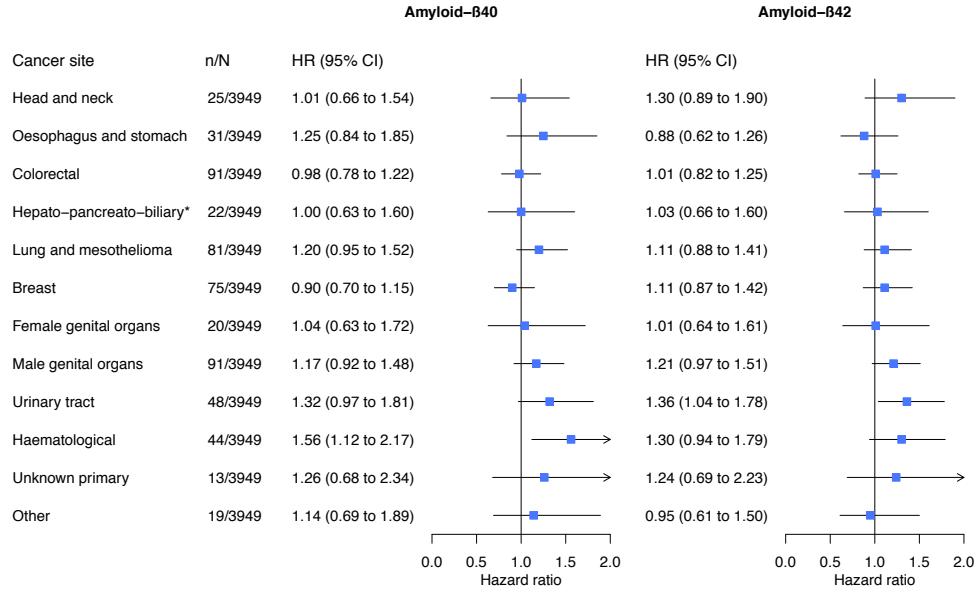

Figure 2 Association between log_2 transformed and standardised plasma amyloid-β40 and amyloid-β42 levels with different cancer types.
Hazard ratios are adjusted for age at blood draw, sex, assay batch number, education, body mass index, hypertension, hypercholesterolaemia, diabetes mellitus, smoking, alcohol use, CES-D sum-score, and systemic immune-inflammation index.
** Thirteen out of 22 participants were diagnosed with pancreatic cancer. Higher levels of amyloid-β40 and amyloid-β42 were associated with a higher risk of cancer (HR for amyloid-β40 = 1.52 [95% CI = 0.83 to 2.79] and amyloid-β42 = 1.51 [95% CI = 0.98 to 2.31]).*
CES-D = Centre for Epidemiologic Studies Depression scale, CI = confidence interval, HR = hazard ratio, n = number of participants with incident cancer, N = total number for participants.

cancer warrant further investigation.

Our finding is in line with a previously conducted cross-sectional study showing that individuals who have been diagnosed with cancer have higher plasma Aβ levels.[23] To further understand this association and its interpretation regarding the link between AD and cancer, it is necessary to first elaborate on the Aβ pathway. Aβ is the product of cleavage of amyloid precursor protein (APP), which is expressed in neuronal and non-neuronal tissues including the kidney, lung, and pancreas. Aβ is formed as product of APP cleavage by α-secretase, followed by β-secretase. APP cleavage can result in different isoforms of Aβ, depending on the number of amino acids (i.e., 38, 40, and 42), with longer isoforms being more prone to aggregation.[35] Although many tissues contain APP, Aβ can only be produced in cells that also express β-secretase, e.g. neuronal cells, muscle cells, platelets, and vascular wall endothelial cells. Plasma Aβ can therefore have different sources.

Against this background, there are different explanations for the association between Aβ

and the risk of cancer. First, the plasma Aβ can be of neuronal origin. Aβ might be reflective of an underlying early-stage AD process and our findings might be pointing towards a shared causal predisposition between cancer and AD. Although effect estimates hardly changed when excluding the first two and five years of follow-up, we cannot fully exclude reverse causation. An alternative explanation therefore is that the blood-brain barrier permeability can increase due to subclinical cancer.[36] Different animal and in vitro studies have shown that systemic inflammation can disrupt the integrity of the blood-brain barrier.[37,38] Because higher inflammatory markers are associated with a higher risk of cancer,[39] the integrity of the blood-brain barrier might be altered by subclinical cancer due to systemic inflammation. This could result in leakage of neuronal Aβ to the peripheral circulation. Such reverse causality is compatible with higher Aβ levels reflective of a higher risk of getting diagnosed with clinical cancer. Second, the plasma Aβ can be of non-neuronal origin. It is conceivable that platelets and vascular wall endothelial cells – the main sources of circulating Aβ[14] – are activated as response to subclinical cancer,[40] resulting in more Aβ production before the cancer is diagnosed. For instance, platelets were indeed more activated in multiple myeloma patients than in healthy controls.[41] In addition, platelets promoted proliferation of multiple myeloma cells and that of acute myeloid leukaemia blasts in vitro.[42] This may partly explain the strong association with haematological cancers, but it should be noted that the group of haematological cancers is composed of different types of leukaemia and lymphoma. In the same context of an extra-neuronal origin of Aβ, Aβ might be produced by organs that express APP, but not β-secretase, for instance pancreas, kidney, and lung.[43] Cancer cells in these organs might be mutated such that β-secretase expression gets enhanced, resulting in Aβ production. We indeed found that the relation between plasma Aβ and cancer risk was stronger for those cancer sites with cells that express APP. Interestingly, APP is upregulated in pancreatic cancer cells.[44] In light of this, we examined the association between Aβ and pancreatic cancer in a post-hoc analysis. Although limited by power, we found that higher plasma Aβ levels were associated with a higher risk of pancreatic cancer. Third, shared mechanisms such as inflammation can cause both higher levels of plasma Aβ and cancer.[23] Higher inflammatory ratios are associated with a higher risk of cancer, indicating a pro-inflammatory state before cancer diagnosis, and interestingly have also been linked with AD.[39,45] This might also explain why the association between plasma Aβ and cancer was more pronounced – albeit not statistically significantly – in *APOE* ε4 carriers than in non-carriers. Human cell studies and animal studies have shown that *APOE4* may predispose cells to inflammation and may promote a greater inflammatory response following immune activation than other *APOE* isoforms, resulting in the secretion of inflammatory factors.[46] **Figure 3** summarises the potential biological mechanisms underlying the association between plasma Aβ and cancer. Lastly, we cannot completely rule out that

the association is partly driven by methodological bias. We have previously shown that lower plasma Aβ levels are associated with a higher risk of AD.[21] Given this association, we might expect that – if cancer and AD are positively associated – lower plasma Aβ levels would be associated with a higher risk of cancer. The finding that higher plasma Aβ levels are related to a higher risk of cancer might be explained because those persons with low plasma Aβ levels are more likely to develop dementia before they might have been diagnosed with cancer. Consequently, higher plasma Aβ levels are associated with a higher risk of cancer.

Some limitations of this study need to be addressed. First, we had no measurements of Aβ within the brain, for instance, measured in cerebrospinal fluid (CSF) or by amyloid positron emission tomography (PET) neuroimaging. However, previous studies have shown correlation between plasma Aβ and CSF or amyloid PET.[16,47] Second, we cannot determine the origin of the measured plasma Aβ levels. Third, although we tried to take the effect of reverse causation into account by excluding the first two and five years of follow-up, we cannot determine the causal effect of Aβ on cancer. The length of the latency period between cancer initiation and manifestation differs per cancer site and can range from five to forty years for solid tumours.[48] Fourth, it should be noted that although the missing and invalid plasma Aβ levels were missing at random, the characteristics of participants with known plasma Aβ levels differed from those of participants with unknown or invalid plasma Aβ levels. This, as well as that most of the included participants were middle class persons of European descent (98.5%), could possibly limit the generalisability of our findings to other populations. Strengths include using Aβ as proxy for preclinical AD to circumvent surveillance and survival bias, the large sample size, and the inclusion of participants with different cancer sites. Although this enabled us to explore the association between plasma Aβ and different cancer sites, the groups of different cancer sites were heterogeneous and analyses were limited by the low number of cases per cancer site.

In conclusion, we found that higher plasma Aβ40 and Aβ42 levels are associated with a higher risk of cancer. This finding may support a potential biological link between AD and cancer. Also, this association may indicate a potential pathophysiological role of Aβ in cancer, outside the context of AD. The causality of this association warrants further investigation, for instance by investigating the trajectory of plasma Aβ levels before cancer diagnosis.

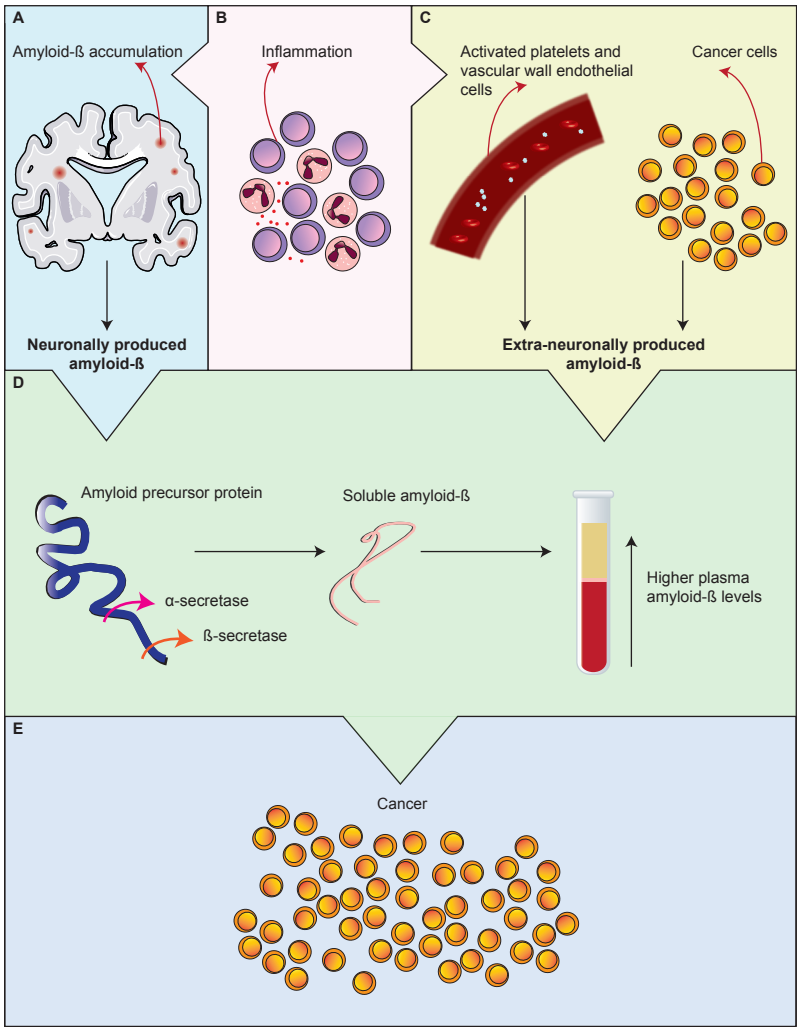

Figure 3 Overview of potential mechanisms underlying the association between plasma amyloid-β and the risk of cancer.

There are different sources of plasma amyloid-β: neuronal cells (A) and non-neuronal cells (B and C). Neuronal amyloid-β production might reflect an underlying AD process (A, this image was modified from Servier Medical Art, licensed under a Creative Common Attribution 3.0 Generic License. http://smart.servier.com/). Extra-neuronal amyloid-β production can be caused by activation of platelets and vascular wall cells (C). This activation might be due to subclinical cancer. In addition, subclinical cancer cells might produce amyloid-β if they also express β-secretase due to mutations (C). Inflammation can stimulate the pathogenesis of both AD and cancer (B), and could therefore indirectly lead to higher plasma amyloid-β levels (D). In turn, higher plasma amyloid-β levels are associated with a higher risk of cancer (E). However, the causality of this association warrants further investigation.

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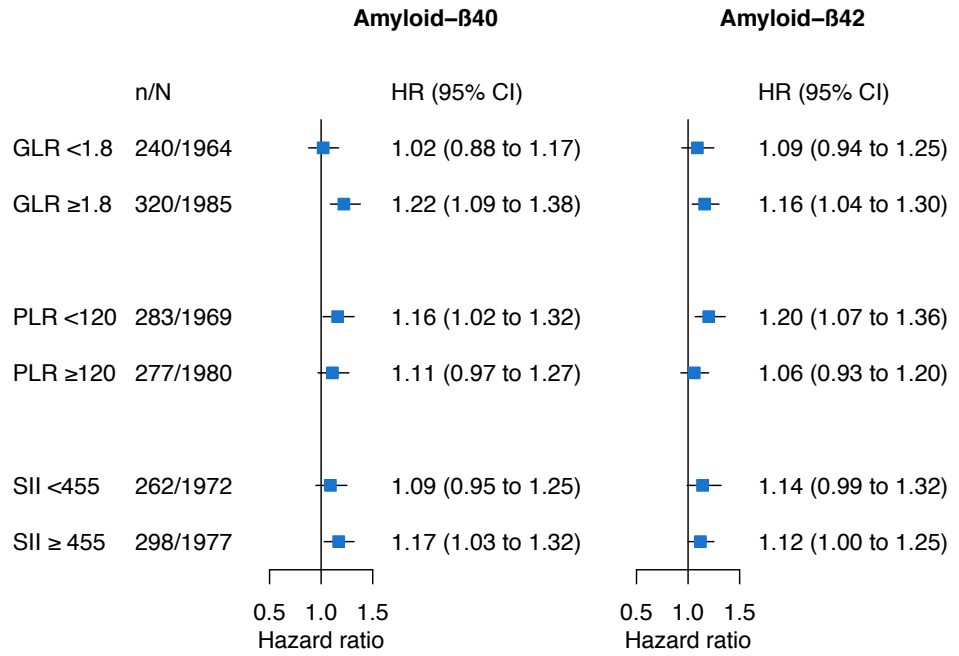
SUPPLEMENTARY MATERIAL



Supplementary Figure 1 Forest plot of association between log_2 transformed and standardised plasma amyloid-β40 and amyloid-β42 levels with risk of cancer, stratified by median granulocyte-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and systemic immune-inflammation index.
Hazard ratios are adjusted for age at blood draw, sex, assay batch number, education, body mass index, hypertension, hypercholesterolaemia, diabetes mellitus, smoking, alcohol use, and CES-D sum-score. CES-D = Centre for Epidemiologic Studies Depression scale, CI = confidence interval, GLR = granulocyte-to-lymphocyte ratio, HR = hazard ratio, n = number of participants with incident cancer, N = total number for participants, PLR = platelet-to-lymphocyte ratio, SII = systemic immune-inflammation index.

Chapter 11

Association between the tumour marker carcinoembryonic antigen and the risk of dementia

van der Willik KD, Schagen SB, Ikram MA

ABSTRACT

Background There is an ongoing debate about how cancer and dementia relate to each other, and whether their relation is biologically determined or caused by surveillance and survival bias.

Methods We aimed to circumvent these biases by determining the relation between the tumour marker carcinoembryonic antigen (CEA) and the risk of dementia in 6692 participants from the population-based Rotterdam Study.

Results We found that higher levels of CEA were associated with a higher risk of dementia (hazard ratio per standard deviation increase in CEA = 1.11 [95% confidence interval = 1.04 to 1.18]).

Conclusions This finding may indicate that cancer and dementia are positively associated, but the mechanisms underlying the relation between CEA and dementia warrant further investigation.

INTRODUCTION

Cancer and dementia are leading causes of morbidity and mortality worldwide.[1,2] Although both diseases are common in the elderly population, their relation is poorly understood.[3] Various observational studies have shown that patients with cancer have a lower risk of developing dementia, and vice versa.[4-8] Different biological explanations underlying this inverse association have been proposed, including genetic predisposition for either promoting or suppressing cell proliferation and cell survival pathways.[9] Yet, patients with cancer or dementia are less likely to be screened for other diseases and have often a limited life expectancy. Therefore, methodological issues including surveillance and survival bias might drive the association towards an inverse direction.

Several studies have tried to tackle these methodological issues, for instance by studying the risk of dementia in patients with different stages of cancer. Patients with advanced stage cancer had the lowest risk of dementia, probably because their mortality risk is higher than that of patients with early stage cancer.[10] In addition, it has been shown that with appropriate model specification, patients with cancer do not have a lower risk of dementia.[11] Lastly, we have shown that persons with mild cognitive impairment (MCI), the transitional stage between normal cognition and dementia, even tended to have a higher risk of cancer than persons with normal cognition.[12] Although MCI and dementia share the same biological underpinnings, the life expectancy of persons with MCI is longer than that of patients with dementia, thereby limiting the effects of the competing risk of mortality. The higher risk of cancer in persons with MCI therefore suggests that the association between cancer and dementia might even be positive rather than inverse.

Studying the preclinical stage of one disease and linking it to the other disease could limit the effects of surveillance and survival bias, because the life expectancy of persons in the preclinical stage of a disease is longer than that of patients with clinically-manifested disease. Censoring for death as competing risk could result in biased effect estimates, if censoring happens to be informative.[13,14] Also, studying the preclinical stage can reduce the effects of selective survival, i.e., those patients who survive cancer are likely to have some protective characteristics that help them to survive.[15] This can further improve our understanding of the biological association between cancer and dementia. Carcinoembryonic antigen (CEA) is an often-used tumour marker, usually in clinical settings to monitor cancer recurrence in curated cancer patients rather than for screening purposes. In this study, we used CEA as marker for preclinical, undiagnosed cancer in community-dwelling individuals free from clinically-diagnosed cancer and related this to the risk of dementia. Using this design, we aimed to

circumvent the effects of the competing risk of mortality on the relation between cancer and dementia, see **Figure 1**.

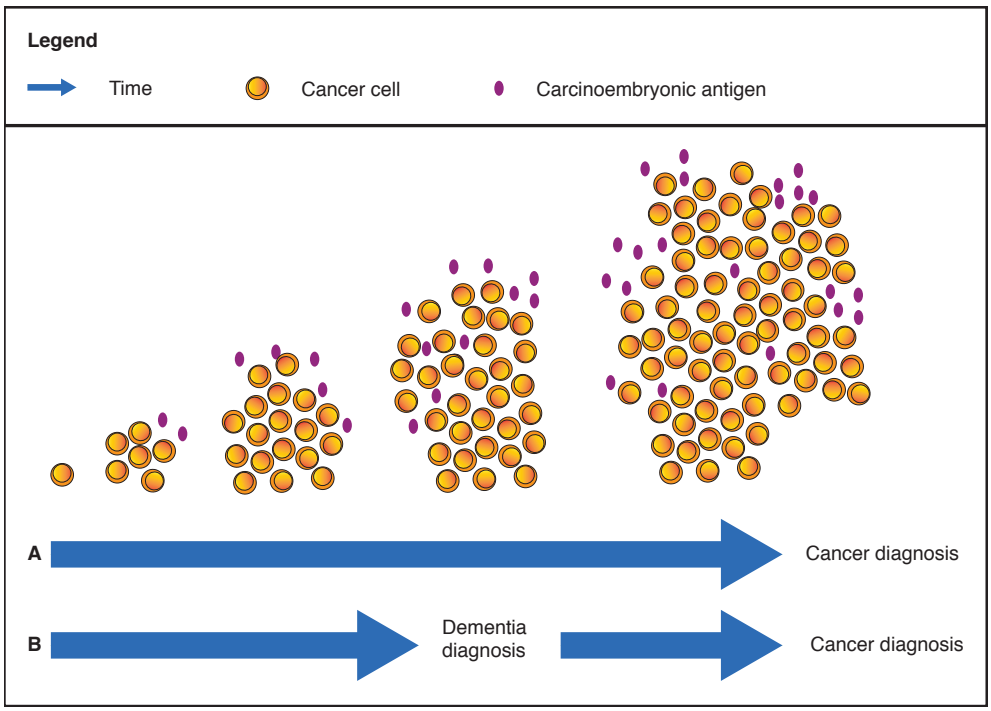


Figure 1 Schematic relation between carcinoembryonic antigen, cancer and dementia.
The time between the first cancer cell and clinical manifestation of the disease can last up to many years. Before clinical manifestation of cancer, cancer cells might already produce different factors including carcinoembryonic antigen. Therefore, carcinoembryonic antigen levels could be elevated before the diagnosis of cancer (scenario A, upper arrow). It is also possible that a person develops both dementia and cancer. Before this person is diagnosed with dementia, this person can already have a preclinical stage of cancer (scenario B, lower arrow). During this preclinical stage of cancer, carcinoembryonic antigen levels might already be elevated. These carcinoembryonic antigen levels could therefore be used to link the preclinical stage of cancer to dementia.

METHODS

Study population

This study is embedded within the Rotterdam Study, a prospective population-based cohort designed to study the occurrence and determinants of diseases in the elderly population. The cohort has been described in detail previously.[16] Briefly, in 1989 all inhabitants aged 55 years

or over from the district Ommoord in Rotterdam, the Netherlands, were invited to participate. This initial cohort (RS-I) comprised 7983 participants and was extended (RS-II) in 2000 with 3011 participants who had become 55 years of age or moved into the study district. In 2006, the cohort was further extended (RS-III) with 3932 participants aged 45 years or over. In total, the Rotterdam Study comprises 14 926 participants (overall response rate 72%).

The Rotterdam Study has been approved by the Medical Ethics Committee of Erasmus Medical Centre and by the board of The Netherlands Ministry of Health, Welfare, and Sports. A written informed consent was obtained from all participants.

CEA levels were determined in plasma samples of 7305 participants that had been obtained during the third visit of RS-I (1997 to 1999) and the first visits of RS-II (2000 to 2001) and RS-III (2006 to 2008). From these 7305 participants, we excluded those with a history of dementia (n=75) or insufficient data to determine cognitive status (n=65). In addition, we excluded participants with a history of cancer (n=425) to study the association between CEA and dementia in a population free from clinically-manifested cancer. It is, however, possible that participants had an undiagnosed, preclinical stage of cancer at the date of blood sample draw. Lastly, we excluded participants without informed consent to access medical records during follow-up (n=48). This resulted in 6692 participants for analyses.

CEA assessment

Blood was sampled in EDTA coated tubes and centrifuged, of which subsequently plasma was aliquoted and frozen at -80^{0}C according to standard procedures. CEA (μg/L) was measured using the Roche Modular P800 Analyser (Roche Diagnostics, Indianapolis, IN, USA). The coefficient of variation for intermediate precision was 17.3%, and for repeatability was 13.9%.

Dementia assessment

Participants were screened for dementia at baseline and subsequent centre visits with the Mini-Mental State Examination and the Geriatric Mental Schedule organic level.[17] Those with a Mini-Mental State Examination score <26 or Geriatric Mental Schedule score >0 underwent further investigation and informant interview, including the Cambridge Examination for Mental Disorders of the Elderly. In addition, the entire cohort was continuously under surveillance for dementia through electronic linkage of the study database with medical records from general practitioners and the regional institute for outpatient mental health care. Available information on clinical neuroimaging was used when required for diagnosis of dementia subtype. A consensus panel led by a consultant neurologist established the final diagnosis according to standard criteria for dementia based on information collected during centre visits and obtained from medical records (Diagnostic and Statistical Manual of Mental Disorders III-

revised). Follow-up until January 1st, 2016 was virtually complete (93.8% of potential person-years observed).

Other assessments

During home interviews, participants provided information on educational level, smoking status, and alcohol use. Educational level was categorised as primary education, lower (lower general education, intermediate general education, or lower vocational education), intermediate (intermediate vocational education or higher general education), or higher (higher vocational education or university). Smoking status was classified into never, current, or former. Alcohol use was categorised as no use or any use. At the research centre, height and weight were measured from which the body mass index (kg/m^2) was computed. Diagnosis of cancer was obtained from general practitioners' medical records (including hospital discharge letters), and through linkage with Dutch Hospital Data, regional histopathology and cytopathology registries, and the Netherlands Cancer Registry.[18] Follow-up of cancer registration was completed up to January 1st, 2015.

Statistical analysis

Characteristics of the study population were stratified by normal (CEA <5.0 μg/L) and high (CEA ≥5.0 μg/L) CEA levels. This cut-off value between normal and high CEA levels has been proposed by the Colorectal Working Group of the American Joint Committee on Cancer.[19]

We used Cox proportional hazards models to obtain hazard ratios (HRs) and 95% confidence intervals (95% CIs) to investigate the association between CEA (continuous and in quartiles) and the risk of dementia. In addition, we compared the risk of dementia in participants with normal CEA levels (CEA <5.0 μg/L) and high CEA (CEA ≥5.0 μg/L) levels. CEA levels were standardised to obtain the effect per one standard deviation (SD) in CEA.

Follow-up time was used as time scale and started at the date of the blood sample draw until the date of dementia diagnosis, death, loss to follow-up, or January 1st, 2016, whichever came first. We verified that the choice of the time scale (follow-up versus age) did not affect the results. HRs were adjusted for age at blood sample draw, sex, education, body mass index, smoking status, and alcohol use. The proportional hazards assumption was checked by visual inspection of the Schoenfeld residuals.

In sensitivity analyses we censored participants after two and five years of follow-up to examine the strength of the association between CEA and dementia when CEA was measured more closely to the date of dementia diagnosis.

Lastly, to support the use of CEA levels as proxy for the preclinical stage cancer, we determined the relation between CEA levels and the risk of cancer using the same models

as those used in the analysis for the relation between CEA levels and the risk of dementia. In this analysis we included the participants with a history of dementia or with insufficient data to determine their cognitive status. Follow-up time started again at the date of the blood sample draw, but ended at the date of cancer diagnosis, death, loss to follow-up, or January 1st, 2015, whichever came first.

Multiple imputation was used for missing covariates (maximum of 0.9%) with five imputed datasets based on other covariates and outcome. Rubin's method was used for pooled HRs and 95% CIs. Statistical analyses were performed using the package 'survival' in RStudio Version 3.3.2.[20]

RESULTS

Table 1 shows the characteristics of participants with normal (n=6238) and high (n=454) CEA levels. The median (interquartile range) age of participants with normal CEA values was 60.3 years (56.3 to 67.9), and that of participants with high CEA values was 60.2 years (56.3 to 67.9). Participants with high CEA levels were more often current smokers than those with normal CEA levels. During a median (interquartile range) follow-up of 8.8 years (7.2 to 14.5), 471 out of 6692 participants were diagnosed with dementia, of whom 25 (5.3%) had high CEA levels.

Higher levels of CEA were associated with a higher risk of dementia (HR per SD increase in CEA level = 1.11 [95% CI = 1.04 to 1.18], **Table 2**). Participants with high CEA levels had a higher risk of dementia than those with normal CEA levels, albeit not statistically significant (HR = 1.14 [95% CI = 0.76 to 1.72]). Compared to participants with CEA levels in the lowest quartile, those with levels in the highest quartile had a higher risk of dementia (HR = 1.28 [95% CI = 0.99 to 1.65]).

Sensitivity analyses yielded slightly higher effect estimates when censoring participants after two years (HR per SD increase in CEA level = 1.16 [95% CI = 1.09 to 1.23]) and five year of follow-up (HR per SD increase in CEA level = 1.13 [95% CI = 1.07 to 1.20], **Table 2**).

Supplementary Table 1 shows the results regarding the relation between CEA levels and the risk of cancer. Higher levels of CEA were associated with a higher risk of cancer (HR per SD increase in CEA level = 1.18 [95% CI = 1.15 to 1.21]). This relation was most pronounced in participants with high CEA levels (HR 2.51 [95% CI 2.00 to 3.13]). The effect estimates hardly changed when censoring participants after two and five years of follow-up.

Table 1 Characteristics of the study population stratified by carcinoembryonic antigen level.

Characteristic	Participants with normal carcinoembryonic antigen levels (<5.0 μg/L) (N=6238)	Participants with high carcinoembryonic antigen levels (≥5.0 μg/L) (N=454)
Age, years, median (IQR)	60.3 (56.3 to 67.9)	60.2 (56.3 to 67.9)
Women, No. (%)	3544 (56.8)	267 (58.8)
Educational level, No. (%)		
Primary	681 (10.9)	66 (14.5)
Lower	2488 (39.9)	201 (44.3)
Intermediate	1766 (28.3)	113 (24.9)
Higher	1250 (20.0)	70 (15.4)
Body mass index, kg/m^2, mean (SD)	27.5 (4.2)	26.6 (4.5)
Smoking status, No. (%)		
Never	1594 (25.6)	33 (7.3)
Former	3213 (51.5)	163 (35.9)
Current	1414 (22.7)	256 (56.4)
Alcohol use, No. (%)	5396 (86.5)	380 (83.7)
Carcinoembryonic antigen, μg/L, median (IQR)	1.7 (1.2 to 2.5)	6.5 (5.6 to 7.9)

Characteristics were measured during the visit of the blood sample draw. Missing values are not imputed and therefore numbers do not sum up to 100%.
IQR = interquartile range, SD = standard deviation.

DISCUSSION

CEA is a tumour marker widely used in oncology for surveillance and to assess treatment response. The observed association between higher levels of CEA and a higher risk of dementia may imply that patients with dementia are more likely to have preclinical cancer prior to their dementia diagnosis. These findings are further supported by a previous study showing that patients with dementia have higher CEA levels than healthy persons.[21]

The current findings indicate the possibility of a positive association between cancer and dementia. Given that higher CEA levels are associated with more advanced stages of cancer,[22] the slightly higher risk of dementia when censoring participants after two and five years of follow-up might suggest that cancer is already more advanced shortly before dementia diagnosis. It has indeed been shown that dementia patients who were subsequently

Table 2 The association between carcinoembryonic antigen and the risk of dementia.

Carcinoembryonic antigen (μg/L)*	Dementia	
	n/N	HR (95% CI)
Continuous†	471/6692	1.11 (1.04 to 1.18)
Cut-off value 5 μg/L		
<5 μg/L	446/6238	1.00
≥5 μg/L	25/454	1.14 (0.76 to 1.72)
Quartiles (range)		
1st quartile (-0.88 to -0.46)	112/1673	1.00
2nd quartile (-0.46 to -0.21)	104/1673	0.92 (0.70 to 1.20)
3rd quartile (-0.21 to 0.17)	124/1673	0.98 (0.76 to 1.28)
4th quartile (0.17 to 38.0)	131/1673	1.28 (0.99 to 1.65)
P for trend		.05
Censored after two years of follow-up		
Continuous†	32/6692	1.16 (1.09 to 1.23)
Censored after five years of follow-up		
Continuous†	124/6692	1.13 (1.07 to 1.20)

Hazard ratios are adjusted for age at blood sample draw, sex, education, body mass index, smoking status, and alcohol use.
** Carcinoembryonic antigen levels were standardised. † Expressed per standard deviation increase.*
CI = confidence interval, HR = hazard ratio, n = number of participants with incident dementia, N = total number of participants.

diagnosed with cancer, had more advanced stages of cancer.[23] Nevertheless, given that observational studies repeatedly find a lower risk of dementia in cancer patients,[4] it is likely that cancer remains undiagnosed in the majority of the patients after dementia diagnosis. Cancer-related symptoms may be obscured by dementia-related frailty (i.e., surveillance bias), or remain subclinical due to early death (i.e., survival bias).[3]

The potential positive link between cancer and dementia may be explained by three proposed underlying mechanisms. Firstly, cancer and dementia share multiple risk factors such as higher age, obesity, lack of activity, smoking, and alcohol use. Secondly, several pathways are involved in the pathogenesis of both diseases, including inflammation, genome instability, and angiogenesis.[24] Thirdly, different proteins that are related to neurodegeneration, including amyloid-β and tau,[25,26] can be elevated in cancer patients, suggesting that these proteins might also be involved in the pathogenesis of cancer.[27-29] In addition, overexpression of the amyloid precursor protein in cancer cells is associated with cell proliferation, migration,

and invasion.[29] Also, the tumour suppression protein BRCA1 has been linked to dementia.[30] Higher amyloid-β burden was associated with BRCA1 dysfunction, resulting in more DNA damage and in deterioration of genomic integrity. Future research on potential underlying mechanisms should further elaborate on the relation between different types of cancer and cancer treatments, and dementia.

However, a word of caution is warranted. Although the tumour marker CEA is often elevated in patients with different types of cancer, including colorectal, pancreatic, and breast cancer,[31] it is primarily used for monitoring recurrence in curatively treated patients with colorectal cancer.[22] Although we found that higher CEA levels were associated with a higher risk of cancer, CEA is not used for screening of cancer in the general, unselected population, because the sensitivity and specificity are limited.[32] CEA can be elevated due to other reasons apart from cancer, such as smoking, inflammation, and hepatic insufficiency.[33,34] Despite the fact that these conditions are partly related to cancer, they offer an alternative explanation for our finding as drivers of the observed association between CEA and dementia.

In conclusion, the relation between CEA and dementia is intriguing and points toward a positive association between cancer and dementia and not to the often postulated inverse association. The mechanisms underlying the association between CEA and dementia and its causality warrant further examination.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1 The association between carcinoembryonic antigen and the risk of cancer.

Carcinoembryonic antigen (*μ*g/L)*	Cancer	
	n/N†	HR (95% CI)
Continuous‡	889/6820	1.18 (1.15 to 1.21)
Cut-off value 5 *μ*g/L		
<5 *μ*g/L	797/6357	1.00
≥5 *μ*g/L	92/463	2.51 (2.00 to 3.13)
Quartiles (range)		
1st quartile (-0.89 to -0.46)	189/1705	1.00
2nd quartile (-0.46 to -0.21)	221/1705	1.25 (1.03 to 1.52)
3rd quartile (-0.21 to 0.18)	220/1705	1.31 (1.08 to 1.60)
4th quartile (0.18 to 38.2)	259/1705	1.87 (1.54 to 2.27)
P for trend		<.001
Censored after two years of follow-up		
Continuous‡	47/6820	1.14 (1.09 to 1.19)
Censored after five years of follow-up		
Continuous‡	122/6820	1.16 (1.12 to 1.19)

Hazard ratios are adjusted for age at blood sample draw, sex, education, body mass index, smoking status, and alcohol use.
** Carcinoembryonic antigen levels were standardised. † Carcinoembryonic antigen levels were measured in 7305 participants. Participants with a history of dementia or insufficient data were included in this analysis. We excluded participants with a history of cancer (n=433) and those without informed consent to access medical records during follow-up (n=52). ‡ Expressed per standard deviation increase.*
CI = confidence interval, HR = hazard ratio, n = number of participants with incident cancer, N = total number of participants.

Chapter 12

Does selection bias explain the inverse relation between cancer and dementia?

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ABSTRACT

Background Observational studies have repeatedly shown that cancer patients have a lower risk of dementia than persons without a history of cancer. To illustrate the potential effects of selection bias on this inverse association between cancer and dementia, we replicated and compared previously used study designs. In addition, we presented alternative approaches to account for selection bias.

Methods Within the setting of the prospective population-based Rotterdam Study, we followed 8899 participants who were dementia-free at baseline over a period of 15 years. We replicated the following four study designs: (i) cohort study with cancer as time-dependent variable; (ii) cohort study with cancer as time-independent variable; (iii) nested case-control study with cancer as time-independent variable; and (iv) cross-sectional case-control study. For (i-iii) we estimated hazard ratios (HRs) with Cox proportional hazards models and for (iv) odds ratios (ORs) based on logistic regression models. Next, we presented the following three different methods to account for immortal time bias: (i) time-dependent cancer; (ii) inverse probability weighting (IPW); and (iii) cloning and censoring. To deal with the competing risk of death, we compared the risk of dementia among participants with and without cancer, as if (i) we could eliminate death; and (ii) regardless of death. We calculated the risk of dementia at each time point using pooled logistic regression.

Results Out of 8899 participants, 1813 (20.4%) were diagnosed with cancer, of whom 68 (3.8%) were subsequently diagnosed with dementia, 183 (10.1%) were lost to follow-up, and 890 (49.1%) died. The risk of dementia in patients with cancer depended on the study design. For instance, when cancer was treated as time-dependent variable the HR for dementia was 0.91 (95% confidence interval [CI] = 0.71 to 1.16]), as time-independent the HR was 0.44 (95% CI 0.35 to 0.56), and in the case-control study the OR for dementia was 0.28 (95% CI = 0.02 to 1.31). When using the alternative methods to deal with immortal time and the competing risk of death, the risk of dementia in participants with cancer was similar to that in participants without cancer.

Conclusions This study indicates that selection bias may drive the inverse association between cancer and dementia. Immortal time bias and competing events should be taken into account by using appropriate analytical methods, because these diseases are strongly related to death. In addition, future studies should further disentangle the processes underlying a cancer diagnosis to estimate the causal effect of cancer on dementia.

INTRODUCTION

Ageing populations worldwide have resulted in an increased prevalence of non-communicable diseases.[1,2] There is a particular interest in the link between the non-communicable diseases cancer and dementia, because these diseases share multiple common risk factors including higher age and smoking. In addition, cancer and dementia have several overlapping pathways such as DNA damage and inflammation, suggesting that these diseases frequently co-occur.[3,4] Many clinical studies have indeed found that patients with cancer often have impaired cognitive function.[5-7] Observational studies, however, have repeatedly shown that patients with cancer have a lower risk of dementia than persons without a history of cancer.[8-21] Several pathophysiological mechanisms underpinning this potential inverse link have been proposed and primarily involve differential expression of cell proliferation and survival pathways.[22]

In addition to these biological mechanisms, selection bias due to shortcomings in previous study designs and analytical decisions may underlie the inverse association between cancer and dementia.[4,23] Selection bias can manifest itself in different ways of which we will highlight three examples, i.e., survival bias, immortal time bias, and bias due to the competing risk of death. Firstly, in cross-sectional studies, participants have to be alive at the moment of assessment to be included in the study. Such conditioning on participants who have survived up to the moment of study assessment can lead to survival bias. Secondly, in longitudinal studies, participants are followed until the date of dementia diagnosis or death. The starting point of follow-up can differ between participants who remain free of cancer during follow-up and those who are diagnosed with cancer during follow-up. Exclusion or misclassification of the time between study entry and cancer diagnosis, i.e., immortal time, may cause differences in baseline characteristics between participants with and without cancer and can induce immortal time bias.[24] Thirdly, most longitudinal studies assume that death occurs at random, whereas in fact, risk factors that are related to death are usually the same that are related to cancer and dementia. This results in that the participant with the worst risk factor profile will die first.[25,26]

In the current study, we illustrate how decisions on study design and statistical analyses may induce selection bias when studying the association between cancer and dementia. In order to do so, we use data from the prospective population-based Rotterdam Study. Firstly, we provide a visual explanation of the potential problem of selection bias due to study design. Secondly, we replicate previous study designs and analytical decisions and show how results vary accordingly. Thirdly, we present three different methods to account for immortal time bias and we provide two alternative approaches to address the competing event of death.

METHODS

Study population

This study is embedded in the Rotterdam Study, a prospective population-based cohort study that was designed to determine causes of diseases in the middle-aged and elderly population.[27] After the pilot phase in 1989, all inhabitants aged 55 years and over of the Ommoord area in Rotterdam, the Netherlands, were invited to participate between 1990 and 1993. This first subcohort comprised 7983 participants (response of 78%) and was extended with the second subcohort between 2000 and 2001 consisting of 3011 participants (response of 67%) who had reached the age of 55 years or who had moved into the study area.

Participants were interviewed at home by a trained research assistant, followed by two visits at the research centre for different examinations including physical examinations, laboratory assessments, and imaging. Follow-up examinations of the first subcohort took place from 1993 to 1995, from 1997 to 1999, from 2002 to 2004, from 2009 to 2011, and from 2014 to 2015. For the second subcohort, follow-up examinations took place between 2004 and 2005, and between 2011 and 2012.

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus Medical Centre and by the Ministry of Health, Welfare and Sport of the Netherlands. Written informed consent was obtained from all participants.

Of the total of 10 994 participants, we excluded those with a history of dementia (n=515) or who were insufficiently screened for history of dementia (n=349), those without informed consent to access medical records during follow-up (n=135), and participants with incomplete data on baseline characteristics including education, smoking, body mass index (BMI), systolic blood pressure, and hypertension (n=1096), resulting in 8899 participants for analyses. Participants who had incomplete baseline characteristics were older at baseline (median age [interquartile range [IQR]] 73.4 years [63.8 to 82.4] versus 65.7 years [60.4 to 73.2]) and were more often women than included participants (68.2% versus 57.4%).

Ascertainment of cancer

Cancer was diagnosed based on medical records of general practitioners (including hospital discharge letters) and through linkage with the Netherlands Cancer Registry, Dutch Hospital Data, and histology and cytopathology registries in the region. Cancer was defined as any primary malignant tumour, excluding non-melanoma skin cancer, that was confirmed by pathology. Diagnoses were coded independently by two physicians according to the International Classification of Diseases, tenth revision (ICD-10). In case of discrepancy,

consensus was sought through consultation with a physician specialised in internal medicine. Date of diagnosis was based on date of biopsy (solid tumours) and laboratory assessment (haematological tumours), or – if unavailable – date of hospital admission or discharge letter. Follow-up was completed up to January 1st, 2015.

Ascertainment of dementia

Participants were screened for dementia at baseline and subsequent centre visits with the Mini-Mental State Examination and the Geriatric Mental Schedule organic level.[28] Those with a Mini-Mental State Examination score <26 or Geriatric Mental Schedule score >0 underwent further investigation and informant interview, including the Cambridge Examination for Mental Disorders of the Elderly. In addition, the entire cohort was continuously under surveillance for dementia through electronic linkage of the study database with medical records from general practitioners and the regional institute for outpatient mental health care. A consensus panel led by a consultant neurologist established the final diagnosis according to standard criteria for dementia (Diagnostic and Statistical Manual of Mental Disorders III-revised). Follow-up was completed up to January 1st, 2016.

Ascertainment of covariates

During home interviews, participants provided information on educational level and smoking habits. Educational level was categorised into lower (primary education or lower vocational education), intermediate (lower secondary education, intermediate vocational education, or general secondary education), or higher (higher vocational education or university). Smoking habits were classified as never, former, or current smoking. At the research centre, height and weight were measured to calculate the BMI (kg/m^2). Systolic and diastolic blood pressure were measured twice on the right arm using a random-zero sphygmomanometer of which the mean was used. Hypertension was defined as a systolic blood pressure of ≥140 mm Hg, a diastolic blood pressure of ≥90 mm Hg, or use of antihypertensive medication.[29] Diabetes mellitus was defined fasting serum glucose level ≥7.1 mmol/L, a random serum glucose level ≥11.1 mmol/L, or use of glucose-lowering medication.[30] History of coronary heart disease (myocardial infarction, percutaneous coronary intervention, or coronary artery bypass grafting) and stroke was assessed by interview and verified by reviewing medical records.[31,32]

Statistical analyses

We first visualised the number of years spent in different transition states using raw data to illustrate the potential problem of survival bias when studying the relation between cancer and dementia.

Replication of previous studies

Next, we replicated the following study designs and statistical analyses that have previously been used to study the association between cancer and dementia. To understand the difference between these studies, we define the time zero as the moment at which we start observing participants based on their exposure level.[24] Given that a cancer diagnosis can occur at any moment in time, previous studies have considered cancer as a time-dependent variable (history of cancer at study entry and incident cancer after cancer diagnosis versus no cancer at study entry and during follow-up) or as a time-independent variable (history of cancer at study entry versus no history of cancer at study entry, or ever versus never cancer). Following these definitions, we replicated (i) cohort study using Cox proportional hazards models and using cancer as time-dependent variable and performing a sensitivity analysis by restricting the study population to participants who survived up to age eighty years during follow-up;[10,13,16] (ii) cohort study using Cox proportional hazards models and using cancer as time-independent variable;[11,15-17,19] (iii) nested case-control study using Cox proportional hazards models and using cancer as time-independent variable (note that this design was previously used to investigate the risk of cancer in patients with dementia);[17] and (iv) cross-sectional case-control study using logistic regression models.[9,12] As previous studies corrected for baseline characteristics, we adjusted models for age, sex, educational level, smoking, BMI, systolic blood pressure, hypertension, diabetes mellitus, history of coronary heart disease, and history of stroke. Follow-up time was used as underlying time scale and was rounded to years. Participants were censored at date of loss to follow-up, death, or after 15 years since start of follow-up. For the nested case-control study, we matched participants who were diagnosed with cancer to two participants who were free from cancer during study follow-up based on the age of cancer diagnosis and sex. For the cross-sectional study, we identified participants who visited at the fourth follow-up round of the first subcohort and the second follow-up round of the second subcohort. In addition, we selected participants who were diagnosed within six months after these follow-up rounds, because dementia is often diagnosed as result of examinations performed during the follow-up round. We subsequently used logistic regression models with history of incident cancer as determinant.

Alternative methods to account for selection bias

In the abovementioned study designs, depending on how time zero was handled, immortal time bias could be inflicted. Immortal time bias can arise when we fail to align the start of follow-up in participants who develop cancer versus those who remain free of cancer during follow-up.[24] Participants who survive longer have a higher probability to be diagnosed with cancer than those who have a shorter survival time. When cancer is treated as a time-

independent variable, such as when the time zero for individuals with cancer is the time of cancer diagnosis, persons who died before cancer diagnosis are by definition excluded from this group. In contrast, when the time at study entry is considered as the time zero in both groups and time of cancer diagnosis is a time-dependent variable, immortal time bias can be reduced. To prevent immortal time bias, we must however consider alternative methods that we will discuss later in this section.[24,33]

For all replications of previous study designs and statistical analyses, death was considered as an uninformative censoring event. A censoring event is an event that prevents observing the true outcome, including the outcome of dementia. For instance, being lost to follow-up is considered as a censoring event, which could be prevented in the study design. We consider a censoring event being uninformative if we assume that persons who are censored are similar to those who remained alive during the study period, given the available covariates. However, by definition, everyone who dies before the outcome, will not develop dementia. This cannot be prevented by the study design, and therefore we can consider death as a competing event.

In the current study, we used the following three alternative approaches to deal with immortal time bias: (i) a naïve approach in which cancer is treated as time-dependent variable; (ii) inverse probability weights (IPW) for the time until cancer diagnosis; and (iii) cloning and censoring.[33] These different approaches are implemented to answer two different questions based on how we include death. The first question is 'what is the risk of dementia among participants who develop cancer versus among those who remain free of cancer during follow-up, considering that we could eliminate death?'. This question reflects the hypothetical scenario were we could prevent death and therefore treat death as a censoring event. We refer to this question as the direct controlled effect.[25] Given that this question relies on the strong assumption of uninformative censoring, we consider time-dependent covariates to simulate a scenario in which censoring for death is uninformative. The second question we propose is 'what is the risk of dementia among participants who develop cancer versus among those who remain free of cancer during follow-up, regardless of death?'. This question does not require any strong assumption on the competing event of death, but the risk of dementia will be affected by the relation between cancer and death.[34] We refer to this question as the total effect.[25] In the following paragraphs, we will describe the technical details of these different approaches.

IPW for the time until cancer diagnosis

We computed weights for the time until cancer diagnosis by fitting a pooled logistic model. The product of the estimated conditional probabilities at each time was subsequently used to estimate the time-dependent weight for each participant at each time point, reflecting the time-

dependent weight inversely proportional to the probability of not being diagnosed with cancer. Weights were fitted considering time until cancer diagnosis in years, cancer, the interaction between time and cancer, sex, age at study entry, cohort, education, and hypertension, and time-updated covariates including smoking, systolic blood pressure, BMI, history of stroke, history of diabetes, and history of coronary heart disease. Time, systolic blood pressure, and BMI were modelled non-linearly using B-spines with three degrees of freedom.

Cloning and censoring

Details of this method have been described previously.[33] In brief, first, we made two copies of each participant. One of the copies was allocated to a 'cancer' arm and the other copy to a 'cancer-free' arm. Participants who were diagnosed with cancer during follow-up were censored at date of end of follow-up in the 'cancer' arm and at date of cancer diagnosis in the 'cancer-free' arm. Those who remained free from cancer during follow-up were censored after a pre-specified period of 15 years in the 'cancer' arm, or if their follow-up time was shorter at date of end of follow-up. In the 'cancer-free' arm, these participants were censored at date of end of follow-up. Censoring is informative and therefore we accounted for this type of censoring using IPW as described above.

Risk of dementia with elimination of death

To "eliminate death", i.e., to account for potential non-differential death between participants who were diagnosed with cancer and those who remained free of cancer, we computed IPW for death by fitting a pooled logistic model that included time-depended covariates. This resulted in a time-depended weight inversely proportional to the probability of not dying for each participant, considering the time-depended covariates that relate to death and dementia, including sex, age at study entry, cohort, education, smoking status, systolic blood pressure, hypertension, BMI, history of stroke, history of diabetes, and history of coronary heart disease. Follow-up time, systolic blood pressure, and BMI were modelled non-linear using B-spines with three degrees of freedom. In addition, we used these time-depended covariates to calculate IPW for loss to follow-up.

Risk of dementia regardless of death

To estimate the risk of dementia regardless of death, we do not rely in the strong assumption of uninformative censoring. We estimated the risk of dementia by the joint probability of incurring either dementia or death at each time point. We calculated the cumulative sum of the probability of surviving both events multiplied by the instantaneous cause-specific hazard of dementia.[25,35] In this approach, we only considered censoring and IPW for loss to follow-up.

Combining the approaches for immortal time bias and the competing risk of death, we performed six different analyses. We created standardised cumulative incidence curves using pooled logistic regression. The 95% confidence intervals (CIs) were obtained by bootstrapping. Statistical analyses were performed using R software Version 3.6.1. The code to run the analyses will be made available on GitHub.

RESULTS

During a median (IQR) follow-up of 13.0 years (7.0 to 15.0), 1813 (20.4%) out of 8899 participants were diagnosed with cancer. Of the participants who were diagnosed with cancer, 68 (3.8%) were subsequently diagnosed with dementia, 183 (10.1%) were lost to follow-up, and 890 (49.1%) died during follow-up. Of the 7086 participants who remained free of cancer, 781 (11.0%) were diagnosed with dementia, 1330 (18.8%) were lost to follow-up, and 1341 (18.9%) died (**Figure 1**). **Table 1** shows the baseline characteristics of the total study population. An overview of the different transition stages among participants who developed either cancer or dementia and died during follow-up is presented in **Figure 2**. The median (IQR) age at cancer diagnosis of these participants was 75.0 years (69.1 to 80.4), whereas the median (IQR) age at dementia diagnosis of these participants was 82.5 years (77.5 to 86.9).

Replication of previous studies

When using cancer as time-dependent variable, the risk of dementia in participants with cancer was similar to that in participants without cancer (hazard ratio [HR] = 0.91 [95% CI = 0.71 to 1.16], **Table 2**). This risk was comparable when restricting the analysis to participants who survived up to at least eighty years (HR = 0.91 [95% CI = 0.67 to 1.26]). Participants with cancer had a lower risk of dementia than those without cancer when using cancer as time-independent variable (HR = 0.44 [95% CI = 0.35 to 0.56]) and in the nested case-control setting (HR = 0.53 [95% CI = 0.41 to 0.69]).

The number of participants that was included in the cross-sectional study design was 5278. Out of these participants, 50 had a dementia diagnosis during the research centre visit. This design is illustrated by **Figure 3**. Participants with a history of cancer had an odds ratio (OR) of 0.28 (95% CI = 0.02 to 1.31) for dementia. When including also participants who were diagnosed with dementia within six months after the research centre visit, the OR was 0.61 (95% CI = 0.26 to 1.24).

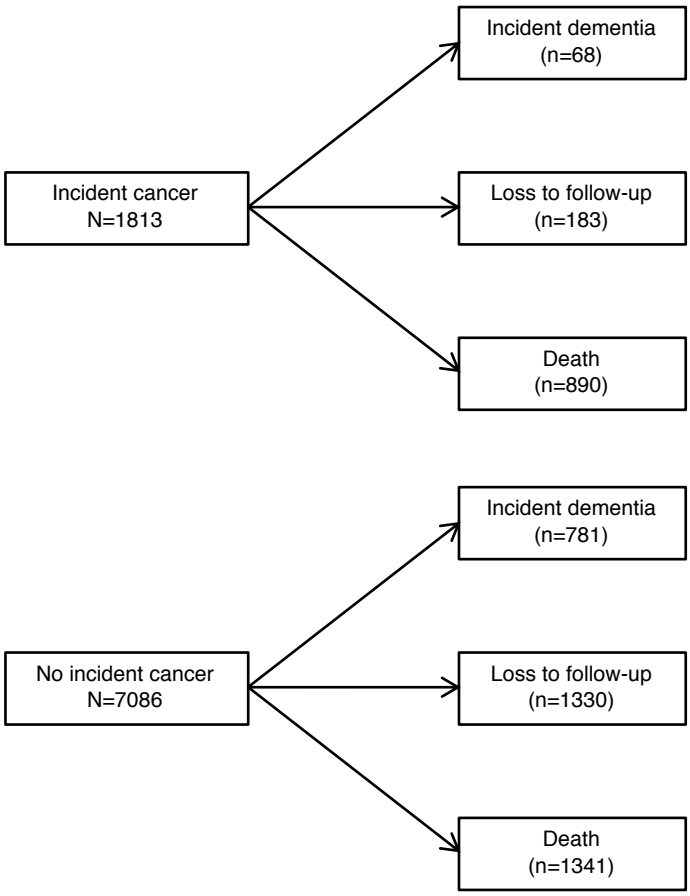

Figure 1 Flowchart of outcomes stratified by incident cancer diagnosis.

Alternative methods to account for selection bias

Figure 4 shows that the risk of death among participants with cancer is higher than among those without cancer. Standardised cumulative incidence curves for the six different analyses are presented in **Figure 5**. When using cancer as time-dependent variable and eliminating death, participants with cancer had a higher risk of dementia than those without cancer, but CIs were overlapping (**Figure 5A**). The risk of dementia regardless of the risk of death was higher in participants with cancer than in those without cancer up to 12 years of follow-up. After 12 years, the difference in the risk of dementia narrows, but CIs largely overlap over the entire follow-up (**Figure 5B**). The risk of dementia was higher when estimating the risk of dementia with elimination of death rather than regardless of death. When we computed IPW for the time until cancer diagnosis, cumulative incidence curves crossed after 12 years of follow-up in both approaches that we used to deal with the competing risk of death (**Figure**

Table 1 Baseline characteristics of study population

Characteristic	Participants (N=8899)
Age, years, median (IQR)	65.7 (60.4 to 73.2)
Women, No. (%)	5112 (57.4)
Educational level, No. (%)	
Lower	4220 (47.4)
Intermediate	3695 (41.5)
Higher	984 (11.1)
Smoking, No. (%)	
Never	2914 (32.7)
Former	3931 (44.2)
Current	2054 (23.1)
Body mass index, kg/m^2, mean (SD)	26.6 (3.8)
Systolic blood pressure, mm Hg, mean (SD)	140.5 (22.2)
Hypertension, No. (%)	5514 (62.0)
Diabetes mellitus, No. (%)	852 (9.6)
History of coronary heart disease, No. (%)	694 (7.8)
History of stroke, No. (%)	254 (2.9)

IQR = interquartile range, N = number of participants, SD = standard deviation.

5C and **5D**). Lastly, when using the cloning and censoring method, we found that cumulative incidence curves for participants with and without cancer were completely overlapping, with a slight deviation after 15 years of follow-up when estimating the risk of dementia regardless of death (**Figure 5E** and **5F**).

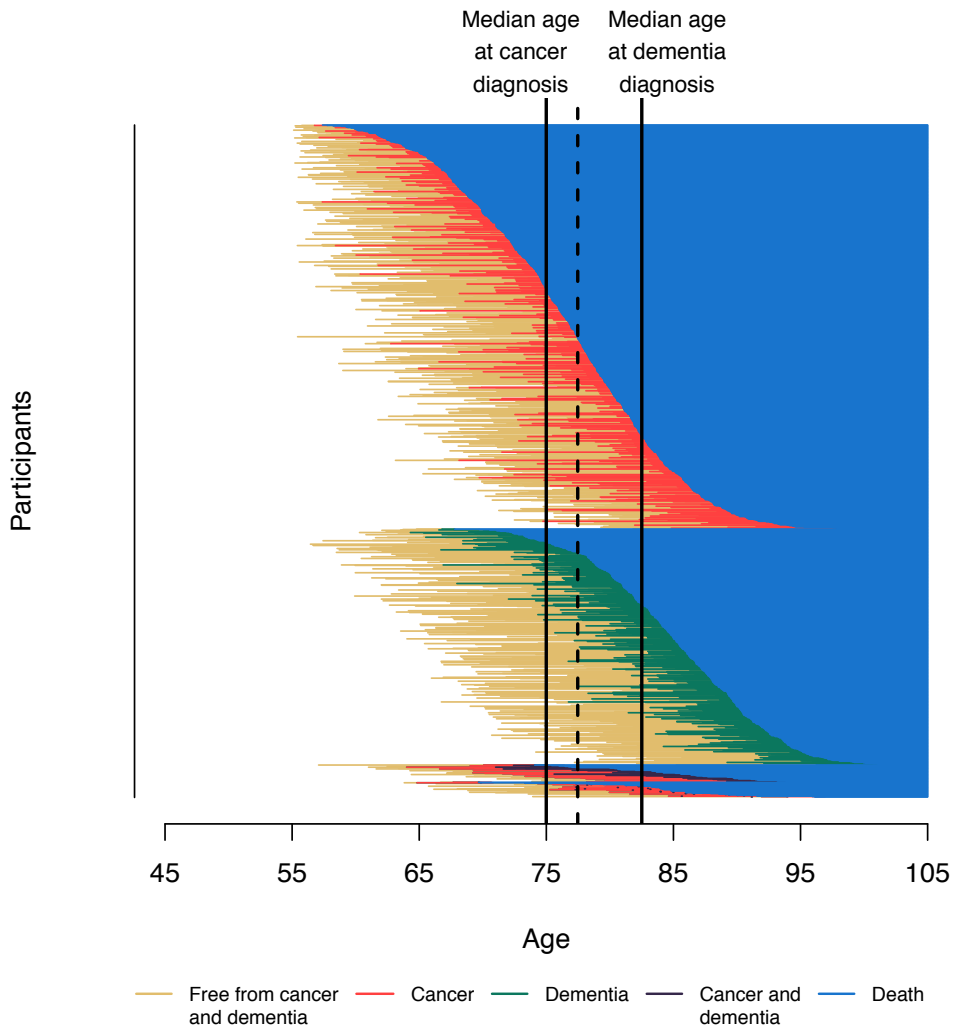

Figure 2 Graphic overview of transition states for participants who were free of cancer and dementia at study entry and who developed cancer or dementia during follow-up and died.
This graph consists of raw data (i.e., participants were not censored after dementia diagnosis and follow-up was not censored after 15 years) of 1556 participants. Out of these participants, 935 (60.1%) were diagnosed with cancer, 546 (36.2%) with dementia, and 75 (4.8%) with both cancer and dementia. The median (interquartile range) age at cancer diagnosis of these participants was 75.0 years (69.1 to 80.4), whereas the median (interquartile range) age at dementia diagnosis of these participants was 82.5 years (77.5 to 86.9). The dotted line indicates the median age at death for participants with cancer (77.5 years [interquartile range = 71.7 to 82.9]).

Table 2 Results of replication of previous study designs and analyses.

Analysis	N cancer	N dementia	N total	HR (95% CI)
Cancer as time-dependent variable	1813	875	8899	0.91 (0.71 to 1.16)
Survived up to at least 80 years	619	523	3423	0.91 (0.67 to 1.26)
Cancer as time independent variable	1813	875	8899	0.44 (0.35 to 0.56)
Nested case-control setting	1805*	409	5414	0.53 (0.41 to 0.69)
				OR (95% CI)
Dementia at ERGO4	359	50	5278	0.28 (0.02 to 1.31)
Dementia <6 months after ERGO4	405	142	5278	0.61 (0.26 to 1.24)

Model is adjusted for baseline measurements of age, sex, education, smoking status, body mass index, systolic blood pressure, hypertension, diabetes mellitus, history of coronary heart disease, and history of stroke.
** Eight out of 1813 participants with cancer could not be matched to a control. One participant with cancer was matched to only one control.*
ERGO4 = fourth visit of the first subcohort and the second visit of the second subcohort, HR = hazard ratio, N = number of participants, OR = odds ratio.

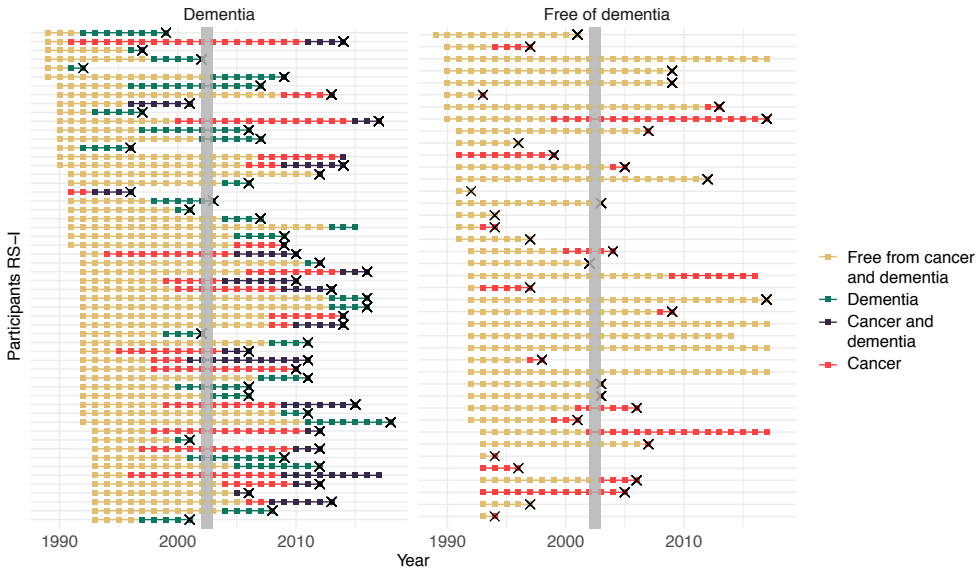

Figure 3 Graphic overview of cross-sectional study design when the assessment took place in 2002 to 2003.
This graph consists of raw data (i.e., participants were not censored after dementia diagnosis and follow-up was not censored after 15 years) of a random sample of participants from the first subcohort of the Rotterdam Study. When assessing participants during the fourth visit round in 2002 and 2003, only those who have survived up to 2002 and 2003 will be included in the study population. Therefore, cross-sectional study designs may result in selection bias.
RS = Rotterdam Study.

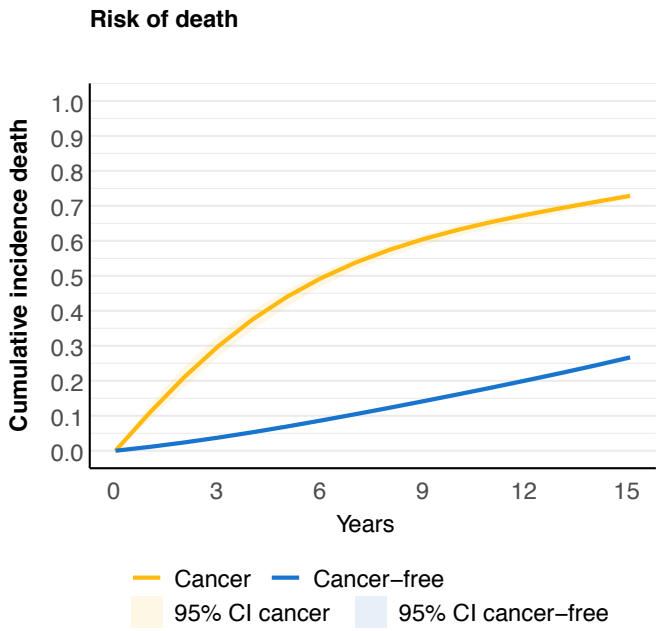

Figure 4 Risk of death for participants who are diagnosed with cancer and those who remain free of cancer during follow-up.
CI = confidence interval.

DISCUSSION

This study shows that the direction and magnitude of the risk of dementia in cancer patients is affected by the characteristics of the study design and statistical analyses. When using appropriate methods to account for immortal time bias and the competing event of death, patients with cancer are not at decreased risk of developing dementia. This suggests that the frequently observed inverse relation between cancer and dementia may be based on selection bias. These findings underline the importance of using appropriate study designs and statistical analyses when studying an exposure and outcome that are strongly related to death.

When we replicated study designs and statistical analyses performed in previous literature, hazard ratios for the risk of dementia in cancer patients varied between 0.44 and 0.91. These effect estimates are comparable to those obtained from previous studies on cancer and dementia.[8-21] The lowest effect estimates were found for cross-sectional study designs. In cross-sectional studies, participants had to survive up to a certain moment in time to be

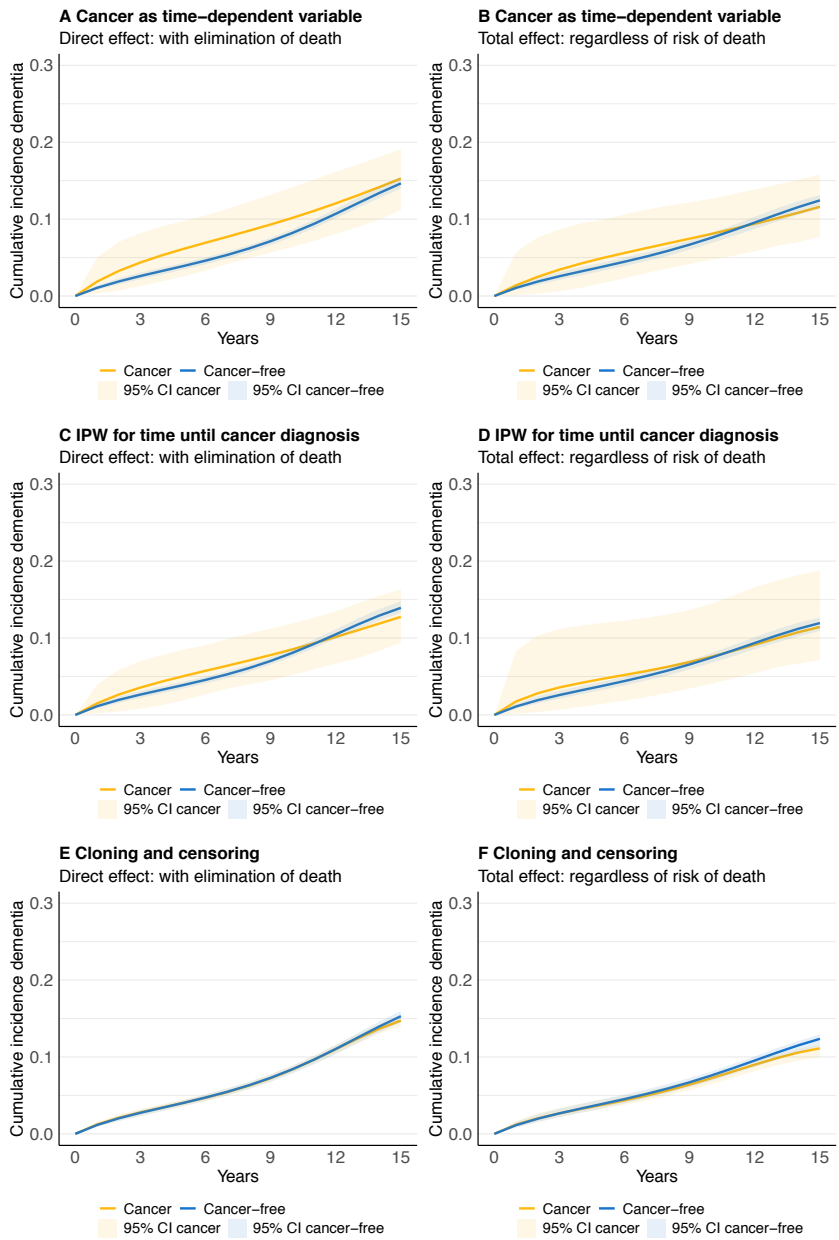

Figure 5 Standardised survival curves for dementia.
Curves for participants with cancer are presented in yellow and for participants without cancer in blue. Panels on the left (A, C, and E) represent the risk of dementia when we eliminated death, i.e., the controlled direct effect. Panels on the right (B, D, and F) represent the risk of dementia regardless of death, i.e., the total effect. Panel A and B show the curves when immortal time is handled by treating cancer as time-depended variable. Panel C and D are the curves obtained after we computed IPW for the time until cancer diagnosis. Panel E and F show the curves after cloning and censoring.
CI = confidence interval, IPW = inverse probability weights.

included in the study. Participants who have survived longer are more likely to be included in a cross-sectional study than those who have a shorter survival time (**Figure 3**). This can result in a selected group of participants who may be healthier and have a longer survival than the general population.

Regarding longitudinal studies, we found a lower risk of dementia in participants with cancer when using cancer as time-independent variable (HR = 0.44 in the cohort setting and HR = 0.53 in the nested case-control setting) than when studying cancer as time-dependent variable (HR = 0.91), which was also observed by Hanson et al.[23] The difference between these outcomes may be explained by selection bias due to immortal time.[34] We have accounted for immortal time using three different methods: (i) using cancer as time-dependent variable; (ii) using IPW for the time until cancer diagnosis; and (iii) cloning and censoring. These three different methods provided similar results. The time-dependent Cox proportional hazards model also reduces immortal time bias, but cannot completely prevent such bias and has two additional shortcomings, i.e., (i) hazard ratios represent a weighted average of the time-dependent hazard ratios of the total follow-up period and may therefore lose information that is preserved by presenting cumulative incidence curves[36]; and (ii) the model assumes that censoring of death is uninformative.

This latter assumption of the Cox proportional hazards model is often not examined. Given that persons need to survive long enough to develop dementia (median age [IQR] of dementia diagnosis in our study was 82.5 years [77.5 to 86.9]), and that dementia shares risks factors related to cardiovascular disease and cancer, they are at increased risk of death. Censoring for death is therefore informative and may result in biased effect estimates.[25] Hanson et al. compared the following two competing risk models to illustrate the effect of the competing risk of death on the relation between cancer and dementia: Fine and Gray and Kalbfleisch and Prentice.[23,37,38] The Fine and Gray model calculates the absolute risk of dementia and allows participants to be at risk for dementia after they have experienced the competing risk of death. The subdistribution hazard for dementia was lower than the cause specific hazard for dementia, indicating that mortality was higher in patients with cancer than in persons without cancer. The Kalbfleisch and Prentice method uses internal time-dependent covariates that are strongly related to the competing risk of death. The significant, positive association between these covariates and dementia indicated non-independence between dementia and death. Other studies have tried to account for the effects of the competing risk of death by studying negative control diseases[39] such as stroke and automobile injuries,[19,40,41] focusing on different cancer types,[8,18,19,41,42] and stratifying follow-up time.[21] In addition, we have previously used the tumour marker carcinoembryonic antigen as a proxy of preclinical cancer and studied the risk with dementia, given that persons with a preclinical stage of the disease have on average a

longer life expectancy than patients with clinically manifested disease.[43] Interestingly, higher levels of the tumour marker carcinoembryonic antigen were associated with a higher risk of dementia suggesting that, from a biological perspective, cancer and dementia might even be positively associated rather than inversely. In the current study, we accounted for the competing risk of death by multiplying the instantaneous hazard of dementia by the probability of being free of any event (i.e., total effect of cancer on dementia) and by computing IPW for death (i.e., direct effect of cancer on dementia, not mediated by death).

It must be noted however, that we, like previous studies, defined cancer as a pathology-confirmed cancer diagnosis. A cancer diagnosis can represent multiple causal pathways as will be explained below. We therefore did not examine the causal effect of cancer when using the alternative methods to account for immortal time bias and the competing risk of death, and as such, we did not consider confounders for the association between cancer, dementia, and death. If future studies aim to understand the causal relation between cancer and dementia, the research question should be redefined, because cancer diagnosis itself does not cause dementia. An ill-defined research question may result in wrong interpretation of the causal effects.[25] Cancer diagnosis may be considered as a proxy for other underlying processes that may cause cognitive impairment and subsequently dementia, see the corresponding directed acyclic graph (DAG) in **Figure 6**. This DAG represents four proposed mechanisms underlying cognitive problems in cancer patients.[44-46] Given that cognitive impairment precedes dementia, we hypothesised that these mechanisms may also underlie a causal association between cancer and dementia. Firstly, shared risk factors for cancer and dementia such as higher age, genetics, and smoking may increase the risk of both cancer and dementia. Secondly, the tumour itself can induce different biological processes including inflammation, vascular changes, oxidative stress, and production of extracellular vesicles that can affect cognitive function.[44] These processes may differ between different types of cancer and disease stages. Thirdly, cancer treatment can accelerate the ageing process by inducing DNA damage, telomere shortening, oxidative stress, inflammation, and changes in hormonal levels.[45,47] In addition, chemotherapy can have direct neurotoxic effects.[46,48] Fourthly, psychological distress including depression, anxiety, stress, and fatigue may be caused by cancer diagnosis and cancer treatment. Such factors can also affect cognitive function and the risk of dementia.[49]

Each of these causal pathways may require adjustment for different confounding structures. For this reason, we recommend future studies to specify the specific pathway of interest. Importantly, measurement error for the proxy of cancer diagnosis may be different for each of these pathways. In addition, even if the causal pathway has been correctly specified, one must assume to have all required information to control for the collider-bias that is induced by condition on surviving (**Figure 6**).

In conclusion, our findings indicate that the type of study design can influence results when studying diseases that are strongly related to death. When taking immortal time bias and the competing risk of death into account, we found that patients with cancer did not have a lower risk of dementia than those without a history of cancer, nor did they have a higher risk. Given the ill-defined definition of cancer diagnosis, we cannot answer the causal effect of cancer on the risk of dementia. Future studies should further disentangle the processes underlying a cancer diagnosis to estimate the causal effect.

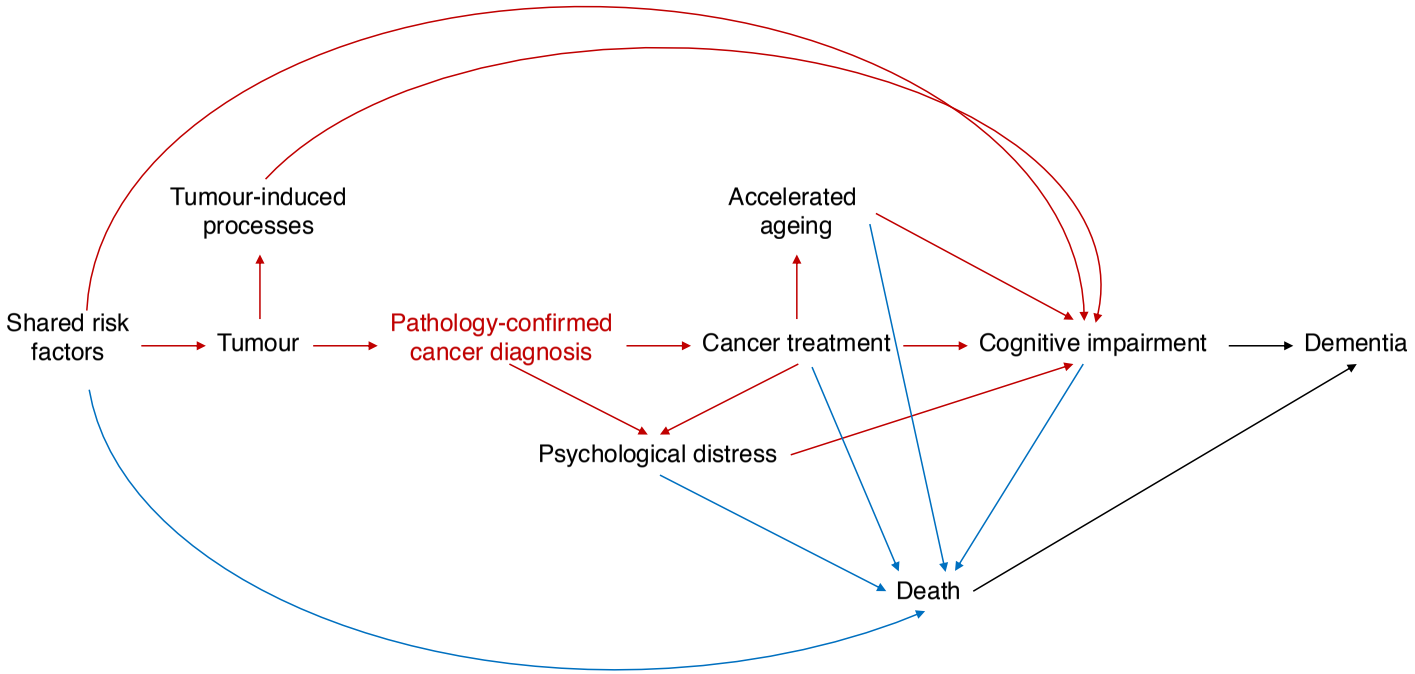

Figure 6 Directed acyclic graph of the relation between cancer and dementia.
When studying the relation between cancer and dementia, cancer is most often defined as cancer diagnosis that is confirmed by pathology. If the question is aetiological, i.e., does cancer cause dementia, then we should redefine this definition of cancer, because the diagnosis itself does not cause dementia. To estimate the causal effect of cancer on dementia, we should take the following four processes into account: (i) shared risk factors for both cancer and dementia such as age, genetics, smoking, and alcohol use; (ii) biological processes induced by the tumour itself including inflammation, vascular changes, oxidative stress, and production of extracellular vesicles; (iii) cancer treatment that may accelerate the ageing process by causing DNA damage, changes in hormonal levels, and oxidative stress; and (iv) psychological distress, including depression, anxiety, stress, and fatigue caused by cancer and cancer treatment. Given that multiple arrows point towards death, conditioning on survival may induce collider bias.

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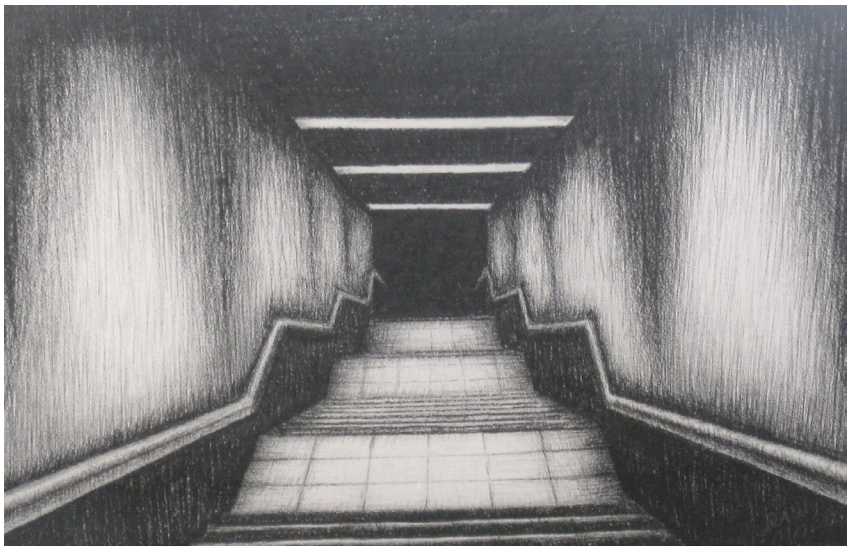
Part IV

Underlying mechanisms



Chapter 13

Inflammation markers and cognitive performance in breast cancer survivors 20 years after completion of chemotherapy

van der Willik KD, Koppelmans V, Hauptmann M, Compter A, Ikram MA, Schagen SB

ABSTRACT

Background Inflammation is an important candidate mechanism underlying cancer and cancer treatment-related cognitive impairment. We investigated levels of blood-cell based inflammatory ratios in breast cancer survivors on average twenty years after chemotherapy and explored the relation between these ratios and global cognitive function.

Methods One hundred sixty-six breast cancer survivors who received post-surgical radiotherapy and six cycles of adjuvant cyclophosphamide, methotrexate, and fluorouracil (CMF) chemotherapy on average twenty years before enrolment were compared with 1344 cancer-free women from a population-based sample (aged between 50 and 80 years). Breast cancer survivors were excluded if they used adjuvant hormonal therapy, or if they developed relapse, metastasis, or second primary malignancies. Systemic inflammation status was assessed by the granulocyte-to-lymphocyte ratio (GLR), platelet-to-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII). Cognitive function was assessed using an extensive neuropsychological test battery from which the general cognitive factor was derived to evaluate global cognitive function. We examined the association between cancer, the general cognitive factor, and inflammatory ratios using linear regression models.

Results Breast cancer survivors had a lower general cognitive factor than non-exposed participants from the comparator group (mean difference = -0.21 [95% confidence interval [CI] = -0.35 to -0.06]). Inflammatory ratios were higher in cancer survivors than in non-exposed participants (mean difference for log[GLR] = 0.31 [95% CI = 0.24 to 0.37], log[PLR] = 0.14 [95% CI = 0.09 to 0.19], log[SII] = 0.31 [95% CI = 0.24 to 0.39]). The association between higher levels of inflammatory ratios and lower general cognitive factor was statistically significant in cancer survivors but not among non-exposed participants. We found a group-by-inflammatory ratio interaction: cancer survivors showed additional lower general cognitive factor per standard deviation increase in inflammatory ratios (*P* for interaction for GLR = .038, PLR = .003, and SII = .033).

Conclusions This is the first study to show that (i) cancer survivors have higher levels of inflammation on average twenty years after treatment; and (ii) these inflammatory levels are associated with lower cognitive function. Although this association needs verification by a prospective study to determine causality, our findings can stimulate research on the role of inflammation in long-term cognitive problems and possibilities to diminish such problems.

INTRODUCTION

Patients with cancer frequently report cognitive problems that can affect their quality of life and daily functioning substantially. Studies have shown that patients with non-central nervous system (non-CNS) cancer can experience cognitive problems during and after completion of treatment including chemotherapy, and a subgroup of patients had cognitive problems up to twenty years after treatment.[1,2]

The cancer survivor population is ageing and growing because of increased life expectancy and more specifically because of advances in cancer treatment and improved screening. In turn, this has resulted in an increasing number of cancer survivors coping with cognitive problems. The driving forces underlying these cognitive problems have not been sufficiently clarified, impeding the approach and process of developing effective interventions. Cognitive problems in cancer patients could be induced by cancer itself, cancer-related treatment, or shared risk factors for the development of both cancer and cognitive problems.[3,4] Disentangling the effects and mechanisms of these causes of disruption of normal cognitive function is challenging. Different mechanisms, including genetic susceptibility, telomere shortening, changes in hormone levels, and inflammation, have been proposed and revealed.[3]

In recent years, inflammation in particular has been suggested as an important and potentially intervenable mechanism in the pathogenesis of cognitive problems in patients with cancer. Higher levels of inflammatory factors such as cytokines are observed in patients with cancer prior to start of any treatment,[5] during chemotherapy,[6-10] and after chemotherapy[11,12] up to five years after treatment initiation.[13] Several studies have found an association between cytokines and cognitive impairment in patients with cancer across different cognitive domains, such as psychomotor speed,[8] executive functioning,[14] and memory.[5,10,11,13] However, these studies did not agree on the involved cytokines or on the affected cognitive domain. Also, because the longest follow-up in these studies was five years, it remains unknown whether inflammation also has a role in long-term or late cognitive problems. Filling this knowledge gap is important as insight into underlying causes of (long-term) cognitive impairment helps to identify those cancer patients at increased risk of developing cognitive problems and opens venues for preventive and therapeutic interventions.

Most studies examined the inflammation status by investigating cytokines using different cytokine panels.[5,6,8-19] In contemporary studies, systemic inflammatory response ratios measured in blood, including the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII), are increasingly used. These ratios have reliable prognostic and predictive value in patients with cancer and can easily

be calculated from readily available standard full blood examination, making them more convenient to use in a clinical setting.[20-24] If related to cognitive problems, these ratios could potentially be used as biomarkers for cancer-related cognitive impairment.

In this study, we investigated global cognitive function, levels of blood cell-based inflammatory ratios, and their relation in breast cancer survivors who had received post-surgical radiotherapy and six cycles of adjuvant cyclophosphamide, methotrexate, and fluorouracil (CMF) chemotherapy on average more than twenty years previously. We furthermore examined whether inflammation and cognitive function were differentially associated between breast cancer survivors and cancer-free women from a population-based sample.

METHODS

Study population

In this study, we selected women who had survived breast cancer and had received adjuvant CMF chemotherapy. We compared them with women from the general population, who were cancer-free and had never received chemotherapy.

Breast cancer survivors

Women with a history of unilateral, invasive breast cancer were identified on the basis of registries of the Netherlands Cancer Institute in Amsterdam and the Daniel den Hoed Cancer Clinic of the Erasmus Medical Centre in Rotterdam as described previously.[2] Briefly, women were selected if they had received post-surgical radiotherapy and six cycles of adjuvant CMF chemotherapy between 1976 and 1995.

Breast cancer survivors were eligible if they were 50 to 80 years old at time of inclusion in 2008, if invasive breast cancer was their first and only malignancy, if they had not developed relapse or distant metastasis, if they had sufficient command of the Dutch language, and if they did not have any contraindications for magnetic resonance imaging (MRI). In addition, ever use of hormonal therapy was applied as an exclusion criterion. Since adjuvant hormonal therapy was not part of the standard treatment for breast cancer patients in the Netherlands until the mid-1990s, only a few women received this treatment. To enhance homogeneity within the group of breast cancer survivors, we included hormone treatment-naive cancer survivors only.

Three hundred fifty-nine breast cancer survivors were assessed for eligibility and 292 were selected. Of these 292 women, 196 agreed to participate and provided informed

consent. We previously reported on cognitive function in these survivors in comparison with cancer-free women identified within the Rotterdam Study.[2] For the current study, the following additional inclusion criteria were defined: availability of blood measurements and completeness of neuropsychological test data to calculate the general cognitive factor. Thirty of the 196 (15.3%) breast cancer survivors were excluded because of missing data on blood measurements (n=5) and incomplete data of neuropsychological tests (n=25, **Figure 1A**). Because breast cancer survivors did not receive an extensive dementia screening, history of dementia was not applied as an exclusion criterion. However, based on the interviews with a trained psychologist, subjective memory complaints, cognitive tests, and brain MRI, it is unlikely that the included breast cancer survivors had dementia at the time of examinations.

Population-based non-exposed participants

Cancer-free women were selected from the Rotterdam Study, an ongoing population-based prospective cohort that started in 1989 in Rotterdam, the Netherlands. The main objective of the Rotterdam Study is to investigate risk factors of diseases in the elderly. By the end of 2008, the Rotterdam Study consisted of three subcohorts, comprising 14 926 individuals. The design of the Rotterdam study was described in detail previously.[25]

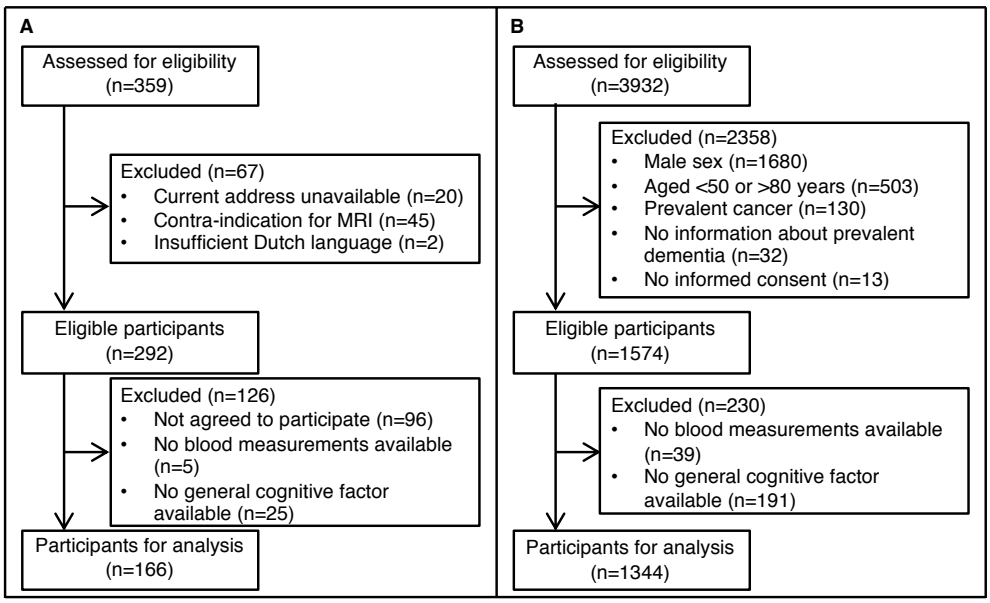


Figure 1 (A) Flowchart for breast cancer survivors, (B) Flowchart for non-exposed participants.

The third subcohort (RS-III) started in 2006 and was the first cohort in which an extensive set of neuropsychological tests was implemented at baseline. For this reason, RS-III was chosen as the reference subcohort, which was composed of 3392 participants (65% out of invitees). From these participants, women between 50 and 80 years old without a history of cancer or dementia were eligible as non-exposed participants (n=1574). This sample comprised the non-exposed participants used in our previous cognitive study.[2] Two hundred thirty persons were additionally excluded because of lack of blood measurements (n=39) and incomplete data of neuropsychological tests (n=191), resulting in 1344 non-exposed participants (**Figure 1B**).

Assessment of inflammatory ratios

All participants had fasting blood samples taken during the research centre visit. Full blood count measurements were performed by using a COULTER® Ac·T diff2™ Haematology Analyser (Beckman Coulter, San Diego, California, USA) directly after the blood sample was drawn. Haematological measurements included absolute granulocyte, lymphocyte, and platelet counts in 10^9 per litre.

We used the granulocyte count as proxy for the neutrophil count because we did not have this measurement available in our sample. Because most of the granulocytes are represented by neutrophils, we believe this did not affect our results.[26,27] For accuracy purposes, we will refer to the granulocyte-to-lymphocyte ratio (GLR) instead of using the term NLR.

The GLR and PLR were calculated as the ratio of granulocyte count to lymphocyte count, and as the ratio of platelet count to lymphocyte count, respectively.[28] The SII was defined as platelet count times the GLR.[22] Because they are either ratios or indices, the derived inflammatory ratios did not have a unit.

Assessment of cognitive function

Cognitive function was evaluated between November 2009 and June 2010 for breast cancer survivors and between February 2006 and December 2008 for non-exposed participants on the same day as the blood sample was drawn. Cognitive function was assessed by a neuropsychological test battery in the research centre of the Rotterdam Study. Six tests were administrated: the Mini Mental State Examination, Letter-Digit Substitution Test (LDST), Word Fluency Test (WFT), Stroop Test (Reading, Naming, and Interference), Purdue Pegboard Test (PPT, right, left, and both hands) and 15-Word Learning Test (15-WLT, Immediate recall, Delayed recall, and Recognition). Global cognitive function was assessed via the general cognitive factor, which was generated by using principal component analysis of the following tests: LDST (total completion time), WFT (number of words), Stroop interference (time in

seconds, adjusted for errors), PPT (total number of pins across three subtasks), and 15-WLT (number of words during delayed recall).[29]

Other assessments

We assessed educational level (primary: primary education, lower: lower general education, intermediate general education, or lower vocational education, intermediate: intermediate vocational education or higher general education, higher: higher vocational education or university) and smoking status (current, former, and never) by interview. Body mass index (BMI, kg/m^2) was computed from measurements of height and weight. Diabetes mellitus was defined as use of antidiabetic medication, a fasting serum glucose level of at least 7.1 mmol/L, or a random serum glucose level of at least 11.1 mmol/L.[30] History of stroke and myocardial infarction were assessed by interview.[31,32] Symptoms of depression were evaluated with the Centre for Epidemiologic Studies Depression scale (CES-D), which was converted to a sum-score.[33] We had no information about anxiety and fatigue and could therefore not control for these symptoms.

Statistical analyses

Linear regression models were used to investigate mean differences in the general cognitive factor and inflammatory ratios between breast cancer survivors and non-exposed participants. Inflammatory ratios were logarithmic transformed because of their skewed distribution. We constructed two nested models: Model I was adjusted for age (continuous) and education (four categories) and Model II was additionally adjusted for smoking status (three categories), BMI (continuous), diabetes mellitus (yes or no), history of stroke (yes or no), history of myocardial infarction (yes or no), and CES-D sum-score (continuous). To investigate whether levels of the general cognitive factor were explained by different inflammatory ratios, we adjusted additionally for each inflammatory ratio separately.

The association between the general cognitive factor and inflammatory ratios was investigated for breast cancer survivors and non-exposed participants using linear regression models. To study whether this association was stronger in breast cancer survivors than in non-exposed participants, we computed interaction terms between history of cancer/cancer treatment and each inflammatory ratio. We explored effect modification by stratifying for mean BMI.

Since mean age was higher in the breast cancer survivors than in the non-exposed participants (**Table 1**), we repeated all analyses using age-matched non-exposed participants to minimise residual confounding. These analyses provided comparable estimates to using all non-exposed participants and therefore are not reported separately.

Multiple imputation was used for missing data on covariates (generally between 0.1% and 0.3% with a maximum of 1.8% for the CES-D sum-score) with five imputed datasets, based on history of cancer/cancer treatment, inflammatory ratios, general cognitive factor, and other covariates (i.e., age, sex, education, BMI, smoking status, presence of diabetes mellitus, history of stroke, history of myocardial infarction, and CES-D sum-score). Rubin's method was used for pooled regression coefficients (β) and 95% confidence intervals (CIs).[34] All analyses were performed by using IBM SPSS Statistics Version 24.0 and RStudio Version 3.3.2. All statistical tests were two-sided, and a *P*-value of less than .05 was considered as statistically significant.

RESULTS

Characteristics of breast cancer survivors and non-exposed participants are presented in **Table 1**. Breast cancer survivors were older than non-exposed participants. Additionally, they generally had completed higher levels of education and had more often had diabetes mellitus and a history of myocardial infarction. Lastly, although the numbers of never smokers were similar between the two groups, breast cancer survivors were more frequently former smokers and less often current smokers.

Inflammatory ratios

Breast cancer survivors had higher median levels of GLR, PLR, and SII than non-exposed participants. History of breast cancer/cancer treatment was associated with higher inflammatory ratios, also after adjustment for age, education, smoking, BMI, diabetes mellitus, history of stroke, history of myocardial infarction, and CES-D sum-score (mean difference for log[GLR] = 0.31, 95% CI = 0.24 to 0.37, log[PLR] = 0.14, 95% CI = 0.09 to 0.19, log[SII] = 0.31, 95% CI = 0.24 to 0.39, **Table 2**). Inflammatory ratios were positively associated with age in both groups.[35]

Cognitive function

Breast cancer survivors had a lower general cognitive factor than non-exposed participants (mean difference = -0.21, 95% CI = -0.35 to -0.06, corresponding with an effect of 3.6 years of age given a decline in general cognitive factor of 0.59 points per 10 years, **Table 2**).[29] Further adjustment for inflammatory factors changed the estimates slightly, indicating that inflammatory ratios explained only a small part of the difference in general cognitive factor

Table 1 Demographics and characteristics of breast cancer survivors and non-exposed participants.

Characteristic	Breast cancer survivors (n=166)	Non-exposed participants (n=1344)	*P*
Age, years, mean (SD)	64.0 (6.7)	57.9 (5.2)	<.001
Educational level, No. (%)			<.001
Primary	14 (8.4)	158 (11.8)	
Lower	59 (35.5)	616 (45.8)	
Intermediate	33 (19.9)	287 (21.4)	
Higher	60 (36.1)	283 (21.1)	
Body mass index, kg/m^2, mean (SD)	26.9 (4.6)	27.4 (4.8)	.18
Smoking status, No. (%)			<.001
Never	57 (34.3)	475 (35.3)	
Former	93 (56.0)	574 (42.7)	
Current	16 (9.6)	295 (21.9)	
Diabetes mellitus, No. (%)	14 (8.4)	54 (4.0)	.01
History of stroke, No. (%)	1 (0.6)	19 (1.4)	.72
History of myocardial infarction, No. (%)	6 (3.6)	11 (0.8)	.001
CES-D sum-score, median (IQR)	6 (4 to 9)	6 (4 to 10)	.08
General cognitive factor, mean (SD)	-0.39 (1.14)	0.05 (0.97)	<.001
Inflammatory ratios, median (IQR)			
GLR	2.06 (1.67 to 2.66)	1.52 (1.20 to 1.92)	<.001
PLR	145 (119 to 176)	124 (102 to 151)	<.001
SII	618 (469 to 796)	443 (328 to 595)	<.001
Age at cancer diagnosis, years, mean (SD)	42.9 (5.6)		
Time since cancer diagnosis, years, mean (SD)	21.0 (4.5)		

CES-D = Centre for Epidemiologic Studies Depression Scale, IQR = interquartile range, GLR = granulocyte-to-lymphocyte ratio, PLR = platelet-to-lymphocyte ratio, SD = standard deviation, SII = systemic immune-inflammation index.

in addition to the effect of history of cancer/cancer treatment (mean difference for history of cancer/cancer treatment after adjustment for log[GLR] = -0.18, 95% CI = -0.33 to 0.02, log [PLR] = -0.21, 95% CI = -0.36 to 0.06, log[SII] = -0.19, 95% CI = -0.34 to 0.03).

Table 2 Association between the general cognitive factor and history of cancer, and inflammatory ratios and history of cancer.

	Model I	Model II
Outcome*	Mean difference (95% CI)	Mean difference (95% CI)
Inflammatory ratio†		
Granulocyte-to-lymphocyte ratio	0.30 (0.24 to 0.36)	0.31 (0.24 to 0.37)
Platelet-to-lymphocyte ratio	0.16 (0.10 to 0.21)	0.14 (0.09 to 0.19)
Systemic immune-inflammation index	0.30 (0.23 to 0.38)	0.31 (0.24 to 0.39)
Cognition‡		
General cognitive factor	-0.18 (-0.34 to -0.03)	-0.21 (-0.35 to -0.06)

Model I is a linear regression of the general cognitive factor or logarithmic transformed inflammatory ratios on cancer status adjusted for age and education. Model II is as Model I plus adjustment for smoking status, body mass index, diabetes mellitus, history of stroke, history of myocardial infarction, and CES-D sum-score.
** All types inflammatory ratios were natural logarithmic transformed. † Mean difference in general cognitive factor between breast cancer survivors and non-exposed participants. ‡ Mean difference in inflammatory ratios between breast cancer survivors and non-exposed participants.*
CI = confidence interval.

Association between cognitive function and inflammatory ratios by cancer status

A lower general cognitive factor was associated with higher inflammatory ratios in breast cancer survivors (**Table 3**). In non-exposed participants, higher inflammatory ratios tended to be associated with a lower general cognitive factor, albeit not statistically significant.

The interaction term between inflammatory ratios and history of cancer/cancer treatment was significant for each inflammatory ratio, indicating that the association between higher inflammation levels and lower general cognitive factor was more pronounced in breast cancer survivors than in non-exposed participants (*P* for interaction between cancer and standardised logarithmic transformed GLR = .038, PLR = .003, and SII = .033, **Figure 2**).

The association between higher inflammatory ratios and lower general cognitive factor differed more between breast cancer survivors and non-exposed participants with a higher BMI than in those with a lower BMI. However, stratified analyses for BMI showed that the effect of one standard deviation increase in inflammatory ratio on general cognitive factor was higher among breast cancer survivors with a BMI below 27.3 kg/m^2 than among those with a higher BMI (**Table 4**).

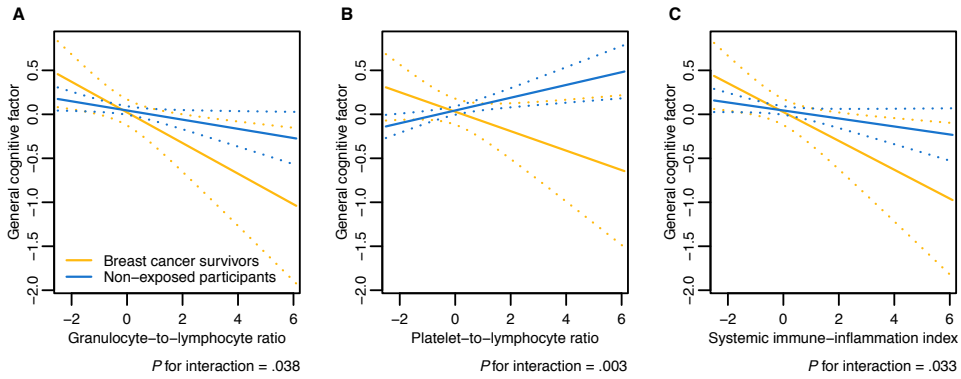


Figure 2 (A) Interaction of log(GLR) and cancer status with the general cognitive factor as outcome. (B) Same as A, for log (PLR). (C) Same as A and B, for log(SII).
Model used for figure is only adjusted for age.
GLR = granulocyte-to-lymphocyte ratio, PLR = platelet-to-lymphocyte ratio, SII = systemic immune-inflammation index.

Table 3 Association between the general cognitive factor and inflammatory ratios in breast cancer survivors and in non-exposed participants.

	Breast cancer survivors	**Non-exposed participants**	
Inflammatory ratio per SD increase*	Mean difference† (95% CI)	Mean difference† (95% CI)	*P* for interaction‡
Model I			
Granulocyte-to-lymphocyte ratio	-0.24 (-0.40 to -0.08)	-0.04 (-0.09 to 0.00)	.06
Platelet-to-lymphocyte ratio	-0.13 (-0.29 to 0.03)	0.05 (0.01 to 0.10)	.003
Systemic immune-inflammation index	-0.22 (-0.38 to -0.07)	-0.03 (-0.08 to 0.01)	.05
Model II			
Granulocyte-to-lymphocyte ratio	-0.23 (-0.39 to -0.08)	-0.02 (-0.07 to 0.02)	.04
Platelet-to-lymphocyte ratio	-0.18 (-0.33 to -0.02)	0.03 (-0.01 to 0.08)	.003
Systemic immune-inflammation index	-0.23 (-0.38 to -0.07)	-0.01 (-0.06 to 0.03)	.03

Model I is a linear regression of the general cognitive factor on each logarithmic transformed inflammatory ratio adjusted for age and education. Model II is as Model I plus adjustment for smoking status, body mass index, diabetes mellitus, history of stroke, history of myocardial infarction, and CES-D sum-score.
** All types inflammatory ratios were natural logarithmic transformed. † Mean difference in general cognitive factor per standard deviation increase in inflammatory ratio. ‡ P-value for interaction term between history of cancer/cancer treatment and inflammatory ratio.*
CI = confidence interval.

Table 4 Association between the general cognitive factor and inflammatory ratios in breast cancer survivors and in non-exposed participants stratified for mean body mass index.

	Breast cancer survivors	Non-exposed participants	
Inflammatory ratio per SD increase*	Mean difference† (95% CI)	Mean difference† (95% CI)	*P* for interaction‡
Body mass index <27.3 kg/m^2	n=104	n=749	
Granulocyte-to-lymphocyte ratio	-0.29 (-0.49 to -0.10)	-0.04 (-0.10 to 0.02)	.48
Platelet-to-lymphocyte ratio	-0.22 (-0.42 to -0.02)	0.01 (-0.05 to 0.08)	.31
Systemic immune-inflammation index	-0.28 (-0.48 to -0.09)	-0.04 (-0.10 to 0.02)	.56
Body mass index <27.3 kg/m^2	n=62	n=595	
Granulocyte-to-lymphocyte ratio	-0.16 (-0.41 to 0.09)	0.01 (-0.06 to 0.08	.01
Platelet-to-lymphocyte ratio	-0.16 (-0.42 to 0.10)	0.05 (-0.02 to 0.12	<.001
Systemic immune-inflammation index	-0.12 (-0.38 to 0.14)	0.02 (-0.05 to 0.09	.005

Model I is a linear regression of the general cognitive factor on each log transformed inflammatory ratio adjusted for age and education. Model II is as Model I plus adjustment for smoking status, diabetes mellitus, history of stroke, history of myocardial infarction, and CES-D sum-score.
** All types inflammatory ratios were natural logarithmic transformed. † Mean difference in general cognitive factor per standard deviation increase in inflammatory ratio. ‡ P-value for interaction term between history of cancer/cancer treatment and inflammatory ratio.*
CI = confidence interval, SD = standard deviation.

DISCUSSION

This study is the first report investigating the association between blood-cell-based inflammatory ratios and cognitive function in breast cancer survivors with an average time since cessation of chemotherapy of more than twenty years. Breast cancer survivors had lower global cognitive function and higher inflammatory ratios compared with women without a history of cancer. The tendency for lower global cognitive function with higher inflammatory ratios was more pronounced in breast cancer survivors, suggesting a potential role for inflammation in the pathophysiology of cognitive problems in cancer survivors. This effect was not modified by BMI. More insight in mechanisms underlying cognitive problems could help identifying those women who are at an increased risk of cognitive problems and developing prevention strategies.

We previously reported on differences in cognitive function between breast cancer survivors and non-exposed participants.[2] In this previous study, we tested between-group performance differences of individual cognitive outcome measures that were currently used to construct the

general cognitive factor and observed that breast cancer survivors performed worse compared to non-exposed participants within several cognitive domains. This suggested that cognitive problems in cancer survivors can be long-lasting. In the present study, we evaluated global cognitive function using the general cognitive factor because we did not expect a specific cognitive domain to be affected by inflammation. We chose to use a robust cognitive summary measure, thereby reducing the number of comparisons.

Interestingly, levels of inflammatory ratios were higher in breast cancer survivors than in non-exposed participants, on average twenty years after cancer treatment. Inflammation plays a critical role in tumorigenesis, tumour progression, and cancer metastasis.[36,37] Research has shown that chronic inflammation is associated with an increased cancer risk.[37] Moreover, different markers of inflammation, such as cytokines, C-reactive protein, and NLR, are often elevated in cancer patients and are associated with poor survival.[9,15-17,24,38] One study investigating inflammation levels after cancer treatment found that C-reactive protein and cytokine levels were elevated up to five years after treatment.[19] Our observation that systemic inflammation ratios are higher in breast cancer survivors than in non-exposed participants on average twenty years after cancer treatment suggests deregulation of the immune system. Whether this is a consequence of cancer or cancer treatment (or both), or a pre-existing deregulation before cancer development cannot be determined with the current study.

The found association of blood-cell based inflammatory ratios and cognitive function in breast cancer survivors is in line with previous observations before, during, and shortly after cancer therapy.[6,17,18] Two studies investigated the link between inflammation and cognitive function prior to start of cancer treatment. The first study showed that elevated levels of interleukin-6 (IL-6) in patients with acute myelogenous leukaemia or myelodysplastic syndrome were associated with poorer executive functioning before cancer treatment.[14] The second study showed that high levels of soluble tumour necrosis factor receptor type II (sTNF-RII) were related to reduced verbal memory function in newly diagnosed breast cancer patients.[5] More studies in breast cancer patients have tried to elucidate the role of inflammation in impaired cognitive function during chemotherapy and two of these studies identified specific cytokines to be involved. Williams et al. focused on sTNR-RII and found that higher levels of this receptor were associated with visual memory function.[10] Cheung et al. observed an association between increased levels of IL-6 and IL-1β, and poorer psychomotor speed function during chemotherapy.[8] Shortly after cancer treatment, higher levels of sTNF-RII were associated with increased memory complaints,[11] and on average five years after cancer treatment, elevated IL-6 and TNFα levels were associated with worse verbal memory.[13] Importantly, the association between inflammation and cognitive function is supported by animal studies. Acute peripheral immune challenges using lipopolysaccharide resulted

in cognitive impairments in a spatial working memory task in mice. Cognitive impairments were observed 1.5-2 hours after injection in tumour-bearing mice but not in tumour-free mice. These cognitive effects could be prevented when using a technique to enhance innate immune reactivity.[39] Together, these results support the hypothesis that inflammation has a role in the complex pathogenesis of both short-term and longer-term cognitive problems in cancer patients.

Owing to our study design, we cannot determine whether the association between inflammation and impaired cognitive function is causal. However, also a causal association could not illuminate the exact underlying mechanisms by which inflammation leads to brain changes and subsequent cognitive problems. Peripheral pro-inflammatory cytokines are able to cross the blood-brain barrier, which may initiate the release of local cytokines.[40] Local cytokine production could result in neurotransmitter deregulation, increased oxidative stress, and decreased neurogenesis and neuroplasticity, which in turn can lead to cognitive dysfunction.[41] It is also possible that inflammation induces epigenetic changes and chromosomal instability, which can be persistent and therefore could be associated with long-term cognitive problems.[42]

Our study has several strengths. First, we have a large sample size of breast cancer survivors who have been treated on average more than twenty years ago, enabling us to investigate long-term effects. Moreover, we used non-exposed participants from a population-based cohort study, who underwent the same examinations as the breast cancer survivors. This design provided standardised ascertainment of outcome and covariates. All participants received a neuropsychological test battery, enabling us to investigate global cognitive function by the general cognitive factor. Lastly, we were able to investigate inflammation status using blood cell-based inflammatory ratios, which are low-cost and easy to use in the clinic.

Study limitations include the design by which we cannot disentangle the effects of cancer and cancer treatment on cognition and levels of inflammatory ratios. Some studies show that patients treated with chemotherapy have higher inflammatory ratios during and after treatment than chemotherapy-naïve patients.[12] However, because inflammatory ratios and cognitive problems can already occur in newly diagnosed cancer patients, it is unlikely that inflammation is only important in chemotherapy-treated patients.[5] Owing to the cross-sectional design, we do not have information about cognitive function and levels of inflammatory ratios before cancer diagnosis and treatment. Moreover, breast cancer patients nowadays receive chemotherapy regimens other than CMF, either with or without adjuvant endocrine therapy, limiting the generalisability to current breast cancer patients. However, cyclophosphamide and 5-fluoroacil are still frequently used in other regimens for adjuvant chemotherapy. Furthermore, we were not able to exclude individuals whose systemic inflammatory ratios may have been elevated due to acute infections and to control for acute-phase reactants such

as C-reactive protein, but we expect that this effect is similar for cancer survivors and non-exposed participants. Lastly, we need to emphasise that by measuring the GLR, PLR, and SII, we cannot identify the exact phenotype of the underlying immune cell populations. Although these ratios are proven to be related to chronic systemic inflammation, it is unknown whether they also reflect higher levels of pro-inflammatory cytokines. In other words, we cannot confirm that observed shifts in the granulocytes, lymphocytes, and platelets cause higher cytokine levels and thereby are functional. To elucidate the exact immune cell populations involved in increases of the GLR, PLR, and SII, determination of different cytokines is needed.

In conclusion, we found that breast cancer survivors who had been treated with chemotherapy on average more than twenty years ago have higher blood cell-based inflammatory ratios than women without a history of cancer. Higher levels of inflammatory ratios tended to be associated with poorer cognitive function in both cancer survivors and cancer-free women, and expression was stronger in breast cancer survivors. This finding suggests that inflammation could have a role in the pathogenesis of long-term cognitive impairment in cancer survivors. Further prospective studies are important to determine the causality of the association and to investigate the effects of lowering inflammation on the development of cognitive problems in cancer patients and survivors, for instance by exercise or anti-inflammatory drugs.

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Chapter 14

Balance between innate versus adaptive immunity and the risk of dementia

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ABSTRACT

Background Immunity has been suggested to be important in the pathogenesis of dementia. However, the contribution of innate versus adaptive immunity in the development of dementia is not clear. In this study, we aimed to investigate (i) the association between components of innate immunity (granulocytes and platelets) and adaptive immunity (lymphocytes) with risk of dementia; and (ii) the association between their derived ratios (granulocyte-to-lymphocyte ratio [GLR], platelet-to-lymphocyte ratio [PLR], and systemic immune-inflammation index [SII]), reflecting the balance between innate and adaptive immunity, with risk of dementia.

Methods Blood cell counts were measured repeatedly between 2002 and 2015 in dementia-free participants of the prospective population-based Rotterdam Study. Participants were followed-up for dementia until January 1st, 2016. Joint models were used to determine the association between granulocyte, platelets, and lymphocyte counts, and their derived ratios with risk of dementia.

Results Of the 8313 participants (mean [standard deviation] age 61.1 [7.4] years, 56.9% women), 664 (8.0%) developed dementia during a median follow-up of 8.6 years. Doubling of granulocyte and platelet counts tended to be associated with an increased risk of dementia (hazard ratio [HR] for granulocytes = 1.22, [95% confidence interval [CI] = 0.89 to 1.67] and for platelets = 1.45 [1.07 to 1.95], respectively). Doubling of the derived ratios GLR, PLR, and SII was associated with an increased dementia risk (HR [95% CI] for GLR = 1.26 [1.03 to 1.53], for PLR = 1.27 [1.05 to 1.53], and for SII = 1.15 [0.98 to 1.34]).

Conclusions GLR, PLR, and SII are associated with an increased risk of dementia in the general population. This supports the role of an imbalance in the immune system towards innate immunity in the pathogenesis of dementia.

INTRODUCTION

Dementia poses a huge burden on societies in terms of financial costs as well as on individual patients and their caregivers regarding suffering and grief.[1] Dementia is a multifactorial disease, in which various pathologies interact during the long preclinical phase, ultimately resulting in its clinical manifestations of cognitive decline and loss of independence. While amyloid depositions, neuronal loss, and vascular damage have long been established as key pathologies underlying dementia,[2] recent findings point towards a key role for the immune system.[3-5] The immune system is a highly complex system involving multiple synergistic and antagonistic substrates, yet broadly can be classified into two components, i.e., innate immunity and adaptive immunity.[6] Innate immunity refers to immune responses present at birth, forming a first line of defence, whereas adaptive immunity is acquired during life by exposure to specific antigens.[7] High activity of innate immunity can lead to disrupted neuronal integrity and ultimately to cell death.[8] Although these components of the immune system work closely together, adaptive immunity is considered to be more neuroprotective than innate immunity, presumably by stimulating phagocytosis of amyloid fibrils.[9,10]

Exact quantification of these opposing components of the immune system is challenging and focus of ongoing research, but recent work from the field of cancer research has suggested that easily obtainable laboratory measurements may in fact capture their relative activity levels to a reliable degree.[11] Measuring granulocytes, including the most abundant subtype neutrophils, and platelets provides important markers of the innate immunity, whereas measuring lymphocytes yields information on the adaptive immunity.[12,13] Furthermore, combining these measurements into ratios, i.e., the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII), is thought to even better reflect the relative balance between innate and adaptive immunity.[11,14-16] Previous work on the link between innate versus adaptive immunity and dementia has shown higher NLR and PLR in dementia patients than in healthy individuals.[17-19] Yet, to really understand the role of the immune system in the risk of developing dementia, it is pivotal to study how these markers change during the preclinical phase of the disease.

We thus investigated the longitudinal association of markers of the innate versus adaptive immune system with the risk of dementia. The underlying hypothesis was that higher activity of the innate versus adaptive immune system would be associated with an increased risk of dementia. A further methodological novelty of our study was the use of joint modelling that enabled us to study the longitudinal evolution of the various markers during the preclinical phase in conjunction with survival analyses.

METHODS

Study population

The present study is embedded in the Rotterdam Study, a prospective population-based cohort study in Rotterdam, the Netherlands. The Rotterdam Study started in 1989 with 7983 persons (response of 78%) aged 55 years and over and residing in the district Ommoord, a suburb of Rotterdam. This first subcohort (RS-I) was extended with a second subcohort (RS-II) in 2000, consisting of 3011 persons (response of 67%) and with a third subcohort (RS-III) in 2006, composed of 3932 persons aged 45 years and over (response of 65%). The design of the Rotterdam Study has been described in detail previously.[20] In brief, participants were examined in detail at study entry and at follow-up visits every three to six years. They were interviewed at home by a trained research nurse, followed by two visits at the research facility for additional interviewing, laboratory assessments, imaging, and physical examinations.

The Rotterdam Study was approved by the Medical Ethics Committee of Erasmus Medical Centre and by the board of The Netherlands Ministry of Health, Welfare, and Sports. A written informed consent was obtained from all participants.

Laboratory tests for granulocytes, platelets, and lymphocytes were introduced from 2002 onwards, corresponding with the following assessment rounds in the Rotterdam Study (baseline in this study): i.e., fourth round of RS-I, second round of RS-II, and first round of RS-III, comprising 9994 participants. From these 9994 eligible participants, we excluded those without complete baseline blood measurements (n=1288). Of the remaining participants, we excluded those with a history of dementia (n=52), participants who were insufficiently screened for dementia (n=62), and those without informed consent to assess medical records during follow-up (n=39). Lastly, we excluded participants with missing apolipoprotein E (*APOE*) genotype (n=240), resulting in 8313 participants for analysis (flowchart of study population is presented in **Figure 1**).

Assessment of blood cell counts and their derived ratios

Fasting blood samples were taken during each visit at the research centre with a maximum of three visits during follow-up. Full blood count measurements were performed using the COULTER® Ac·T diff2™ Haematology Analyser (Beckman Coulter, San Diego, California, USA) directly after blood sample drawn. Laboratory measurements included absolute granulocyte, platelet, and lymphocyte counts in 10^9 per litre. Since neutrophil counts were not available, we used granulocyte count as a reliable proxy given that these are the most abundant subtype of neutrophils.[21,22]

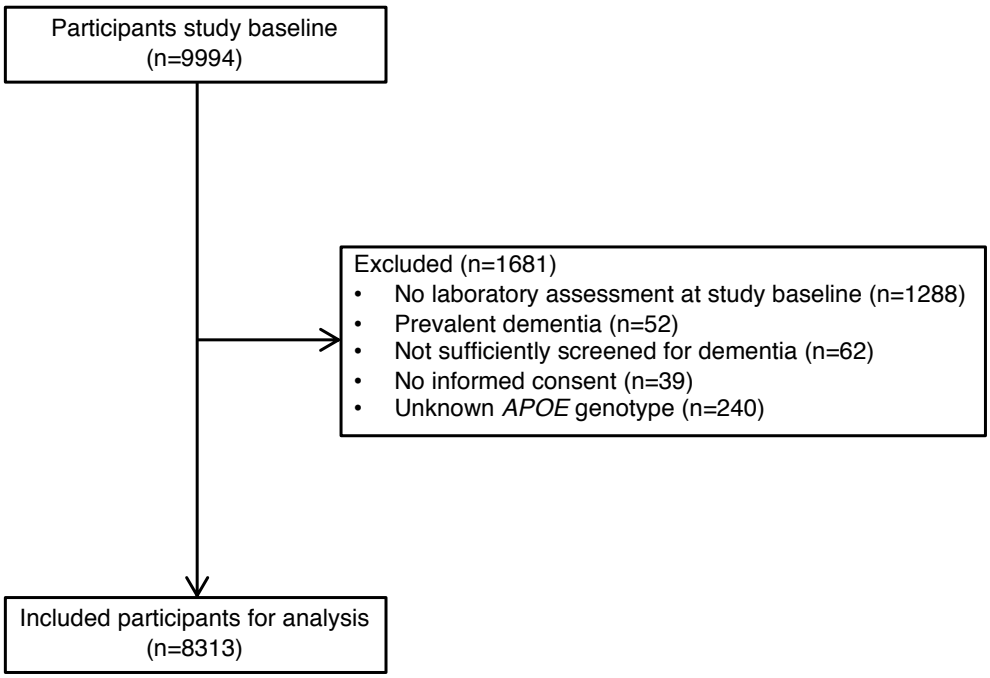

Figure 1 Flowchart participants for analysis association between blood cell counts and their derived ratios, and dementia.
APOE = apolipoprotein E.

The granulocyte-to-lymphocyte ratio (GLR) and PLR were calculated as the ratio of granulocyte count to lymphocyte count, and as the ratio of platelet count to lymphocyte count, respectively. The SII was defined as platelet count times the GLR.

Assessment of dementia

Participants were screened for dementia at baseline and subsequent centre visits with the Mini-Mental State Examination and the Geriatric Mental Schedule organic level.[23] Those with a Mini-Mental State Examination score <26 or Geriatric Mental Schedule score >0 underwent further investigation and informant interview, including the Cambridge Examination for Mental Disorders of the Elderly. The entire cohort was continuously under surveillance for dementia through electronic linkage of the study database with medical records from general practitioners and the regional institute for outpatient mental health care. Available information on clinical neuroimaging was used when required for diagnosis of dementia subtype. A consensus panel led by a consultant neurologist established the final diagnosis according to standard criteria for dementia (Diagnostic and Statistical Manual of Mental Disorders III-revised), Alzheimer's disease (AD, National Institute of Neurological and Communicative Disorders and Stroke and

the Alzheimer's Disease and Related Disorders Association), and vascular dementia (National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherché et l'Enseignement en Neurosciences). Follow-up until January 1st, 2016 was virtually complete (93.8% of potential person-years observed).

Other measurements

We assessed education and smoking by interview. Educational level was classified into primary education, lower (lower general education, intermediate general education, or lower vocational education), intermediate (intermediate vocational education or higher general education), or higher (higher vocational education or university). Smoking status was categorised as never, former, or current smoker. Body mass index (BMI) was computed from measurements of height and weight (kg/m^2). Diabetes mellitus was defined as use of antidiabetic medication, fasting serum glucose level ≥7.1 mmol/L, or random serum glucose level ≥11.1 mmol/L.[24] History of stroke was assessed by interview and verified by reviewing medical records.[25] *APOE* genotype was determined using polymerase chain reaction on coded DNA samples in RS-I and with a bi-allelic TaqMan assay in the two extensions (RS-II and RS-III).[26,27] *APOE* ε4 carrier status was defined as carrier of one or two *APOE* ε4 alleles.

Statistical analysis

We associated the different blood cell counts and their derived ratios with the risk of all-cause dementia using the framework of joint models for longitudinal and survival data. In this way, we are able to account for the endogenous nature (i.e., blood cell counts can be measured with error during follow-up and their values at any time point can be affected by an event occurring at an earlier time point) [28] and the correlations in the repeated measurements of granulocyte, platelet, and lymphocyte counts.[29]

In order to normalise the skewed distribution of granulocyte, platelet, and lymphocyte counts, and their derived ratios, we used a natural logarithmic transformation. Hazard ratios (HRs) with 95% confidence intervals (CIs) were obtained from the joint models, using the piecewise-constant baseline hazard, and multiplied with log(2), providing a HR for doubling of the blood cell counts and their ratios. We computed two nested models: Model I was adjusted for baseline age (continuous, centred as age minus mean age) and sex; Model II was additionally adjusted for education, smoking status, BMI (continuous), diabetes mellitus, history of stroke, and *APOE* ε4 carrier status. For assessment of the association between the individual components of the ratios and dementia, we repeated analyses with adjustment for the baseline blood cell counts of the remaining two blood cell types (for instance, the association of granulocyte count with dementia was adjusted for platelet and lymphocyte

counts). Follow-up time was used as time scale and started at the first laboratory assessment until date of all-cause dementia diagnosis, death, loss to follow-up, or January 1st, 2016, whichever came first. Censoring participants at date of death allowed us to compute cause-specific HRs.

In sensitivity analyses, we repeated all analyses using age as time scale instead of follow-up time to account for potential residual confounding by age and to minimise potential effects of left truncation. We additionally censored for stroke events during follow-up to preclude that the observed effect may be driven by incident strokes that occurred before dementia diagnosis. Moreover, we investigated the association between the ratios and AD or vascular dementia separately. Lastly, we explored effect modification by stratifying by median age, sex, smoking status, diabetes mellitus, and *APOE* ε4 carrier status.

Multiple imputation was used for missing covariates (maximum of 0.99%), with five imputed datasets based on other covariates and the outcome. Rubin's method was used for pooled HRs and 95% CIs.[30] Two-sided $P<.05$ was considered statistically significant. Statistical analyses were performed using the R packages 'survival', 'nlme', 'JM', and 'JMbayes' in RStudio Version 3.3.2.[28,29,31,32]

RESULTS

Characteristics of included and excluded study participants are presented in **Table 1**. An overview of the median blood cell counts and blood cell-based ratios per assessment round is shown in **Supplementary Table 1**. Mean age of included study participants was 61.1 years and 56.9% were women. During a median follow-up of 8.6 years (70 273 person-years), 664 participants developed all-cause dementia (543 AD, 31 vascular dementia) with an incidence rate (95% CI) of 9.4 (8.7 to 10.2) per 1000 person-years.

Higher levels of granulocytes reflecting higher innate immunity were associated with an increased risk of dementia, but only after correcting for the platelet and lymphocyte counts (HR for doubling granulocyte count [95% CI] = 1.33 [0.99 to 1.79], **Table 2**). Doubling of platelets was associated with an increased risk of dementia (HR [95% CI] = 1.48 [1.11 to 1.96]). Regarding adaptive immunity, higher levels of lymphocytes were associated with a decreased risk of dementia (HR for doubling lymphocyte count [95% CI] = 0.80 [0.64 to 0.99]).

Higher levels of GLR, PLR, and SII were associated with an increased dementia risk (HR [95% CI] for doubling GLR = 1.34 [1.10 to 1.63], for PLR = 1.29 [1.08 to 1.55], and for SII = 1.18 [1.02 to 1.39], respectively [**Table 2**]). Risk estimates were comparable when using the

Table 1 Baseline characteristics of the included and excluded study participants.

Characteristic	Included participants (n=8313)	Excluded participants (n=528)*	
		No blood measurements (n=1288)	Unknown *APOE* genotype (n=240)
Age, years, mean (SD)	61.1 (7.4)	72.6 (11.8)	61.7 (8.2)
Women, No. (%)	4729 (56.9)	845 (65.6)	160 (66.7)
Educational level, No. (%)			
Primary	908 (10.9)	233 (18.1)	25 (10.4)
Lower	3329 (40.0)	537 (41.7)	95 (39.6)
Intermediate	2429 (29.2)	336 (26.1)	57 (23.8)
Higher	1588 (19.1)	161 (12.5)	37 (15.4)
Body mass index, kg/m^2, mean (SD)	27.6 (4.3)	27.6 (4.5)	28.2 (4.8)
Smoking status, No. (%)			
Never	2479 (29.8)	403 (31.3)	70 (29.2)
Former	4191 (50.4)	550 (42.7)	106 (44.2)
Current	1595 (19.2)	308 (23.9)	57 (23.8)
Diabetes mellitus, No. (%)	501 (6.0)	136 (10.6)	15 (6.3)
History of stroke, No. (%)	305 (3.7)	54 (4.2)	11 (4.6)
APOE ε4 carrier, No. (%)	2328 (28.0)	244 (18.9)	
Blood cell types, 10^9/L, median (IQR)			
Granulocytes	3.8 (3.1 to 4.7)		4.0 (3.2 to 4.9)
Platelets	263 (223 to 307)		277 (232 to 319)
Lymphocytes	2.2 (1.8 to 2.6)		2.3 (1.9 to 2.8)
Blood cell-based ratios, median (IQR)			
Granulocyte-to-lymphocyte ratio	1.7 (1.4 to 2.3)		1.7 (1.4 to 2.2)
Platelet-to-lymphocyte ratio	120 (96 to 151)		119 (96 to 150)
Systemic immune-inflammation index	455 (339 to 619)		473 (339 to 651)

Values are shown before multiple imputation and therefore not always add up to 100%.
** Excluded participants in this table only include those participants who were excluded due no complete blood measurements or unknown APOE ε4 carrier status.*
APOE = apolipoprotein E, IQR = interquartile ratio, n = number of participants, SD = standard deviation.

Table 2 Association between blood cell counts and derived ratios, and risk of all-cause dementia.

Laboratory assessment*	All-cause dementia (n/N = 664/8313)	
	Model I HR (95% CI)	Model II HR (95% CI)
Blood cells		
Granulocytes	1.14 (0.87 to 1.50)	1.07 (0.80 to 1.43)
Corrected for platelets and lymphocytes	1.33 (0.99 to 1.79)	1.22 (0.89 to 1.67)
Platelets	1.48 (1.11 to 1.96)	1.43 (1.08 to 1.90)
Corrected for granulocytes and lymphocytes	1.48 (1.10 to 2.00)	1.45 (1.07 to 1.95)
Lymphocytes	0.80 (0.64 to 0.99)	0.81 (0.64 to 1.03)
Corrected for granulocytes and platelets	0.76 (0.61 to 0.96)	0.78 (0.62 to 1.00)
Inflammatory ratios		
Granulocyte-to-lymphocyte ratio	1.34 (1.10 to 1.63)	1.26 (1.03 to 1.53)
Platelet-to-lymphocyte ratio	1.29 (1.08 to 1.55)	1.27 (1.05 to 1.53)
Systemic immune-inflammation index	1.18 (1.02 to 1.39)	1.15 (0.98 to 1.34)

Model I is adjusted for age and sex. Model II is adjusted for age, sex, education, smoking status, body mass index, diabetes mellitus, history of stroke, and APOE4 ε4 carrier status.
** All types of blood cells and their derived ratios were natural logarithmic transformed.*
APOE = apolipoprotein E, CI = confidence interval, HR = hazard ratio, n = number of incident dementia events, N = number of participants for analysis.

adjusted model and when using age as time scale instead of follow-up time.

Censoring for stroke did not meaningfully change the risk estimates (**Table 3**). Higher levels of platelets showed a slightly stronger association with AD than with all-cause dementia, whilst the association with granulocytes was less pronounced for AD. Risk estimates for all-cause dementia and AD were comparable for the ratios. For vascular dementia, risk estimates regarding the individual blood cell components and their derived ratios were more pronounced than for all-cause dementia, but small numbers led to wider confidence intervals (n=31).

Stratified analyses showed that the association between the ratios and dementia was particularly pronounced in participants aged below the median age of 65.4 years, women, and non-smokers (**Figure 2**). However, formal interaction terms did not reach statistical significance. Also, no significant effect modification was observed across different strata of these variables for the association between granulocyte, platelet, and lymphocyte counts, and risk of dementia (**Figure 3**).

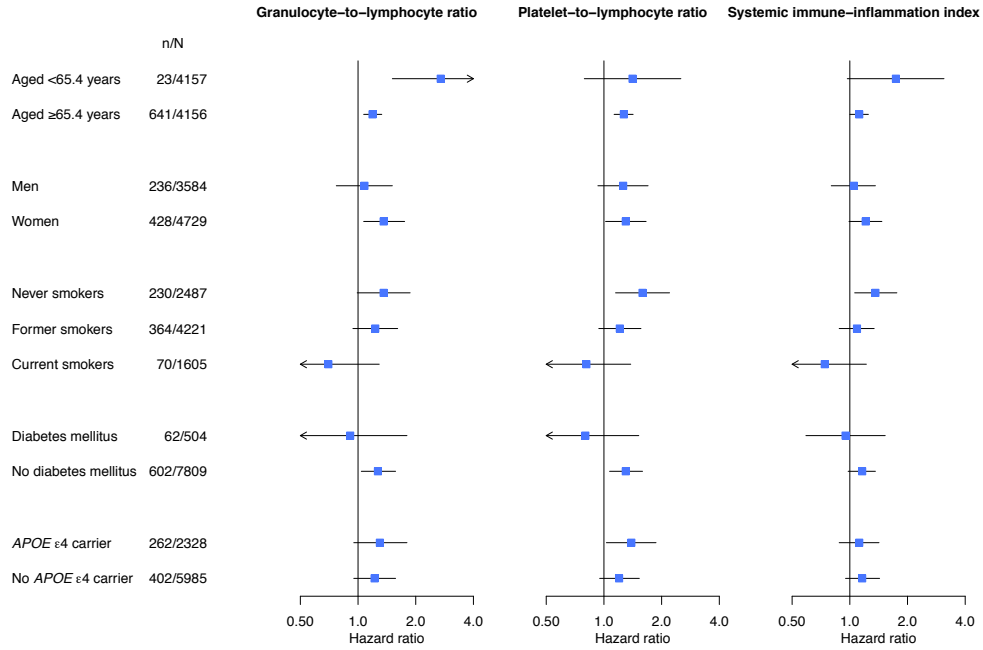

Figure 2 Forest plots of the association of the granulocyte-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and systemic immune-inflammation index, and risk of dementia.
Hazard ratios are shown in logarithmic scale with stratification by median age, sex, smoking status, diabetes mellitus, and APOE ε4 carrier status.
APOE = apolipoprotein E, n = number of incident dementia events, N = number of participants for analysis.

DISCUSSION

In this population-based study, we found that higher levels of granulocyte and platelet counts are related to an increased risk of dementia, whereas a higher lymphocyte count is associated with a decreased dementia risk. Furthermore, higher levels of their derived ratios, i.e., GLR, PLR, and SII are associated with an increased risk of all-cause dementia, including its subtype AD and even more with vascular dementia.

Activation of the immune systems can result in inflammation by production of different cytokines.[33] These cytokines can act as a link between the innate and the adaptive immune system, having pro- or anti-inflammatory effects depending on the type of cytokine.[34] A recent meta-analysis of 175 studies has suggested that AD is accompanied by an inflammatory response and that this can be reflected by a variety of systemic cytokines, for instance interferon-γ, interleukin-2 (IL-2), and in particular IL-6, of which dysregulation has been associated with multiple chronic inflammatory diseases.[35,36] It is now recognised that systemic

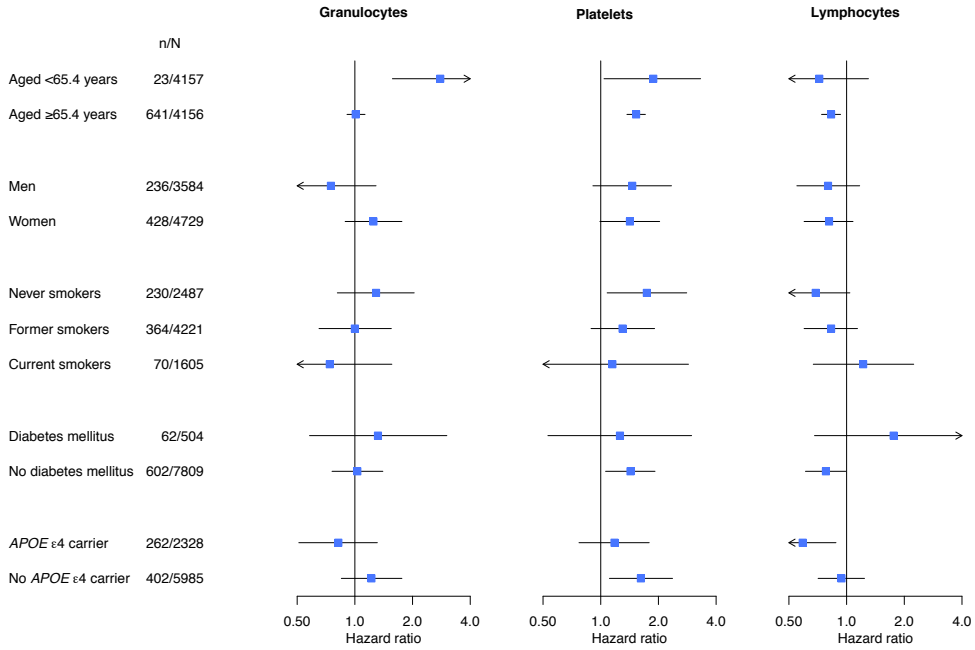

Figure 3 Forest plots of the association of granulocytes, platelets, and lymphocytes, and the risk of dementia.
Hazard ratios are shown in logarithmic scale with stratification by median age, sex, smoking status, diabetes mellitus, and APOE ε4 carrier status.
APOE = apolipoprotein E, n = number of incident dementia events, N = number of participants for analysis.

inflammation can trigger or exacerbate the inflammatory environment of the brain, thereby contributing to chronic neuro-inflammation and neurodegeneration.[37] A plausible explanation for the occurrence of this chronic neuro-inflammation in (pre)demented individuals involves a disruption of a process called resolution.[38] Resolution is an active process that halts the acute phase of inflammation and restores tissue homeostasis. The acute inflammatory phase is usually initiated in response to infection, neoplasia, tissue injury, or other major homeostatic stressors. This phase is accompanied by the increased release of pro-inflammatory mediators such as prostaglandins, leading to leukocyte recruitment. Normally, resolution would clear the recruited granulocytes.[39] However, it has been shown that failure of resolution, induced by any chronic inflammatory state, is associated with an overactive innate immune system, resulting in the development of chronic inflammation, which could subsequently lead to AD.[38,40,41] Our finding that an increase in the granulocyte count, resulting in a higher GLR and SII, is associated with an increased risk of dementia could therefore support the role of insufficient resolution in the pathogenesis of dementia.

Table 3 Association between blood cell counts derived ratios and risk of all-cause dementia and dementia subtypes.

Laboratory assessment*	All-cause dementia, censored for stroke (n/N = 579/8008)†	Alzheimer's disease (n/N = 543/8313)	Vascular dementia (n/N = 31/8313)
	HR (95% CI)	HR (95% CI)	HR (95% CI)
Blood cells			
Granulocytes	1.13 (0.83 to 1.56)	1.03 (0.75 to 1.42)	1.99 (0.52 to 7.55)
Corrected for platelets and lymphocytes	1.36 (0.96 to 1.93)	1.12 (0.79 to 1.58)	1.92 (0.44 to 8.41)
Platelets	1.45 (1.07 to 1.96)	1.59 (1.17 to 2.17)	3.86 (1.02 to 14.6)
Corrected for granulocytes and lymphocytes	1.47 (1.07 to 2.02)	1.63 (1.18 to 2.27)	3.39 (0.84 to 13.7)
Lymphocytes	0.80 (0.62 to 1.02)	0.85 (0.66 to 1.10)	0.76 (0.25 to 2.30)
Corrected for granulocytes and platelets	0.76 (0.58 to 0.98)	0.81 (0.62 to 1.06)	0.64 (0.20 to 2.03)
Inflammatory ratios			
Granulocyte-to-lymphocyte ratio	1.33 (1.07 to 1.65)	1.17 (0.95 to 1.46)	1.85 (0.74 to 4.62)
Platelet-to-lymphocyte ratio	1.31 (1.07 to 1.60)	1.30 (1.06 to 1.60)	1.99 (0.82 to 4.81)
Systemic immune-inflammation index	1.19 (1.01 to 1.41)	1.15 (0.97 to 1.37)	1.77 (0.87 to 3.63)

Models are adjusted for age, sex, education, smoking status, body mass index, diabetes mellitus, history of stroke, and APOE4 ε4 carrier status.
** All types of blood cells and their derived ratios were natural logarithmic transformed. † Number of participants for analysis is 8313 minus participants with a history of stroke (n=305).*
APOE = apolipoprotein E, CI = confidence interval, HR = hazard ratio, n = number of incident dementia events, N = number of participants for analysis.

Only few studies have examined the interplay between the innate and adaptive immunity by studying levels of these blood cell-based ratios in dementia patients. Two cross-sectional studies have shown that NLR and PLR were elevated in AD patients compared to dementia-free controls.[17,18] In contrast, a longitudinal study assessing the trajectory of NLR has found no difference in its longitudinal evolution between AD patients and dementia-free participants.[19] Although they have examined differences between AD patients and dementia-free controls, they did not investigate the risk of developing dementia in dementia-free participants in relation to their levels of NLR. In the present study, we did take the time until dementia into account by a joint modelling approach and were therefore able to assess the risk of dementia in relation

to the change in blood cell counts and their derived ratios.

Interestingly, recent evidence has shown that the NLR and PLR are partly genetically determined with 36% estimated heritability for NLR and 64% for PLR in a healthy population.[42] Moreover, different single nucleotide polymorphisms (SNPs) identified through genome-wide association study (GWAS) were significantly related to the PLR phenotype, but not to NLR.[43] Importantly, some but not all of these SNPs were also related to platelets, indicating that these SNPs capture the interplay between platelets and lymphocytes. Thus far, no GWAS for SII has been performed. Exploring the dementia risk by genetically predicted blood cell-based ratios may provide more insight in the causal role of immunity in dementia.

Strengths of our study include the population-based setting and the thorough follow-up for dementia. Another strength is the prospective design of this study, with the blood cell counts being measured at multiple time points. Using an innovative statistical method, we combined these repeated measurements with dementia as survival outcome. Moreover, we used blood cell counts and their derived ratios, which are low-cost and easy to implement in the clinic and other research settings. Although these ratios are proven to be associated with chronic systemic inflammation, we need to emphasise that it is unknown whether higher levels of GLR, PLR, and SII are functional and cause higher levels of pro-inflammatory cytokines. To identify the actual involved immune cell populations determination of different cytokines is still needed. In addition, the innate and adaptive immune systems are overlapping, making it difficult to completely distinguish their separate effects. Also, we used the granulocyte count as proxy for the neutrophil count. Although the relative proportion of neutrophils compared to eosinophils and basophils may be lower in persons with several specific diseases such as parasitic infections, asthma, or immune diseases, neutrophils are generally the most important subtype of granulocytes. If anything, misclassification of the granulocytes would be non-differential and would therefore lead to underestimation of the estimates.[11] In addition, we cannot rule out reversed causality, i.e., that dementia is subclinical at time of the laboratory assessments and causes higher levels of GLR, PLR, and SII. Lastly, we did not have the power to study other neurodegenerative diseases beyond dementia, such as Parkinson's disease or amyotrophic lateral sclerosis. It would be interesting for future studies to also investigate the relation between inflammation and these diseases.

In conclusion, higher levels of the ratios GLR, PLR, and SII are associated with an increased risk of developing dementia in the general population. Higher activation of the innate immune system reflected by higher levels of granulocytes and platelets is associated with an increased dementia risk, while the adaptive immune system is suggested to be more neuroprotective. These findings support the role of dysregulation of the immune systems in the pathogenesis of dementia. Further studies are warranted to assess during which phase of

the pathogenesis of dementia immunity is involved and to assess causality in order to develop prevention and therapeutic strategies.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1 Overview of median blood cell counts and blood cell-based ratios measured per Rotterdam Study assessment round.

Laboratory assessment	First assessment round* (N=8313)	Second assessment round† (N=5663)	Third assessment round‡ (N=1886)
Blood cell types, 10^9/L, median (IQR)			
Granulocytes	3.8 (1.6)	4.0 (1.6)	3.6 (1.5)
Platelets	263 (84)	262 (83)	224 (75)
Lymphocytes	2.2 (0.8)	2.2 (0.8)	1.9 (0.9)
Blood cell-based ratios, median (IQR)			
Granulocyte-to-lymphocyte ratio	1.7 (0.9)	1.8 (0.9)	1.9 (1.1)
Platelet-to-lymphocyte ratio	120 (55)	116 (53)	117 (59.1)
Systemic immune-inflammation index	455 (280)	461 (283)	421 (290)

** The first measurement corresponds with the fourth round of RS-I, second round of RS-II, and first round of RS-III. † The second measurement corresponds with the fifth round of RS-I, third round of RS-II, and second round of RS-III. ‡ The third measurement corresponds with the sixth round of RS-I and the fourth round of RS-II.*
IQR = interquartile ratio, N = number of participants, RS = Rotterdam Study.

Chapter 15

Aortic arch calcification and the risk of cancer

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ABSTRACT

Background Atherosclerosis and cancer share multiple disease pathways. Yet, it is unclear if atherosclerosis is associated with a subsequent higher cancer risk. We determined the association of atherosclerotic calcification in the aortic arch, as proxy for systemic atherosclerosis, with the risk of cancer.

Methods Between 2003 and 2006, 2404 participants (mean age: 69.5 years, 52.5% women) from the prospective population-based Rotterdam Study underwent computed tomography to quantify calcification in the aortic arch. Participants were followed for the onset of cancer, death, loss to follow-up, or January 1st, 2015, whichever came first. We computed sex-specific tertiles of aortic arch calcification volumes. Next, we examined the association between the volume and severity (i.e., tertiles) of aortic arch calcification and the risk of cancer using Cox proportional hazards models.

Results During a median (interquartile range) follow-up of 9.6 years (8.9 to 10.5), 348 participants were diagnosed with cancer. Participants with the greatest severity of aortic arch calcification had a higher risk of cancer (hazard ratio [95% confidence interval] for the third tertile compared to the first tertile of aortic arch calcification volume in the total population is 1.39 [1.04 to 1.86]).

Conclusions Individuals with the most severe aortic arch calcification had a higher risk of cancer. While this could reflect the impact of long-term exposure to shared risk factors, it might also point towards the co-occurrence of both conditions.

INTRODUCTION

Cardiovascular diseases and cancer remain the leading causes of morbidity and mortality worldwide.[1,2] Several studies have shown that patients with cancer are at higher risk of developing cardiovascular disease.[3,4] This may be due to a cancer-induced hypercoagulable state,[5] or as a consequence of the detrimental effects of cancer treatment on the vascular system.[4,6,7] Additionally, it has also been proposed that atherosclerosis, the most important underlying condition of cardiovascular events, and cancer may share a common pathophysiology.[8]

Common risk factors such as age, smoking, obesity, and genetic susceptibility are known to contribute to the risk of both atherosclerosis and cancer. Specific molecular pathways leading to atherosclerosis, including inflammation, oxidative stress, and uncontrolled cell proliferation, are also involved in the pathogenesis of cancer.[9,10] As such, both diseases are likely to co-occur. Although many studies have focused on the presence of atherosclerosis after cancer diagnosis, less is known about the presence and extent of atherosclerosis before cancer manifestation. This is of particular interest, since the first atherosclerotic lesions may already develop during infancy.[11] Understanding the sequence and timing between these potentially interconnected diseases is pivotal as it may help to identify high-risk patients and to develop preventive strategies for both diseases.

Due to the central anatomical location in the arterial system, the presence and amount of atherosclerosis in the aortic arch may provide an easy measurable proxy of the systemic burden of atherosclerosis within an individual. As such, aortic arch atherosclerosis has repeatedly been linked to mortality, in particular also of non-cardiovascular origin, of which cancer represents a substantial part.[12,13] Hence, to further investigate the link between atherosclerosis and cancer, we determined the association between aortic arch calcification – as proxy for systemic atherosclerosis – with the subsequent risk of cancer within the setting of a large prospective population-based study.

METHODS

Setting

This study is embedded within the Rotterdam Study, a prospective population-based cohort study that investigates determinants and occurrence of chronic diseases in the middle-aged and elderly population. The design of the Rotterdam Study has been described in detail previously.[14] At study entry, all participants were interviewed at home by a trained research assistant, followed by two visits to the research facility to undergo different examinations including laboratory assessments and imaging. Follow-up examinations take place every three to six years.

Study population

For the present study, we used the follow-up visit between 2003 and 2006 as baseline, because during this period, participants who visited the research centre were invited to undergo non-contrast multi-detector computed tomography (MDCT) scanning of the aortic arch as part of a project of visualising arterial calcification.[15] We scanned 2524 participants (response rate, 78%). Out of 2524 scans, six scans were ungradable for aortic arch calcification because of the presence of image artefacts, leaving a total of 2518 complete examinations with information on calcification. We excluded participants with a history of cancer (n=114), resulting in 2404 participants for analysis. The follow-up for cancer took place continuously and was completed for this study until January 1st, 2015.

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl) and into the WHO International Clinical Trials Registry Platform (ICTRP; www.who.int/ictrp/network/primary/en/) under shared catalogue number NTR6831. All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

Assessment of aortic arch calcification

We used a 16-slice or 64-slice MDCT scanner (Somatom Sensation 16 or 64; Siemens, Forchheim, Germany) to perform non-contrast CT scanning. We scanned the aortic arch using an extra-cardiac scan that reached from the aortic arch to the intracranial vasculature (1 cm above the sella turcica). Detailed information on both scans is provided elsewhere.[16,17] As proxy

of aortic arch atherosclerosis, we quantified aortic arch calcification on the extra-cardiac scan by including all calcification from the origin of the aortic arch (defined as the image in which the ascending and descending aorta merge into the inner curvature of the aortic arch) to the first 1 cm of the branches originating from the arch.[18] Calcification volumes were calculated using dedicated software (Syngo Calcium Scoring; Siemens, Forchheim, Germany) and were expressed in mm^3.

Assessment of cancer

Diagnoses of cancer were based on medical records of general practitioners (including hospital discharge letters) and through linkage with Dutch Hospital Data, the Netherlands Cancer Registry, and histology and cytopathology registries in the region. Incident cancer was defined as any primary malignant tumour, excluding non-melanoma skin cancer. Only pathology-confirmed cancers were included in analysis to exclude the possibility of false-positive cancer diagnoses. Diagnoses were coded independently by two physicians according to the International Classification of Diseases, tenth revision (ICD-10). In case of discrepancy, consensus was sought through consultation with a physician specialised in internal medicine. Date of diagnosis was based on date of biopsy (solid tumours) and laboratory assessment (haematological tumours), or – if unavailable – date of hospital admission or discharge letter. Follow-up of cancer registration was complete until January 1st, 2015.

Measurement of covariates

Information on educational level, smoking behaviour, and use of antidiabetic-, antihypertensive-, lipid-lowering-, and antithrombotic medication was obtained by trained interviewers. Educational level was classified into primary education, lower education (lower or intermediate general education, or lower vocational education), intermediate (intermediate vocational education or higher general education), or higher (higher vocational education or university). Smoking status was categorised as never, current, or former. At the research centre, height and weight were measured from which the body mass index (BMI, kg/m^2) was computed. In addition, systolic and diastolic blood pressures were measured twice on the right arm with a random-zero sphygmomanometer of which the mean was used for analyses. Hypertension was defined as a systolic blood pressure of ≥140 mm Hg, a diastolic blood pressure of ≥90 mm Hg, or use of antihypertensive medication.[19] Hypercholesterolaemia was defined as use of lipid-lowering medication or serum total cholesterol >6.5 mmol/L. Diabetes mellitus was defined as use of antidiabetic medication, fasting serum glucose level ≥7.1 mmol/L, or random serum glucose level ≥11.1 mmol/L.[20] We defined history of cardiovascular disease as history of myocardial infarction, stroke, percutaneous transluminal coronary angioplasty, or coronary artery bypass graft.[21-23] Granulocyte count was measured using the COULTER®

Ac·T diff2™ Haematology Analyser (Beckman Coulter, San Diego, California, USA).

Statistical analysis

Considering the skewed distribution of aortic arch calcification volumes, we performed a natural log-transformation and added 1 mm^3 to each non-transformed volume to deal with calcium scores of 0 (Ln[calcification volume+1.00mm^3]). First, we used Cox proportional hazards models to determine the association between aortic arch calcification (per 1-standard deviation [SD] increase) and the subsequent risk of cancer. Model I was adjusted for age at MDCT scan and sex. To investigate to which extent any association would be driven by shared risk factors, Model II was additionally adjusted for shared risk factors including educational level, smoking status, BMI, hypertension,[24] hypercholesterolaemia,[25] diabetes mellitus, history of cardiovascular disease, and granulocyte count. We chose to use granulocyte count as markers of inflammation, since these blood cells in particular are related to larger volumes of arterial calcification.[26]

Second, we computed tertiles of calcification severity and investigated associations with the risk of cancer, using the same two Cox proportional hazards models as described above and using the first tertile as reference category. As calcification volumes were larger in men than in women, the tertiles were computed sex-specifically. Also, considering differences in risk factors for atherosclerosis and cancer between men and women,[27] we performed analyses stratified by sex.

For all Cox proportional hazards models, follow-up time was used as time scale and started at date of MDCT scan until date of incident cancer, death, loss to follow-up, or January 1st, 2015, whichever came first. Censoring unexposed participants at date of death allowed us to compute cause-specific hazard ratios (HRs). The proportional hazards assumption was met for all analyses (Schoenfeld residuals test, all *P*-values >.05).

To explore the robustness of our findings, we performed several sensitivity analyses to assess potential bias associated with mortality given the strong association between atherosclerosis and mortality.[13] First, we restricted the analyses to shorter follow-up periods to limit the number of mortality events. To this end, participants with longer follow-up duration were censored at respectively two, three, four, and five years after the MDCT-scan. Second, we repeated the continuous analyses for the most common cancer types, i.e., breast cancer (among women), prostate cancer (among men), colorectal cancer, and lung cancer. Third, we stratified analyses by use of lipid-lowering and antithrombotic medication, and by median granulocyte count. In addition, we formally tested interaction by adding multiplicative interaction terms to the model.

To account for missing data of covariates (maximum amount of missing data: 5.9%) we

used multiple imputation (n=five imputations) by chained equations along with age, sex, calcification volumes, cardiovascular risk factors, and cancer incidence. Statistical analyses were performed using STATA v.15 (StataCorp).

RESULTS

Characteristics of participants at time of MDCT scan are presented in **Table 1**. In addition, population characteristics stratified by tertiles of aortic arch calcification volumes are shown in **Supplementary Table 1**. The mean (SD) age was 69.5 years (6.8) and 52.5% were women. Among participants in the highest tertile of aortic arch calcification, a higher prevalence of cardiovascular risk factors was observed than in participants in the lowest tertile. During a median (interquartile range) follow-up of 9.6 years (8.9 to 10.5), 348 out of 2404 participants were diagnosed with cancer, and 463 participants died. The most frequently diagnosed cancer types were prostate (39.2% among men), breast (34.4% among women), colorectal (16.1% overall), and lung (11.5% overall).

We found no statistically significant association between continuous volumes of aortic arch calcification and the risk of cancer (**Table 2**). When investigating tertiles of calcification, we found that severe calcification was associated with a higher risk of cancer in the total population and in men (adjusted HR [95% confidence interval [CI]] for the third tertile compared to the first tertile of aortic arch calcification in total population = 1.39 [1.04 to 1.86] and in men = 1.44 [1.00 to 2.09]). This association was also observed in women, albeit not statistically significant (HR = 1.33 [95%CI = 0.83 to 2.13]). Effect estimates were slightly attenuated when we corrected for different cardiovascular risk factors.

When censoring participants with a follow-up duration longer than two years, we found that effect estimates were higher for both continuous aortic arch calcification volume as well as for severe calcification, albeit not statistically significant (adjusted HR [95%CI] per 1-SD increase in aortic calcification = 1.24 [0.90 to 1.71], and adjusted HR [95%CI] for the third tertile compared to the first tertile of aortic arch calcification = 1.53 [0.75 to 2.13]). The effect estimates decreased with inclusion of longer follow-up duration (**Table 3**).

Table 1 Characteristics of the study population.

Characteristic	All participants (N=2404)	Men (N=1143)	Women (N=1261)
Age, years, mean (SD)	69.5 (6.8)	69.6 (6.6)	69.5 (6.9)
Educational level, No. (%)			
Primary	198 (8.2)	70 (6.1)	128 (10.2)
Lower	1023 (42.6)	298 (26.1)	725 (57.5)
Intermediate	737 (30.7)	444 (38.8)	293 (23.2)
Higher	446 (18.6)	331 (29.0)	115 (9.1)
Body mass index, kg/m^2, mean (SD)	27.7 (4.1)	27.4(3.5)	27.9 (4.5)
Smoking, No. (%)			
Never	687 (28.6)	157 (13.7)	530 (42.0)
Former	1339 (55.7)	777 (68.0)	562 (44.6)
Current	378 (15.7)	209 (18.3)	169 (13.4)
Hypertension, No. (%)	1800 (74.9)	860 (75.2)	940 (74.5)
Hypercholesterolaemia, No. (%)	1017 (42.3)	422 (36.9)	595 (47.2)
Lipid-lowering medication use, No. (%)	598 (24.9)	292 (25.5)	306 (24.3)
Antithrombotic medication use, No. (%)	591 (24.6)	349 (30.5)	242 (19.2)
Diabetes mellitus, No. (%)	328 (13.6)	170 (14.9)	158 (12.5)
History of cardiovascular disease, No. (%)	296 (12.3)	204 (17.8)	92 (7.3)
Granulocyte count, median (IQR)	3.8 (3.0 to 4.6)	4.0 (3.2 to 4.8)	3.6 (2.9 to 4.4)
Aortic arch calcification mm^3, median (IQR)	263.8 (46.5 to 883.2)	296.9 (52.9 to 1009.5)	228.5 (41.8 to 825.0)

Characteristics were measured at time of MDCT-scan. Numbers are shown after multiple imputation. IQR = interquartile range, SD = standard deviation.

Regarding specific cancer types, we found that among women, aortic arch calcification was associated with a lower risk of breast cancer (HR [95%CI] per 1-SD increase in aortic arch calcification = 0.71 [0.52 to 0.98]) and with a higher risk of lung cancer in the total population (HR [95% CI] = 2.35 [1.39 to 3.96], **Table 4**).

Table 2 The association between aortic arch calcification and the risk of cancer.

	Aortic arch calcification		Risk of cancer	
		n/N	Model I HR (95% CI)	Model II HR (95% CI)
Total population	Per 1-SD increase*	348/2404	1.12 (0.99 to 1.27)	1.09 (0.96 to 1.24)
	Tertiles			
	T1	107/802	1.00	1.00
	T2	98/801	0.91 (0.69 to 1.20)	0.89 (0.67 to 1.18)
	T3	143/801	1.48 (1.12 to 1.95)	1.39 (1.04 to 1.86)
Women	Per 1-SD increase*	131/1261	1.10 (0.90 to 1.33)	1.05 (0.88 to 1.28)
	Tertiles			
	T1	41/421	1.00	1.00
	T2	35/420	0.85 (0.54 to 1.34)	0.82 (0.52 to 1.30)
	T3	55/420	1.45 (0.93 to 2.27)	1.33 (0.83 to 2.13)
Men	Per 1-SD increase*	217/1143	1.14 (0.97 to 1.33)	1.12 (0.96 to 1.32)
	Tertiles			
	T1	66/381	1.00	1.00
	T2	63/381	0.95 (0.67 to 1.35)	0.94 (0.66 to 1.34)
	T3	88/381	1.49 (1.04 to 2.12)	1.44 (1.00 to 2.09)

Hazard ratios in Model I are adjusted for age (and sex in the total population). Hazard ratios in Model II are adjusted for covariates in Model I plus adjustment for education, smoking status, body mass index, hypertension, hypercholesterolaemia, diabetes mellitus, history of cardiovascular disease, and granulocyte count.

**Ln(calcification volume+1 mm^3)–transformed volumes.*

CI = confidence interval, HR = hazard ratio, n = number of participants with incident cancer, N = total number for participants, SD = standard deviation, T = tertile.

No effect modification was observed by lipid-lowering and antithrombotic medication use and by median granulocyte count. All interactions were tested on the multiplicative scale and did not reach statistical significance (P>0.05).

Table 3 The association between aortic arch calcification and the risk of cancer while censoring at different time-points.

	Aortic arch calcification		Risk of cancer	
		n/N	Model I HR (95% CI)	Model II HR (95% CI)
Restricted to two years of follow-up	Per 1-SD increase*	62/2404	1.25 (0.92 to 1.70)	1.24 (0.90 to 1.71)
	Tertiles			
	T1		1.00	1.00
	T2		1.15 (0.59 to 2.27)	1.13 (0.57 to 2.24)
	T3		1.59 (0.80 to 3.15)	1.53 (0.75 to 2.13)
Restricted to three years of follow-up	Per 1-SD increase*	103/2404	1.13 (0.90 to 1.43)	1.12 (0.88 to 1.42)
	Tertiles			
	T1		1.00	1.00
	T2		1.02 (0.60 to 1.72)	1.01 (0.60 to 1.72)
	T3		1.45 (0.86 to 2.46)	1.43 (0.83 to 2.47)
Restricted to four years of follow-up	Per 1-SD increase*	137/2404	1.04 (0.86 to 1.27)	1.04 (0.85 to 1.27)
	Tertiles			
	T1		1.00	1.00
	T2		0.83 (0.53 to 1.29)	0.82 (0.53 to 1.28)
	T3		1.08 (0.69 to 1.69)	1.06 (0.67 to 1.69)
Restricted to five years of follow-up	Per 1-SD increase*	183/2404	1.02 (0.87 to 1.20)	1.02 (0.87 to 1.21)
	Tertiles			
	T1		1.00	1.00
	T2		0.80 (0.55 to 1.17)	0.81 (0.55 to 1.18)
	T3		1.11 (0.75 to 1.62)	1.11 (0.74 to 1.65)

Hazard ratios in Model I are adjusted for age (and sex in the total population). Hazard ratios in Model II are adjusted for covariates in Model I plus adjustment for education, smoking status, body mass index, hypertension, hypercholesterolaemia, diabetes mellitus, history of cardiovascular disease, and granulocyte count.

**Ln(calcification volume+1 mm^3)–transformed volumes.*

CI = confidence interval, HR = hazard ratio, n = number of participants with incident cancer, N = total number for participants, SD = standard deviation, T = tertile.

Table 4 The association between aortic arch calcification and the risk of different cancer types.

		Risk of cancer	
	n/N	Model I HR (95% CI)*	Model II HR (95% CI)*
Breast cancer	45/1261	0.75 (0.55 to 1.00)	0.71 (0.52 to 0.98)
Prostate cancer	72/1143	1.08 (0.82 to 1.39)	1.07 (0.81 to 1.40)
Colorectal cancer	56/2404	1.18 (0.86 to 1.63)	1.04 (0.76 to 1.43)
Lung cancer	40/2404	2.87 (1.72 to 4.78)	2.35 (1.39 to 3.96)

Hazard ratios in Model I are adjusted for age. Hazard ratios in Model II are adjusted for covariates in Model I plus adjustment for education, smoking status, body mass index, hypertension, hypercholesterolaemia, diabetes mellitus, history of cardiovascular disease, and granulocyte count.
** HR is expressed per 1SD increase in aortic arch calcification volume. Volumes were transformed as Ln(calcification volume+1 mm^3).*
CI = confidence interval, HR = hazard ratio, n = number of participants with incident cancer, N = total number for participants, SD = standard deviation.

DISCUSSION

In this population-based study, we found that only individuals with the most severe aortic arch calcification had a higher risk of cancer, in particular in the short term.

It has previously been shown that atherosclerosis and cardiovascular events occur after cancer diagnosis potentially as a result of cancer itself – by inducing a hypercoagulable state – and cancer treatment. Based on the shared risk factors and pathophysiology, we hypothesised that atherosclerosis is also associated with a subsequent higher risk of cancer and that the strength of this association would diminish after adjustment for shared risk factors. When investigating the association between the amount of aortic arch calcification and the risk of cancer, we found only a slightly higher risk, which was not statistically significant. However, when targeting the group of individuals in the highest tertile of calcification, we found 39% increase of the risk of cancer compared to those with the lowest tertile. Further investigation of this association demonstrated that the effect of atherosclerosis on cancer seems to be largest in the short term (during the first two years of follow-up). Although this indicates that severe atherosclerosis may be present before cancer diagnosis, it might also reflect reverse causation. It is possible that subclinical cancer development already influences the course of atherosclerosis. Since both atherosclerosis and cancer are conditions with a long preclinical phase, we cannot prove causality, nor can we rule out reverse causation.

Overall, we found no prominent differences in the association between atherosclerosis and cancer before and after adjustment for cardiovascular risk factors. This suggests that overall, traditional cardiovascular risk factors do not fully explain potential co-occurrence of atherosclerosis and cancer. This could point towards other factors, such as genetic variation or exogenous factors that may explain the differential susceptibility to either atherosclerosis or cancer. However, exposure to shared risk factors might explain the higher risk of cancer in those individuals with the most severe aortic arch calcification and the strong association with lung cancer. It is likely that individuals who have been exposed to shared risk factors for a long period, and in high amounts, have both the largest volumes of aortic arch calcification and the highest risk of cancer. This suggests that the co-occurring deterioration of atherosclerosis and development of cancer is due to long and severe exposure to risk factors. However, also in these individuals, the size of the effects estimates only slightly diminished after correction for shared risk factors.

Sex differences – reflected by differences in hormonal levels – may also influence the apparent different impact of shared risk factors on the development of atherosclerosis and cancer. Aortic arch calcification was associated with a lower risk of breast cancer in women. It has previously been proposed that an inverse association between aortic atherosclerosis and cancer holds in particular for cancers that are hormone-dependent or highly affected by genetics rather than for those caused by exogenous factors.[28] Also, aortic arch calcification in particular is strongly associated with mortality, indicating that the inverse relation between aortic arch calcification and cancer may partly be due to residual survival bias.[13] More in-depth inquiry on this topic is required.

Several strengths of our study are worth mentioning. Our study is a large prospective population-based study and therefore less vulnerable to selection and information bias than retrospective ones. In addition, we have prospective and unbiased collection of many risk factors which are not available in healthcare databases. All cancers were pathology proven, which excludes the chance of misclassification. Also, we had an image-based assessment of calcification volumes and standardised ascertainment of cancer incidence.

Yet, some potential limitations need to be addressed. First, despite sufficient power to detect a large effect size of 1.5 for all-cancers (α=0.05, β=0.80), we acknowledge the lack of statistical power to elaborate on specific cancer types. Second, strong associations of atherosclerosis with the risk of cardiovascular events and mortality may have weakened any potential association of atherosclerosis with cancer. Nevertheless, our finding that the most severe aortic calcification is associated with a higher risk of cancer – while these persons have the highest risk of mortality – might indicate that the effect of survival is limited. Third, the burden of atherosclerosis may influence the prognosis and course of cancer rather than the development itself. Ideally, measures of atherosclerosis at multiple time points are needed to

also assess changes in atherosclerotic burden before cancer diagnosis. Future studies are needed to unravel differences in the aetiology between atherosclerosis and cancer explained by other factors such as genetic and exogenous factors. Lastly, calcification is only a part of the atherosclerotic plaque. Non-calcified parts of the plaques cannot be visualised with non-enhanced CT. Nevertheless, it has been shown that calcification volume is an adequate measure for the total underlying plaque burden.[29]

We found that only individuals with the most severe aortic arch calcification had a higher risk of cancer, potentially through long-term parallel exposure to shared risk factors. Other factors, such as genetic variation or exogenous factors, may further explain susceptibility to either atherosclerosis or cancer.

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SUPPLEMENTARY MATERIAL

Table 1 Characteristics of the study population stratified by tertiles of aortic arch calcification volumes.

Characteristic	Tertile 1	Tertile 2	Tertile 3
Age, years, mean (SD)	66.1 (4.8)	69.1 (6.0)	73.4 (7.2)
Educational level, No. (%)			
Primary	32 (4.0)	56 (7.0)	110 (13.7)
Lower	348 (43.4)	329 (41.1)	346 (43.2)
Intermediate	237 (29.6)	270 (33.7)	230 (28.7)
Higher	185 (23.1)	146 (18.2)	115 (14.4)
Body mass index, kg/m^2, mean (SD)	27.6 (3.9)	27.8 (4.0)	27.7 (4.3)
Smoking, No. (%)			
Never	279 (34.8)	239 (29.8)	169 (21.1)
Former	425 (53.0)	445 (55.6)	469 (58.6)
Current	98 (12.2)	117 (14.6)	163 (20.3)
Hypertension, No. (%)	520 (64.8)	599 (74.8)	681 (85.0)
Hypercholesterolaemia, No. (%)	284 (35.4)	348 (43.4)	385 (48.1)
Lipid-lowering medication use, No. (%)	140 (17.5)	202 (25.2)	256 (32.0)
Antithrombotic medication use, No. (%)	113 (14.1)	183 (22.8)	295 (36.8)
Diabetes mellitus, No. (%)	84 (10.5)	105 (13.1)	139 (17.4)
History of cardiovascular disease, No. (%)	45 (5.6)	82 (10.2)	169 (21.1)
Granulocyte count, median (IQR)	3.6 (2.9 to 4.4)	3.7 (3.0 to 4.6)	4.0 (3.2 to 4.9)
Aortic arch calcification, mm^3, median (IQR)	15.9 (0.6 to 46.6)	263.9 (161.0 to 389.4)	1356.2 (884.4 to 2491.9)

Characteristics are measured at time of MDCT-scan. Numbers are shown after multiple imputation. IQR = interquartile range, SD = standard deviation.

Chapter 16

Long-term effects of adjuvant treatment for breast cancer on carotid plaques and brain perfusion

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ABSTRACT

Background Breast cancer treatment has been associated with vascular pathology. It is unclear if such treatment is also associated with long-term cerebrovascular changes. We studied the association between radiotherapy and chemotherapy with carotid pathology and brain perfusion in breast cancer survivors.

Methods We included 173 breast cancer survivors exposed to radiotherapy and chemotherapy, assessed on average 21.2 years after cancer diagnosis, and 346 age-matched cancer-free women (1:2) selected from the population-based Rotterdam Study. Outcome measures were carotid plaque score, intima-media thickness (IMT), total cerebral blood flow (tCBF), and brain perfusion. Additionally, we investigated the association between inclusion of the carotid artery in the radiation field (no, small, or large part), tumour location, and these outcome measures within cancer survivors.

Results No statistically significant differences were observed between cancer survivors and cancer-free women regarding plaque score or IMT. Cancer survivors had lower tCBF (-19.6 mL/min, 95% confidence interval [CI] = -37.3 to -1.9) and brain perfusion (-2.5 mL/min per 100 mL, 95% CI = -4.3 to -0.7) than cancer-free women. Among cancer survivors, a large versus a small part of the carotid artery in the radiation field was associated with a higher IMT (0.05, 95% CI = 0.01 to 0.09). Also, survivors with a right-sided tumour had lower left carotid plaque score (-0.31, 95% CI = -0.60 to -0.02) and higher brain perfusion (3.5 mL/min per 100 mL, 95% CI = 0.7 to 6.2) than those with a left-sided tumour.

Conclusions On average two decades post-diagnosis, breast cancer survivors had lower tCBF and brain perfusion than cancer-free women. Also, survivors with a larger area of the carotid artery within the radiation field had a larger IMT. Future studies should confirm if these cerebrovascular changes underlie the frequently observed cognitive problems in cancer survivors.

INTRODUCTION

Breast cancer patients are at an increased risk of developing cardiovascular diseases due to effects of cancer treatment on the vascular system.[1-3] Adjuvant chemotherapy has been associated with vascular damage,[4] specifically with narrowing of the vascular lumen as a result of thickening of the vessel wall through endothelial damage.[5] In addition, it has been related to cardiotoxicity through injury of cardiac myocytes and antimetabolites, which is associated with myocardial ischemia.[6] Adjuvant radiotherapy for breast cancer has also been linked to vascular pathology,[7] including carotid stenosis,[8,9] and carotid stiffness,[10] as well as to an increased risk of stroke.[11] In addition, breast cancer patients may have a higher risk of congestive heart failure and myocardial infarction that can persist up to twenty years after treatment.[3,12-14] However, the synergistic effects of chemotherapy and radiotherapy on vascular pathology in breast cancer patients that may arise due to accumulation of vascular damage remain largely unknown.

Cardiovascular diseases including carotid pathology can result in changes in total cerebral blood flow (tCBF).[15] In turn, a preclinical study has shown that disrupted tCBF in mice can lead to cognitive deficits[16] and lower tCBF has been associated with accelerated cognitive decline and dementia in humans.[17] As yet, it is unknown if the potential cardiovascular side effects of breast cancer itself and breast cancer treatment are associated with disruptions in tCBF and therefore with brain perfusion. Such knowledge is of particular interest, because it could contribute to the understanding of the well-documented brain structural alterations and cognitive deficits that are prevalent in about 20% to 30% of cancer survivors.[18]

We have previously shown that such structural brain alterations including reductions in total brain volume and grey matter volume, and cognitive deficits can occur up to twenty years after cessation of cancer treatment in breast cancer survivors who were treated with radiotherapy and subsequent CMF (Cyclophosphamide-Methotrexate-Fluorouracil) chemotherapy.[19,20] In the current study that uses the same study population, we characterised the combined effects of cancer itself, radiotherapy, and chemotherapy, on atherosclerotic carotid disease and tCBF by comparing these breast cancer survivors to a 1:2 age-matched population-based, cancer-free reference group. To gain further insight into the contribution of regional radiotherapy on carotid pathology, we assessed the association between carotid atherosclerosis and radiation fields. The prevalence of cardiovascular diseases may differ between patients with left and right-sided breast tumours.[3] Also, due to left-right differences in anatomy, a larger part of the left carotid artery may lie in the radiation field than of the right artery. We therefore determined the association between tumour location (left or right side) and carotid plaque score.

METHODS

Participants

Breast cancer survivors

We identified women with a history of unilateral, invasive breast cancer from the registries of the Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital and the Daniel den Hoed Cancer Clinic of the Erasmus Medical Centre. Women were selected for the current study if they had been treated with both post-surgical radiotherapy and six cycles of adjuvant CMF chemotherapy (Cyclophosphamide 100 mg/m^2 on days 1-14; Methotrexate 40 mg/m^2 on days 1 and 8; 5-Fluorouracil 600 mg/m^2 on days 1 and 8) between 1976 and 1995. The radiotherapy regimen depended on type of surgery and disease stage and was classified into one or more of the following fields: axillary, breast, chest wall, internal mammary chain, McWhirter, or supraclavicular radiation.

Breast cancer survivors were eligible if they were between 50 and 80 years of age at time of selection, if invasive breast cancer was their first and only malignancy, if they had remained cancer-free since treatment for breast cancer, and if they had sufficient command of the Dutch language. Exclusion criteria were use of adjuvant endocrine therapy and magnetic resonance imaging (MRI) contraindications.

A detailed overview of the participant inclusion has been described previously.[21] In short, 195 (67.0%) of the 291 eligible breast cancer survivors agreed to participate and were assessed between October 2008 and October 2009. Of the 195 women who participated, in four of them no tCBF data was available because they had not completed the MRI examination due to claustrophobia. In another four participants the ultrasound images of the carotid arteries of either one or two vessel beds were unusable. Hence, total plaque score could not be calculated for these participants. Lastly, intima-media thickness (IMT) was not measured for five participants. Finally, 182 participants were available for analyses.

Population-based reference women

Women without a history of cancer were selected from the prospective population-based Rotterdam Study.[22,23] As of 2008, the study includes 14 926 participants. By the end of the inclusion period of chemotherapy-exposed breast cancer survivors (October 2009), 1337 female participants of the Rotterdam Study had undergone complete carotid artery ultrasound assessment and a brain MRI.[23] Each breast cancer survivor was randomly matched to two out of these 1337 cancer-free women based on age at time of carotid artery ultrasound (age range +/- 4 years). Nine out of 182 breast cancer survivors could not be matched. We chose

to match two cancer-free women per breast cancer survivor to limit the number of unmatched participants, which might induce selection bias.

Methods

All examinations for both the breast cancer survivors and the reference women took place at the research centre of the Rotterdam Study and were conducted by the same technicians. Breast cancer survivors were assessed between October 2008 and October 2009, and reference women were examined between April 2006 and August 2009.

Carotid artery ultrasound

Ultrasonography of both carotid arteries was performed with a 7.5-MHz linear-array transducer and a duplex scanner (EnVisor; Philips Medical Systems Nederland B.V., Eindhoven, Netherlands). Plaques, defined as focal widenings of the vessel wall relative to adjacent segments with protrusion into the lumen composed of either only calcified deposits or a combination of calcified and non-calcified material, were examined at six sites for both the left and right side including: the anterior (near) and posterior (far) walls of the (i) internal carotid artery; (ii) carotid bifurcation; and (iii) common carotid artery.[24] A weighted plaque score ranging from 0 to 6 was computed by adding the number of sites at which a plaque was detected, divided by the total number of sites for which an ultrasonographic image was available and multiplied by 6 (the maximum number of sites). IMT was measured on a longitudinal, two-dimensional (2D) ultrasound image of the distal common carotid artery, on which the near and far walls were displayed as two bright white lines separated by a hypoechogenic space.[25] IMT was defined as the distance between the leading edge of the far wall – displayed as the first bright line – and the leading edge of the near wall, i.e., the second bright line. The mean IMT was calculated as the average of three measurements of both the left and right carotid arteries.

MRI acquisition and processing

MRI was performed on a 1.5-Tesla MRI scanner (General Electric Healthcare, Milwaukee, Wisconsin). During the study period, no software or hardware upgrades were performed. Breast cancer survivors and reference women were scanned using the same MRI scanner.

Our full scan protocol has been described in detail previously.[23] In short, for tCBF measurement, 2D phase-contrast imaging was performed. First, a sagittal 2D phase-contrast MRI angiographic scout image was performed. On this scout image, a transverse imaging plane perpendicular both to the precavernous portion of the internal carotid arteries and to the middle part of the basilar artery was chosen for an axial 2D phase contrast image (repetition

time = 20 ms, echo time = 4 ms, field of view = 19 cm^2, matrix = 256×160, flip angle = 8º, number of excitations = 8, bandwidth = 22.73 kHz, velocity encoding = 120 cm/sec, slice thickness = 5 mm).

As previously described, we calculated flow from the phase-contrast images using interactive data language-based custom software (Cinetool version 4, General Electric Healthcare, Milwaukee, WI, USA).[23] Regions of interest (ROIs), encompassing the entire lumen of the vessel, were drawn manually around both carotids and the basilar artery at the level of the clinoid segment on the phase-contrast images. The mean signal intensity in each ROI reflects the flow velocity in the vessel (cm/seconds). Flow (in mL/seconds) was calculated by multiplying the average velocity with the cross-sectional area of the vessel. To calculate tCBF (mL/min), flow rates for the carotid arteries and the basilar artery were summed and multiplied by 60 seconds/minute. Two independent, experienced technicians performed all manual ROI drawing and flow measurements (inter-rater correlations >0.94 for all vessels).

Total CBF strongly depends on the amount of brain tissue.[26] To account for this, we calculated brain perfusion (in mL/min per 100 mL) by dividing tCBF (mL/min) by brain volume (mL) and multiplying the obtained result by 100. Brain volume was automatically obtained from three high-resolution axial MRI sequences that were acquired for each participant: (i) a T1-weighted three-dimensional fast radio frequency spoiled gradient recalled acquisition in steady state with an inversion recovery prepulse (FASTSPGR-IR); (ii) a proton density-weighted sequence; and (iii) a fluid attenuated inversion recovery sequence (FLAIR). Pre-processing steps and the classification algorithm that were used to extract total brain volume (TBV) from these three sequences have been described in detail elsewhere.[27] In short, voxels were segmented into either: grey matter, white matter, cerebrospinal fluid, or background. The number of grey matter and white matter voxels were summed up and multiplied by the volume per voxel in mm^3 to obtain total brain volume.

Demographics

Information on potential confounders was collected for all participants. Body mass index (BMI) was calculated from height and weight measurements (kg/m^2). Sitting diastolic and systolic blood pressures (mm Hg, average of two assessments) were measured on the right arm with a random-zero sphygmomanometer.[26] Self-reported data on age at menopause, diabetes mellitus, smoking status (current, former, and never), and educational level (primary education, lower education [lower or intermediate general education, or lower vocational education], intermediate [intermediate vocational education or higher general education)], and higher [higher vocational education or university]) were obtained. In addition, information on use of antihypertensive medication, anticoagulant medication, and lipid-lowering medication

was collected.

Statistical analyses

Analysis of variance (ANOVA, continuous variables) and chi-square tests (categorical variables) were used to compare characteristics of breast cancer survivors and population-based reference women.

We used negative binomial regression to compare the distribution of plaque scores between groups (count variable, range 0 to 12), and linear regression models to compare groups on IMT (continuous), tCBF (continuous), and brain perfusion (continuous). Even though we matched the breast cancer survivors and controls on age at carotid artery ultrasound, we corrected all analyses for age to account for potential residual confounding by age. In addition, all analyses were corrected for BMI. In an extended model, Model II, we additionally corrected for prevalence of diabetes mellitus, smoking status, use of anticoagulant medication and lipid-lowering medication, and educational level. We adjusted for educational level because of the different distribution of educational level between groups in the current study and its association with cardiovascular diseases in general.[28] Note that all potential confounders were measured at time of assessment of the outcomes and not at time of cancer diagnosis and treatment. We therefore considered age at menopause, systolic and diastolic blood pressure, and use of antihypertensive medication as potential mediators rather than potential confounders, and did therefore not correct for these factors.[29,30] Lastly, for the analyses on tCBF and brain perfusion we additionally corrected for total plaque score and total IMT in Model III to determine whether any association was explained by plaque score or IMT.

Within the group of breast cancer survivors, we investigated if the degree to which the carotid artery was included in the radiation field was associated with plaque scores (negative binomial regression analysis), IMT, tCBF, or brain perfusion (linear regression analysis). We therefore classified field of radiotherapy in: (i) carotid artery was not in the radiation field (only axillary, breast, or chest wall radiation); (ii) a small part of the carotid artery was in the radiation field (internal mammary chain radiation, with or without axillary, breast, or chest wall radiation); and (iii) a large part of the carotid artery was in the radiation field (McWhirter or supraclavicular with or without internal mammary chain, axillary, breast, or chest wall radiation). For these analyses, we used the same models as used when comparing breast cancer survivors with cancer-free reference women. Survivors with a small part of the carotid artery in the radiation field were selected as the reference group because this was the most common type of treatment (see **Table 1**).

We subsequently looked at the association of breast tumour side with plaque score by comparing carotid plaque scores of the left and right carotid artery within survivors. Here too,

we used negative binomial regression models to investigate differences in carotid plaque scores. Linear regression models were used to determine the difference in IMT (total, left, and right), tCBF, and brain perfusion.

Statistical analyses were performed in R Version 3.3.2.

RESULTS

Characteristics of breast cancer survivors and population-based cancer-free reference women are presented in **Table 1**. Breast cancer survivors had been diagnosed on average (standard deviation [SD]) 21.1 years (4.4) before participation in this study, at a mean (SD) age of 42.6 years (5.4). They had a higher systolic and diastolic blood pressure, a younger age at menopause (mean age 44.1 versus 49.3 years), a higher educational level, and were less often current smokers than women from the reference group.

Breast cancer survivors had a similar total carotid plaque score (adjusted beta = -0.01 [95% CI = -0.20 to 0.18]) and total IMT score (adjusted beta = 0.00 [95% CI = -0.02 to 0.03]) as cancer-free reference women (**Table 2**). Regarding brain perfusion, breast cancer survivors had a statistically significantly lower mean tCBF (adjusted beta = -19.6 mL/min [95% CI = -37.3 to -1.9]) and brain perfusion (adjusted beta = -2.5 mL/min per 100 mL [95% CI = -4.3 to -0.7]) than the cancer-free reference women (**Table 2**). Effect estimates for tCBF and brain perfusion hardly changed after including total mean IMT and total plaque score in the model (**Table 2**, Model III).

Within the group of breast cancer survivors, no difference was found between participants with the carotid artery partly in the radiation field and those without the carotid artery in the radiation field (**Table 3A**). Survivors who underwent radiotherapy with a larger portion of the carotid artery in the radiation field had slightly higher carotid plaque scores, lower mean tCBF, and lower mean perfusion than those with a smaller portion of the carotid artery in the radiation field, albeit not statistically significant (**Table 3B**). Also, they had a statistically significantly higher total and right IMT score (adjusted beta for total IMT score = 0.05 [95% CI = 0.01 to 0.09] and for right IMT score = 0.09 [95% CI = 0.01 to 0.16], **Table 3B**). Right-sided breast cancer survivors had a statistically significantly lower left carotid plaque score (adjusted beta = -0.31 [95% CI = -0.60 to -0.02]) and a higher brain perfusion (adjusted beta = 3.5 mL/min per 100 mL [95% CI = 0.7 to 6.2]) than participants who survived a left-sided tumor (**Table 4**).

Table 1 Baseline characteristics of the study population.

Characteristic	Breast cancer survivors (n=173)	Cancer-free reference women (n=346)	*P*
Age, years, mean (SD)	63.8 (6.5)	61.7 (6.2)	<.001
Body mass index, kg/m^2, mean (SD)	26.7 (4.6)	27.4 (4.6)	.11
Systolic blood pressure, mm Hg, mean (SD)	139 (19)	131 (19)	<.001
Diastolic blood pressure, mm Hg, mean (SD)	84 (10)	81 (11)	.03
Age at menopause, years, mean (SD)	44.1 (5.1)	49.3 (5.8)	<.001
Diabetes mellitus, No. (%)	13 (7.5)	12 (3.5)	.07
Smoker status, No. (%)			.003
Never	60 (34.7)	132 (38.2)	
Former	95 (54.9)	95 (41.4)	
Current	18 (10.4)	70 (20.3)	
Antihypertensive medication, No. (%)	60 (34.7)	1 (0.3)	<.001
Anticoagulant medication, No. (%)	16 (9.2)	39 (11.4)	.54
Lipid-lowering medication, No. (%)	31 (17.9)	85 (24.9)	.09
Educational level, No. (%)			.002
Primary	15 (8.7)	45 (13.0)	
Lower	67 (38.7)	172 (49.9)	
Intermediate	36 (20.8)	67 (19.4)	
Higher	15 (31.8)	61 (17.7)	
Total brain volume, mL, mean (SD)	902 (76)	900 (80)	.72
Age at cancer diagnosis, years, mean (SD)	42.6 (5.4)		
Time since diagnosis in years, mean (SD)	21.1 (4.4)		
Side of tumour, right side, No. (%)	89 (51.4)		
Radiation field, No. (%)			
Carotid artery not in radiation field*	17 (9.9)		
Carotid artery partly in radiation field†	102 (59.6)		
Carotid artery in radiation field‡	52 (30.4)		

** Axillary, breast, or chest wall radiation. † Internal mammary chain radiation. ‡ McWhirter (supraclavicular and axillary) or supraclavicular radiation.*
Side of tumour and radiation field was missing for one breast cancer survivor.
SD = standard deviation.

Table 2 Association between breast cancer survivors and carotid plaque scores, intima-media thickness, total cerebral blood flow, and brain perfusion.

Outcome	Cancer-free reference women (n=346)	Breast cancer survivors (n=173)	Model I β (95% CI)*	Model II β (95% CI)*	Model III β (95% CI)*
Total carotid plaque score, median (IQR)	2.0 (1.0 to 3.0)	1.0 (0.0 to 3.0)	-0.12 (-0.31 to 0.07)	-0.01 (-0.20 to 0.18)	
Intima-media thickness, mean (SD)	0.83 (0.14)	0.84 (0.14)	0.00 (-0.02 to 0.02)	0.00 (-0.02 to 0.03)	
Total cerebral blood flow, mL/min, mean (SD)	547 (97)	520 (90)	-19.5 (-36.6 to -2.4)	-19.6 (-37.3 to -1.9)	-19.7 (-37.2 to -2.1)
Total brain perfusion, mL/min per 100 mL, mean (SD)	60.9 (9.5)	57.6 (9.0)	-2.8 (-4.6 to -1.1)	-2.5 (-4.3 to -0.7)	-2.5 (-4.2 to -0.7)

Model I = adjusted for age and body mass index; Model II = as Model I, plus: prevalence of diabetes mellitus, smoking status, use of anticoagulant medication, use of lipid-lowering medication, and education; Model III = as Model II, plus: total mean intima-media thickness and total plaque score.

** Difference in median plaque score, mean intima-media thickness, mean cerebral blood flow, or mean brain perfusion between cancer-free women (reference) and breast cancer survivors.*

CI = confidence interval, IQR = interquartile range, SD = standard deviation.

Table 3A Association between radiation to the carotid artery and carotid plaque scores, intima-media thickness, total cerebral blood flow, and brain perfusion in breast cancer survivors.

Outcome	Small part carotid artery in radiation field (n=102)	Carotid artery not in radiation field (n=17)	Model I β (95% CI)*	Model II β (95% CI)*	Model III β (95% CI)*
Carotid plaque score, median (IQR)					
Total	1.0 (0.0 to 3.0)	2.0 (1.0 to 4.0)	0.35 (-0.18 to 0.89)	0.22 (-0.29 to 0.74)	
Left	1.0 (0.0 to 2.0)	1.0 (0.0 to 2.0)	0.15 (-0.44 to 0.71)	0.05 (-0.53 to 0.60)	
Right	1.0 (0.0 to 2.0)	1.0 (0.0 to 3.0)	0.53 (-0.04 to 1.09)	0.39 (-0.15 to 0.91)	
Intima-media thickness, mean (SD)					
Total	0.82 (0.13)	0.84 (0.17)	0.01 (-0.06 to 0.08)	0.00 (-0.06 to 0.07)	
Left	0.83 (0.15)	0.84 (0.16)	0.03 (-0.03 to 0.08)	0.03 (-0.02 to 0.08)	
Right	0.81 (0.14)	0.85 (0.18)	0.03 (-0.02 to 0.08)	0.02 (-0.02 to 0.07)	
Cerebral blood flow, mL/min, mean (SD)					
Total	524 (91)	505 (62)	-21.6 (-66.7 to 23.5)	-21.7 (-67.5 to 24.2)	-16.4 (-61.7 to 28.8)
Brain perfusion, mL/min per 100 mL, mean (SD)					
Total	57.5 (9.1)	56.9 (6.6)	-1.2 (-5.8 to 3.5)	-1.2 (-5.9 to 3.5)	-0.6 (-5.2 to 4.0)

Model I = adjusted for age and body mass index; Model II = as Model I, plus: prevalence of diabetes mellitus, smoking status, use of anticoagulant medication, use of lipid-lowering medication, and education; Model III = as Model II, plus: total mean intima-media thickness and total plaque score.

** Difference in median total plaque score, mean intima-media thickness, mean total cerebral blood flow, or mean brain perfusion between breast cancer survivors treated with carotid artery partly in radiation field (i.e., internal mammary chain radiation, reference group) and those without carotid artery in radiation field.*

CI = confidence interval, IQR = interquartile range, SD = standard deviation.

Table 3B Association between radiation to the carotid artery and carotid plaque scores, intima-media thickness, total cerebral blood flow, and brain perfusion in breast cancer survivors.

Outcome	Small part carotid artery in radiation field (n=102)	Carotid artery in radiation field (n=52)	Model I β (95% CI)*	Model II β (95% CI)*	Model III β (95% CI)*
Carotid plaque score, median (IQR)					
Total	1.0 (0.0 to 3.0)	1.5 (1.0 to 4.3)	0.31 (-0.05 to 0.67)	0.27 (-0.07 to 0.62)	
Left	1.0 (0.0 to 2.0)	1.0 (0.0 to 2.0)	0.31 (-0.07 to 0.68)	0.28 (-0.09 to 0.64)	
Right	1.0 (0.0 to 2.0)	1.0 (0.0 to 2.0)	0.31 (-0.09 to 1.09)	0.22 (-0.17 to 0.61)	
Intima-media thickness, mean (SD)					
Total	0.82 (0.13)	0.89 (0.14)	0.06 (0.01 to 0.10)	0.05 (0.01 to 0.09)	
Left	0.83 (0.15)	0.87 (0.14)	0.05 (-0.02 to 0.11)	0.05 (-0.02 to 0.11)	
Right	0.81 (0.14)	0.90 (0.17)	0.10 (0.03 to 0.17)	0.09 (0.01 to 0.16)	
Cerebral blood flow, mL/min, mean (SD)					
Total	524 (91)	511 (92)	-17.3 (-47.2 to 12.5)	-13.7 (-44.9 to 17.5)	-16.4 (-61.7 to 28.8)
Brain perfusion, mL/min per 100 mL, mean (SD)					
Total	57.5 (9.1)	57.6 (9.3)	-0.5 (-3.6 to 2.5)	-0.5 (-3.7 to 2.7)	-0.1 (-3.3 to 3.1)

Model I = adjusted for age and body mass index; Model II = as Model I, plus: prevalence of diabetes mellitus, smoking status, use of anticoagulant medication, use of lipid-lowering medication, and education; Model III = as Model II, plus: total mean intima-media thickness and total plaque score.

** Difference in median total plaque score, mean intima-media thickness, mean total cerebral blood flow, or mean brain perfusion between breast cancer survivors treated with carotid artery partly in radiation field (i.e., internal mammary chain radiation, reference group) and those with carotid artery in radiation field (i.e., McWhirter or supraclavicular lymph node radiation).*

CI = confidence interval, IQR = interquartile range, SD = standard deviation.

Table 4 Association between tumour location (left or right-sided breast cancer) and carotid plaque scores, intima-media thickness, total cerebral blood flow, and brain perfusion in breast cancer survivors.

Outcome	Left-sided breast cancer (n=83)	Right-sided breast cancer (n=89)	Model I β (95% CI)*	Model II β (95% CI)*
Carotid plaque score, median (IQR)				
Total	2.0 (1.0 to 3.5)	1.0 (0.0 to 3.0)	-0.24 (-0.56 to 0.09)	-0.15 (-0.36 to 0.05)
Left	1.0 (0.0 to 2.0)	1.0 (0.0 to 2.0)	-0.34 (-0.68 to -0.00)	-0.31 (-0.60 to -0.02)
Right	1.0 (0.0 to 2.0)	1.0 (0.0 to 2.0)	-0.07 (-0.43 to 0.29)	-0.01 (-0.36 to 0.35)
Intima-media thickness, mean (SD)				
Total	0.85 (0.13)	0.84 (0.15)	-0.02 (-0.05 to 0.02)	-0.01 (-0.05 to 0.03)
Left	0.85 (0.15)	0.84 (0.15)	-0.02 (-0.06 to 0.02)	-0.01 (-0.05 to 0.04)
Right	0.84 (0.14)	0.84 (0.17)	-0.01 (-0.06 to 0.03)	-0.01 (-0.06 to 0.03)
Cerebral blood flow, mL/min, mean (SD)				
Total	507 (84)	530 (92)	26.2 (0.29 to 52.1)	25.9 (-0.90 to 52.7)
Brain perfusion, mL/min per 100 mL, mean (SD)				
Total	56.0 (8.1)	59.0 (9.4)	3.2 (0.6 to 5.9)	3.5 (0.7 to 6.2)

Model I = adjusted for age and body mass index; Model II = as Model I, plus: age at menopause, prevalence of diabetes mellitus, smoking status, use of anticoagulant medication, use of lipid-lowering medication, and education.

** Difference in median total plaque score, mean intima-media thickness, mean total cerebral blood flow, or mean brain perfusion between breast cancer survivors treated with left-sided cancer (reference group) and right-sided cancer.*

CI = confidence interval, IQR = interquartile range, SD = standard deviation.

DISCUSSION

This study shows that on average twenty years after treatment with chemotherapy and radiotherapy, breast cancer survivors have lower tCBF and brain perfusion than aged-matched cancer-free women. Our results within breast cancer survivors indicate that radiotherapy on a larger part of the carotid artery is associated with a greater IMT. Lastly, we found that plaque scores in the left carotid artery were significantly lower in participants with a right-sided tumour than in those with a left-sided tumour.

We found that breast cancer survivors had lower tCBF and brain perfusion than cancer-free women, which was not completely explained by carotid pathology. In contrast, it has previously been shown that one year after completion of chemotherapy, brain perfusion was increased in breast cancer survivors, which might reflect a temporary compensatory mechanism for chemotherapy-induced damage.[31] In addition, brain perfusion was decreased in the frontal and parietal parts of the brain, which was associated with lower grey matter density.[32] The lower brain perfusion in our study might therefore underlie the cognitive deficits and alterations in brain volumes that we previously observed in this group of cancer survivors,[19,20] and which are observed in breast cancer survivors who have completed chemotherapy in general.[33] We have explored this hypothesis in post-hoc analyses and indeed found that the relation between global cognitive function and brain perfusion differed between breast cancer survivors and reference women (**Supplementary Material**). In addition, lower brain perfusion is associated with a higher risk of transient ischemic attack (TIA) in the general population.[34] Although it has been shown that breast cancer survivors have a non-statistically significant higher risk of TIA,[35] it might be relevant to focus on those survivors with altered brain perfusion.

We did not find a difference in carotid pathology between the total group of breast cancer survivors and the cancer-free reference women. However, within breast cancer survivors, we found that more radiotherapy on the carotid artery was associated with a greater IMT. Carotid IMT is a marker for atherosclerosis. Although carotid plaques are a stronger predictor of cardiovascular disease than IMT in the general population,[36] greater IMT is also associated with cardiovascular events independent of major cardiovascular risk factors including carotid plaques.[37] Therefore, greater IMT in breast cancer survivors treated with radiotherapy on the carotid artery might explain the higher risk of cardiovascular events in those breast cancer survivors who were treated with radiotherapy.[3] A potential explanation for the fact that we did not find differences between breast cancer survivors and cancer-free controls might be that cancer survivors had adopted a healthier lifestyle after their diagnosis and treatment. This may limit the damaging effects of chemotherapy and radiotherapy on the vascular system. This

hypothesis is supported by a higher rate of former smokers in our group of cancer survivors, which might suggest that these women stopped smoking after their cancer diagnosis.

Our observation of higher left plaque score in breast cancer survivors with left-sided cancer than those with right-sided cancer may reflect an interaction between a generally higher rate of plaques in the left versus the right carotid artery and radiotherapy. In the general population, the prevalence of left-sided plaques is twice as high as right-sided plaques.[38] Also, left-sided plaques are predominantly composed of intraplaque haemorrhage and fibrous tissue and are therefore more vulnerable to plaque rupture and subsequent thromboembolic complications than right-sided plaques.[38] In addition, the left carotid artery may be exposed to higher arterial pressure due to left-right differences in anatomy. For instance, the left carotid artery is directly connected to the aortic arch, whereas the right carotid artery is connected to the brachiocephalic artery.[39] It is therefore possible that radiotherapy accelerates the number of plaques on the left side. Previously, our group has reported that breast cancer survivors who had received radiotherapy for left-sided breast cancer had higher risks for myocardial infarction (hazard ratio (HR) = 1.77) and congestive heart failure (HR = 1.41) than breast cancer survivors with right-sided tumours, although these effects were not significant.[3] This higher risk might be explained by a higher radiation exposure of the heart in left-sided cancer patients. Together, these findings emphasise the importance of cardiovascular risk screening in breast cancer survivors, in particular in those with left-sided breast cancer.

Strengths of our study are the sample of almost two hundred breast cancer survivors with a long interval since radiotherapy and chemotherapy, the homogeneous study population with regard to the cytotoxic agents received (regimen and cycles), and the comparison with population-based reference women without a history of cancer who underwent the same examinations as the breast cancer survivors.

CMF chemotherapy has a high likelihood of inducing early menopause. Age at menopause was therefore considered a mediating variable in the between-group analyses. Because of this, it is impossible to separate the direct effects of chemotherapy, and the effects through menopause. Samples with sufficient numbers of subjects who did and did not reach early menopause due to chemotherapy are necessary to separate the effects of chemotherapy and menopause on vascular pathology and brain perfusion.

A limitation is that the included breast cancer survivors did not receive endocrine therapy. Endocrine therapy was not part of the standard treatment for patients with breast cancer in the Netherlands until the mid-1990s. However, nowadays, patients frequently receive endocrine therapy, and it has been shown that this therapy is associated with the presence of carotid plaques.[40] Also, the CMF regimen is no longer considered an optimal adjuvant chemotherapy regimen for breast cancer, but it has been the standard regimen worldwide up to the 1990s.[39]

It is therefore currently the only regimen that enables the investigation of the very late effects of chemotherapy in sufficiently large numbers of persons. Current regimens often include individual components of the CMF regimen, including cyclophosphamide and 5-fluorouracil. Therefore, the current findings may also be relevant for breast cancer survivors that are treated with contemporary chemotherapy regimens. Also, there is still a large group of women who have been treated with CMF in the past of whom some women may now experience the negative cerebral consequences. Lastly, the observed associations may be less pronounced in breast cancer patients who are currently treated with radiotherapy, as radiotherapy for breast cancer is usually given to more limited target volumes and radiotherapy techniques have improved leading to lower doses to the carotid arteries[41] and the heart.[42]

Breast cancer survivors have lower brain perfusion on average twenty years post-treatment which may be part of the mechanism underlying the well-known cognitive sequelae of chemotherapy. Radiotherapy on the carotid artery is associated with a larger IMT, which in turn might result in more cardiovascular disease. Therefore, cardiovascular risk management of breast cancer survivors is important.

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SUPPLEMENTARY MATERIAL

Supplementary methods

Cognitive function

Cognitive function was assessed by a neuropsychological test battery administered at the research centre. The following cognitive tests were administrated: Letter-Digit Substitution Test, Word Fluency Test, Stroop Test (Reading, Naming, Interference), Purdue Pegboard Test (right, left, both hands), and 15-Word Learning Test (Immediate recall, Delayed recall, Recognition).[1-5]

Global cognitive function was assessed by the general cognitive factor based on Letter-Digit Substitution Test, Word Fluency Test, Stroop Test: Interference, sum-score of individual Purdue Pegboard Tests, and Word Learning Test: Delayed recall. The general cognitive factor was identified as the first unrotated component of a principal component analysis, which explained at least 44.6% of the total variance in individual cognitive tests.[6] The general cognitive factor was only computed if all five individual tests were completed. Therefore, the general cognitive factor could not be computed for 22 breast cancer survivors (12.7%) and 39 reference women (11.3%). Excluded breast cancer survivors and reference women were slightly older than those who had all tests completed (mean age for breast cancer survivors was 65.7 versus 63.5 years, mean age for reference women was 63.1 versus 61.5 years). Most excluded breast cancer survivors had one missing test result (51.3%), 25.6% had two missing test results, and 23.1% had three or more missing test results. Of the excluded reference women, 68.1% had one missing test result, 22.7% had two missing test results, and 9.1% had three or more missing test results.

Statistical analyses

In post-hoc analyses, we focused on the outcomes that were statistically significantly different between breast cancer survivors and reference women (i.e., total cerebral blood flow and brain perfusion). We explored whether the association between general cognitive function and total cerebral blood flow or brain perfusion differed between breast cancer survivors and reference women. In order to do so, we used linear regression models with the general cognitive factor as outcome and computed interaction terms between cancer status and total cerebral blood flow or brain perfusion. These models included the same covariates as Model II that we used to test differences in total cerebral blood flow and brain perfusion between breast cancer survivors and reference women, including: age, body mass index, prevalence of diabetes mellitus, smoking status, use of anticoagulant medication and lipid-lowering medication, and

educational level.

Supplementary results

Results regarding the difference in total cerebral blood flow and brain perfusion between breast cancer survivors and reference women were similar in this smaller study population (n=458) to those obtained from the total study population (n=519).

We found that per mL/min increase in total cerebral blood flow, the general cognitive factor increased with 0.001 units. This association was stronger in breast cancer survivors than in reference women: per mL/min increase in total cerebral blood flow their general cognitive factor increased with an additional 0.002 units (95% confidence interval = 0.000 to 0.004, *P* for interaction = .03).

For each mL/min per 100 mL increase in brain perfusion, the general cognitive factor increased with 0.003 units. In breast cancer survivors, the general cognitive factor increased with an additional 0.025 units per mL/min per 100mL increase in brain perfusion (95% confidence interval = 0.006 to 0.044, *P* for interaction = .01).

Given the linear associations, a decrease in either total cerebral blood flow or brain perfusion will result in a stronger decrease in the general cognitive factor in breast cancer survivors than in reference women. These findings may indicate that the relation between changes in cerebral blood flow, brain perfusion, and general cognitive factor differ between breast cancer survivors and reference women.

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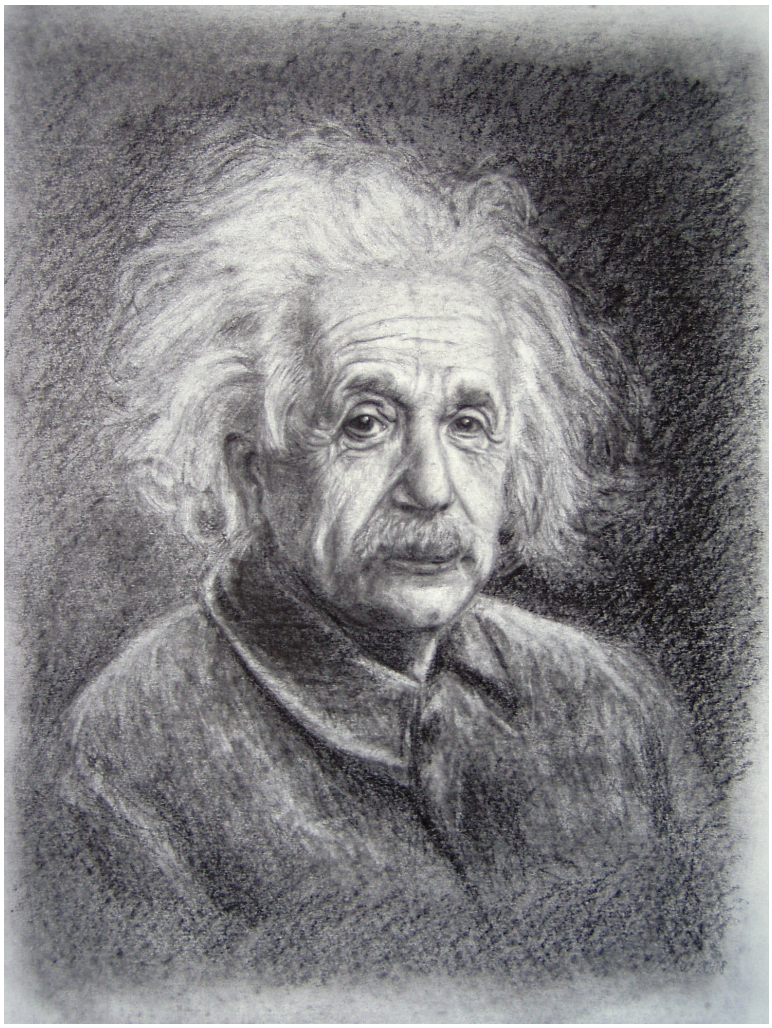
Part V

General discussion



The aim of this thesis was to better understand the origin and course of cognitive decline in non-central nervous system (CNS) cancer patients and survivors, their risk of dementia, and the mechanisms underlying cognitive problems and dementia in cancer patients. In this last Part, I bring the main findings of this thesis together and place these findings in a broader perspective. Next, I discuss the methodological considerations, outline the clinical implications, and provide suggestions for future studies.

REVIEW AND INTERPRETATION OF MAIN FINDINGS

Part I - Cancer registration

The Netherlands Cancer Registry aims to register all cancers in the Netherlands and has nationwide coverage since 1989.[1] Optimal cancer registration is necessary for studying cancer statistics and cancer aetiology. To improve the quality of cancer registration, different studies have determined the completeness and accuracy of national cancer registries.[2-13] For instance, completeness of cancer registration by the Netherlands Cancer Registry was estimated at 98.7% in 1990[2] and at 96.2% in 1993[3] based on linkage with data from general practitioners. The comparison of cancer registration by national cancer registries and population-based studies remains however scarce. In **Chapter 2** we therefore determined the potential added value of population-based studies by linking participants in the Rotterdam Study to patients in the Netherlands Cancer Registry.[14] Although there is no golden standard of cancer registration, we considered this comparison as the best alternative. Two findings stood out: (i) completeness of registered pathology-confirmed cancers was >95.0% in both registries, whereas completeness of cancers that were not confirmed by pathology was only 40.0% in the Netherlands Cancer Registry compared to 97.7% in the Rotterdam Study; and (ii) the date of diagnosis was more often inaccurately registered by the Rotterdam Study (11.8% of the cancers) than by the Netherlands Cancer Registry (4.8% of the cancers).

In **Chapter 3**, we took a closer look at the group of cancers that was often not registered by the Netherlands Cancer Registry, i.e., cancers that were not confirmed by pathology. Although we cannot rule out that non-pathology-confirmed tumours are in fact benign lesions, these patients have undergone the same diagnostic work-up – apart from pathological confirmation – as patients with pathology-confirmed cancer. Pathological confirmation of tumours can be omitted if patients are vulnerable, if they have other major health concerns, or if confirmation has no therapeutic consequences. We found that 11.7% of all cancers in the Rotterdam Study were not confirmed by pathology.[15] Patients with non-pathology-confirmed cancers were older,

had more comorbidities, and had more aggressive cancer types than patients with pathology-confirmed cancers. Importantly, the overall survival of patients with non-pathology-confirmed cancer (32.6% at one year after diagnosis) was substantially lower than that of patients with pathology-confirmed cancer (63.4% at one year after diagnosis).

Main message Part I

The main message that emerges from this Part, is that combining multiple sources of cancer registration is necessary to improve the quality of cancer registration by population-based studies and national cancer registries. More effort is needed to register non-pathology-confirmed cancers, in particular as these cancers are related to patient and tumour characteristics. We therefore suggest to include non-pathology-confirmed cancers in sensitivity analyses to minimise bias. Implications for cancer registries will be discussed in the Implications section.

Part II - Cancer and cognition

Several studies have shown that non-CNS cancer patients can have impaired cognitive function.[16-22] Most of these studies have focused on the effects of chemotherapy on the brain. In addition, few studies have found that some patients have already impaired cognitive function before start of cancer treatment.[23-28] Although psychological factors that accompany a cancer diagnosis cannot be completely ruled out, animal studies have confirmed these findings by showing that tumour-bearing, treatment-naive rodents can have impaired memory function.[29,30,31] Together, these findings suggest that cancer treatment is not the only cause of cognitive problems in cancer patients and that cancer itself, psychological factors, or shared risk factors for both cancer and cognitive impairment may also affect cognitive function.

Before exploring the origin and course of cognitive function in cancer patients in a population-based setting, we first determined in **Chapter 4** the trajectories of cognitive and motor function in the general population. Many studies have shown that cognitive and motor function decline during ageing,[32-44] but the natural course of decline in these functions as well as their temporal relation in the general population is poorly understood. In a population free from neurodegenerative diseases, we found that cognitive and motor function declined linearly between the ages 45 and 65 years, followed by steeper decline. The decline in cognitive and motor function was similar – i.e., decline in cognitive function did not precede decline in motor function and vice versa – but there was a high variation in the rate of decline in individual tests. This indicates that some cognitive and motor functions, such as inhibition and psycho-motor speed, might be more vulnerable to normal ageing than for instance memory.

These established trajectories of cognitive and motor function could be used as standard to identify persons who deviate from the natural course of decline. I have illustrated this

application in **Figure 1** by showing the cognitive trajectories of participants in the Rotterdam Study who were diagnosed with dementia during study follow-up and of those were diagnosed with cancer. Before dementia diagnosis, participants deviated from the expected natural course of decline, whereas before cancer diagnosis, participants followed the same course of decline as the general population.

Although **Figure 1** suggests that persons who will be diagnosed with cancer do not deviate from the expected natural course of cognitive decline, it does not indicate whether their cognitive function deviates from this course more closely towards the clinical manifestation of cancer. To further elucidate the change in cognitive function before cancer diagnosis, we studied in **Chapter 5** the trajectory of cognitive decline in participants who were diagnosed with cancer during study follow-up and compared this with the trajectory of cognitive decline in participants who remained free of cancer during follow-up.[45] Instead of age, we used follow-up time as underlying time scale to study the change in cognitive function towards diagnosis. This design enabled us to study purely the impact of cancer itself and shared risk factors on cognitive function while circumventing the potential effects of psychological factors and cancer treatment. We found that the trajectory of cognitive function in participants prior to cancer diagnosis was similar to that observed in participants who remained free of cancer.[45] Although this study had some limitations such as the long interval between cognitive assessments and the limited number of assessments directly preceding cancer diagnosis, this finding suggests that – if anything – the effects of cancer itself and shared risk factors on cognitive function are limited before clinical manifestation of the disease.

Changes in cognitive function correlate moderately with changes in brain structure.[46,47] For instance, it has been shown that persons without cognitive impairment, but with lower volumes of the hippocampi or temporal lobes are at a higher risk of dementia than persons with normal brain tissue volumes.[48] To further explore the potential impact of cancer itself and shared risk factors on the brain, we determined in **Chapter 6** the relation between different measurements of brain structure and the risk of cancer. We found no association between brain structure and the risk of cancer, indicating that before cancer diagnosis, patients do not have more brain abnormalities than persons who remain free of cancer. Our findings therefore do not support that cancer affects the brain before clinical manifestation of the disease. Although we could not examine brain structure more closely towards cancer diagnosis, our findings indicate that, if anything, the impact of cancer and shared risk factors on brain structure before cancer diagnosis is very subtle.

In **Chapter 7** we further extended the trajectories of cognitive function in cancer patients by including cognitive assessments after cancer diagnosis to study the cognitive trajectories from before to after cancer diagnosis. Although cognitive function in cancer patients after diagnosis

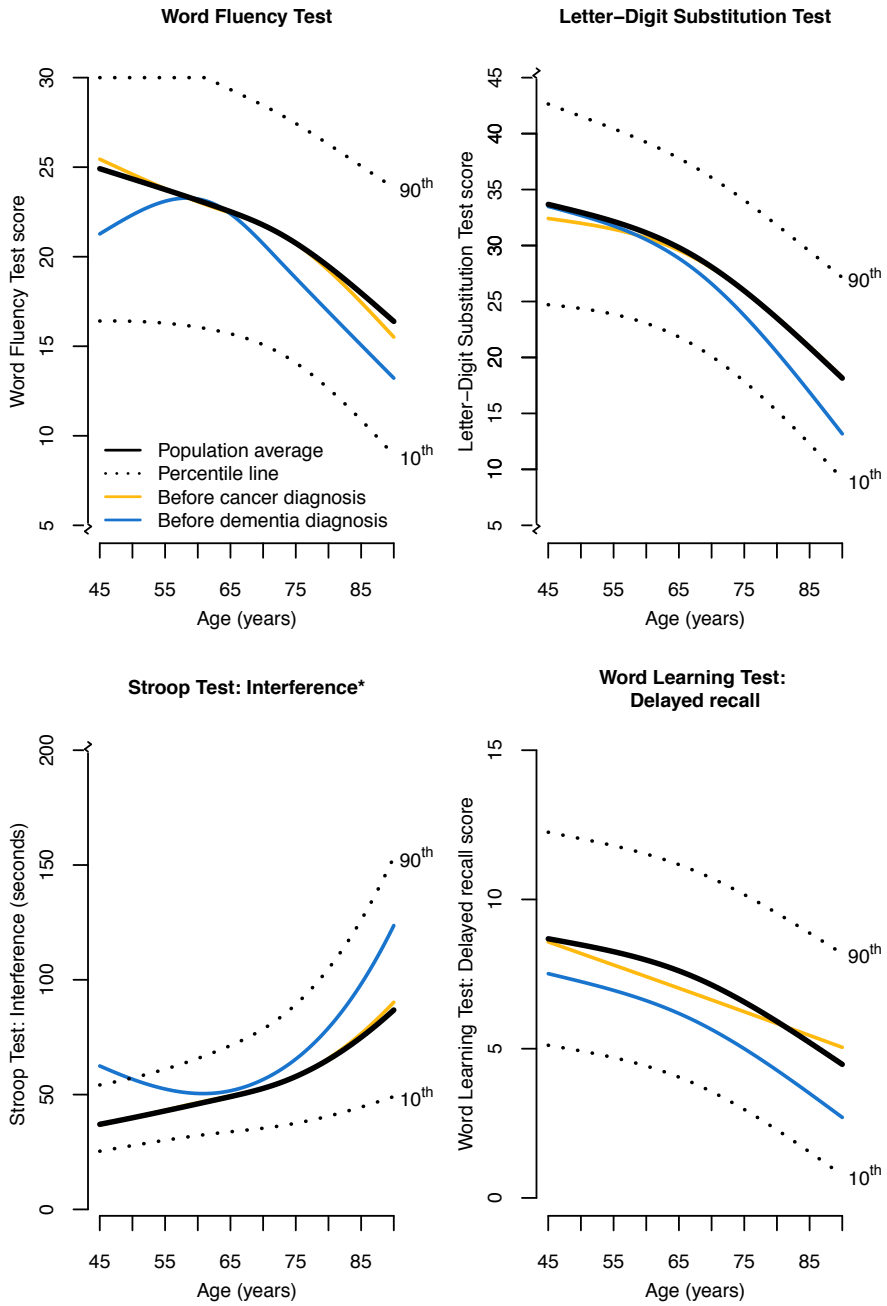


Figure 1 Trajectories of cognitive test scores.
The trajectories of the total population are shown in black. The trajectories of participants who were diagnosed with dementia during follow-up are shown in blue and of those who were diagnosed with cancer during follow-up are shown in yellow. The confidence intervals are not shown for clarity.
** Higher score indicates worse performance.*

has been extensively studied, clinical studies have not performed cognitive assessments prior to cancer diagnosis, have focused on a subgroup of cancer patients and intensive treatments, and may have been biased by selection of patients.[49] Therefore, the course of cognitive function after cancer diagnosis and treatment in the general population of cancer patients is still poorly understood. We found that cognitive function in cancer patients from before to after cancer diagnosis declined with a similar rate as that in cancer-free persons. It must be noted that a large number of cancer patients was excluded from this study, because the majority of the patients (64.3%) did not have cognitive assessments after cancer diagnosis. Consequently, we have probably selected the healthiest patients with favourable cancer types. For instance, only 3.6% of the included patients had lung cancer – which is the second most common cancer type in the Netherlands when excluding non-melanoma skin cancers – and only 11.3% were treated with chemotherapy. Larger numbers are therefore needed to assess cognitive change in patients who underwent systemic treatments. Our findings indicate that in general, cognitive function in cancer patients changes similarly to that in persons without a history of cancer. This suggests that the effect of cancer itself on cognitive function is limited and underlines the necessity to identify high risk patients.

Main message Part II

In conclusion, the findings from the studies in this Part indicate that cognitive function and brain structure are not affected before cancer diagnosis. Also, at a population-level, the course of cognitive function in non-CNS cancer patients after diagnosis is similar to that in cancer-free persons. These findings indicate that, if anything, effects of cancer itself and shared risk factors on cognitive function and brain structure are very subtle. The implications of these findings will be discussed in the Implications section.

Part III - Cancer and dementia

Having studied the change in cognitive function in cancer patients, we subsequently focused on their risk of dementia. At a population-level, we found that the change in cognitive function in the general population of cancer patients was similar to that in cancer-free persons. In addition, previous clinical studies have shown that patients with specific types of cancer or cancer treatment often have cognitive impairment. Against this background, we hypothesised that the risk of dementia in cancer patients is either similar or higher to the dementia risk in persons without a history of cancer. This hypothesis is supported by various biological processes that are involved in the pathogenesis of cancer and dementia, including inflammation, angiogenesis, oxidative stress, and DNA damage.[50] In addition, a genome-wide association study has found a positive genetic correlation between cancer and dementia

genes, implying that cancer and dementia share some genetic background.[51]

In contrast to our hypothesis, studies have repeatedly shown that cancer patients have a lower risk of developing dementia than cancer-free persons.[52-64] Interestingly, it has also been found that patients with dementia have a lower risk of developing cancer,[59-67] suggesting an inverse link between cancer and dementia. This inverse link has been found for different cancer types, including non-melanoma skin cancer.[54,55] Although different biological mechanisms underpinning this inverse link have been proposed,[68-70] methodological issues including surveillance and survival bias as potential drivers of the inverse direction of this association have not been sufficiently investigated.[71,72] The studies in this Part aimed to circumvent these biases in order to elucidate the biological relation between cancer and dementia.

First, in **Chapter 8**, we studied Alzheimer's disease (AD), the most common type of dementia, as a multistep process using multistage models that have originated from cancer research.[73] The underlying theory is that a healthy stem cell transforms into a malignant cell through sequential mutations. Multistage models have established that seven successive mutations – equivalent to seven steps – are needed before a healthy cell becomes malignant.[74] We showed that AD complied with the multistep process and that 14 steps were required before clinical manifestation of the disease. Interestingly, genetically predisposed persons needed less steps before clinical manifestation, which was also observed in cancer.[75] This indicates that these persons have already inherited one of the required steps, which can provide additional insight in the pathogenesis of dementia. These findings further support some biological similarity between cancer and AD.

Next, we studied the preclinical stage of either cancer or dementia, and linked it to the other disease. We hypothesised that if there is a biological link between cancer and dementia, this would extent across all preclinical stages of the disease. By using this approach, we could limit the effects of surveillance and survival bias, because persons with a preclinical stage of a disease have often a longer life expectancy than those with clinically-manifested disease. Although not all persons with a preclinical stage of a disease will develop clinically-manifested disease, it may still be informative from a biological perspective.

In **Chapter 9** we first verified that patients with dementia have a lower risk of developing cancer than persons without dementia.[76] Next, we determined the relation between mild cognitive impairment – a preclinical stage of dementia – and the risk of cancer. In contrast to patients with dementia, persons with mild cognitive impairment had a higher risk of developing cancer than cognitively normal persons. Mild cognitive impairment is defined as having both objective cognitive impairment and subjective memory complaints. In addition, persons have to be at least 60 years old. Therefore, although we found in **Chapter 5** and **6** no relation between cognitive function and the risk of cancer, the association between mild cognitive

impairment and cancer might partly be explained by the combination of objective cognitive impairment and subjective memory complaints, rather than only deficits in objective cognitive function, or by the different study population (i.e., participants were aged at least 60 years when studying mild cognitive impairment). Subsequently, we studied in **Chapter 10** the relation between plasma amyloid-β – one of the earliest detectable changes in preclinical dementia, even before the onset of mild cognitive impairment – and the risk of cancer and found that higher levels of plasma amyloid-β were related to a higher risk of cancer. Lastly, in **Chapter 11** we determined the relation between the tumour marker carcinoembryonic antigen and the risk of dementia and found that higher levels of carcinoembryonic antigen were associated with a higher risk of dementia.

Although these associations might suggest a positive relation between cancer and dementia, one can argue the validity of using such markers as preclinical stages of a disease. Also, we cannot fully rule out methodological bias by studying preclinical stages of a disease. Therefore, we provided in **Chapter 12** an alternative approach to deal with selection bias.

Since the date of cancer diagnosis is time-dependent, we first accounted for immortal time (**Box 1** in Methodological considerations) by using the following three methods: (i) studying cancer as time-dependent variable; (ii) using inverse probability weights for the time until cancer diagnosis; and (iii) by cloning and censoring our dataset. Cancer is also related to death and therefore, part of the effect of cancer on dementia is through death. Censoring for death is informative and can result in spurious effect estimates. **Figure 2** shows the corresponding directed acyclic graph. To deal with the competing risk of death and loss to follow-up, we used inverse probability weighting. Using this method, we found that cancer patients do not have a higher risk of dementia than persons without cancer. This emphasises the importance of using the correct statistical methods when studying an association in the presence of the competing risk of death.

Main message Part III

Taken together, the association between cancer and dementia remains complicated. A substantial amount of bias may influence the direction of this association. We have used different approaches to circumvent such bias. Our results do not support an inverse association between non-CNS cancer and dementia. In fact, cancer patients had a similar risk of developing dementia as persons without a history of cancer when using appropriate statistical models. If anything, our findings based on preclinical disease stages may stimulate future studies to explore a positive, biological relation between non-CNS cancer and dementia. Suggestions for such exploration are provided in the Directions for future research section.

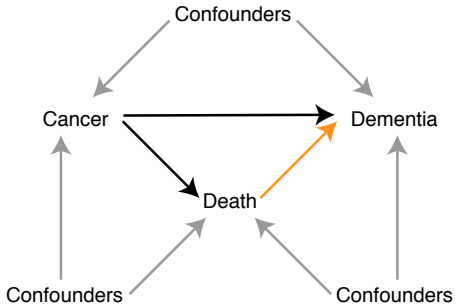


Figure 2 Directed acyclic graph for the relation between cancer and the risk of dementia.
We were interested in the relation between cancer and the risk of dementia. Both cancer and dementia are strongly associated with death. Therefore, part of the effect of cancer on dementia goes through death (orange arrow). We must take the competing risk of death into account when studying this relation. In addition, confounders (grey arrows) for the relation between cancer and dementia, cancer and death, and dementia and death, might affect the relation between cancer and dementia and should therefore be taken into account.

Part IV - Underlying mechanisms

Lastly, we investigated potential mechanisms underlying cognitive problems – and possibly dementia – in cancer patients. Different proposed mechanisms are the release of extracellular vesicles, inflammation, oxidative stress, vascular changes, mitochondrial dysfunction, changes in hormonal levels, and telomere shortening.[77-79] The studies in this thesis focused on inflammation and vascular factors.

In **Chapter 13** and **16**, we studied breast cancer survivors who were treated with surgery, radiotherapy, and chemotherapy on average twenty years before assessment. Although these breast cancer survivors did not participate in the Rotterdam Study, they underwent identical assessments in the same research centre as participants in the Rotterdam Study. This enabled us to use participants in the Rotterdam Study without a history of cancer as controls. We have previously shown that these breast cancer survivors have worse cognitive function, less grey matter volume, less white matter integrity, and more cerebral microbleeds than cancer-free controls.[21,80,81] Note that, although we think that the effect of cancer itself on cognitive function is limited based on our findings in **Part II**, we cannot disentangle the effects of cancer and cancer treatment in these breast cancer survivors. I will therefore refer to the potential underlying mechanisms of both cancer and cancer treatment.

In these breast cancer survivors, we investigated in **Chapter 13** the association between cognitive function and inflammation by quantifying the granulocyte-to-lymphocyte ratio (GLR), platelet-to-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII).[82] These inflammatory ratios can broadly capture the balance between the two main components of the immune system: innate immunity, referring to the immune responses present at birth,

and adaptive immunity, reflecting the immune responses acquired during life.[83] Granulocytes and platelets are primarily involved in innate immunity, whereas lymphocytes are related to adaptive immunity. Therefore, a higher GLR, PLR, and SII reflect an imbalance in the immune system towards innate immunity.

We found that breast cancer survivors had – twenty years after cancer treatment – higher inflammatory ratios than cancer-free controls. In addition, the relation between higher inflammatory ratios and lower general cognitive function was stronger in breast cancer survivors than in cancer-free controls. The SII may already be higher before cancer diagnosis.[84] Therefore – and because of our study design – we could not determine causality. Nevertheless, these findings suggest that an imbalance in the immune system towards innate immunity may underlie late cognitive problems in cancer patients. Interestingly, we found in **Chapter 14** that this imbalance may also be involved in the pathogenesis of dementia.[85] It would therefore be interesting to further follow the breast cancer survivors and study their risk of dementia.

Activation of the immune system can result in inflammation. Although we used the GLR, PLR, and SII as proxy for inflammation, it must be noted that we could not identify the phenotype of the underlying immune cell populations. Therefore, it is unknown if the underlying blood cells are functional and hence cause higher levels of pro-inflammatory cytokines. Few studies have tried to identify cytokines that may be involved in cognitive impairment in cancer patients, but findings are heterogeneous in terms of the involved cytokines and affected domains of cognitive function.[25,86-90] Animal studies have further supported involvement of pro-inflammatory cytokines and have shown that administration of low-dose aspirin in tumour-bearing, treatment-naive mice resulted in improved memory function without affecting the tumour burden.[91] If the higher inflammatory ratios truly reflect a higher production of pro-inflammatory cytokines, these cytokines may cross the blood-brain barrier and activate microglial cells, thereby initiating the release of neuronal cytokines. This can result in neurotransmitter deregulation, decreased neurogenesis, and lower neuroplasticity.[78,92,93] Interestingly, it has been shown that amyloid-β may also activate microglial cells and thereby stimulate the production of pro-inflammatory cytokines in the brain.[94] Given that higher levels of plasma amyloid-β are associated with a higher risk of cancer, amyloid-β might also have a role in cognitive problems in cancer patients.

Next, in **Chapter 15** and **16** we focused on the potential role of vascular factors in cognitive problems in cancer patients. Cancer and cancer treatment are associated with vascular changes including hypercoagulable state, atherosclerosis, and injury to cardiac myocytes, resulting in a higher risk of cardiovascular diseases.[95-99] Moreover, cancer and cardiovascular diseases share different pathways, including inflammation and oxidative stress,[100,101] which may

also contribute to the higher risk of cardiovascular diseases in cancer patients. In **Chapter 15**, we studied presence of atherosclerotic calcification in the aortic arch before cancer diagnosis. We found that persons with the highest amount of aortic arch calcification had a higher risk of cancer than persons with the lowest amount of aortic arch calcification. Despite the fact that we cannot determine causality, this finding indicates that vascular changes can occur even before cancer diagnosis. In **Chapter 16** we studied vascular factors after cancer diagnosis and treatment, and found no difference in presence of carotid plaques and intima-media thickness between breast cancer survivors and cancer-free controls. It is possible that cancer survivors adopt a healthier lifestyle,[102] thereby limiting the potential damaging effects of cancer treatment. Also, cancer patients or survivors with the highest amount of atherosclerosis may have died of cardiovascular disease before study enrolment, resulting in survival bias. Within breast cancer survivors, radiotherapy on the carotid artery was associated with a greater intima-media thickness. In addition, breast cancer survivors had lower brain perfusion than cancer-free controls. In the general population, lower brain perfusion has been associated with an accelerated decline in cognitive function and with a higher risk of dementia.[103] Lower brain perfusion can result in hypoxia, which is associated with microglial cell activiation.[104] In turn, microglial cells can produce pro-inflammatory cytokines, resulting in neuronal damage.[105] In addition, hypoxia might result in accelerated amyloid-β production and less amyloid-β clearance.[106] Therefore, our findings suggest that cancer and cancer treatment are related to vascular changes that might underlie cognitive problems in cancer patients.

Main message Part IV

In summary, our findings indicate that inflammation and vascular factors – which are also interrelated[107] – may both contribute to cognitive problems and potentially dementia in non-CNS cancer patients. Although these factors are also associated with cognitive problems and dementia in persons without a history of cancer, the impact might be different in non-CNS cancer patients and survivors potentially due to acceleration of ageing processes, differences in the involved immune cell populations, longer exposure to inflammation, or disruptions in the blood-brain barrier.[77,108,109] These mechanisms might be used as targets for prevention and intervention strategies.

METHODOLOGICAL CONSIDERATIONS

The shortcomings and strengths of the individual studies presented in this thesis have been discussed in the corresponding Chapters. In this section, I will elaborate on several methodological considerations that generally apply to the research described in this thesis.

Study setting

The majority of the studies in this thesis were embedded in the Rotterdam Study, a large prospective population-based cohort study.[110] We chose to study cognitive function in cancer patients in a population-based setting, because of the following three advantages that will be discussed in more detail: (i) limiting selection of cancer patients; (ii) studying changes in cognitive function from before to after cancer diagnosis; and (iii) including a large population of cancer patients with different cancer types and treatments.

Firstly, the design of the Rotterdam Study increases generalisability of the findings to the general population and reduces the possibility of selection bias. We hypothesised that selection of participants in clinical settings might have unduly influenced the prevalence and severity of cognitive problems in cancer patients, because cancer patients with cognitive problems might be more willing to participate in a study on cognitive function. Therefore, despite the lack of ethnic and socioeconomic diversity in the Rotterdam Study, studying participants in a population-based setting increases the generalisability of findings to the general population. Nevertheless, also in population-based studies some selection is inevitable. For instance, persons with poorer health status are less likely to participate in observational studies.[111] Such selection may have resulted in the inclusion of relatively healthy participants and may therefore have affected the obtained effect estimates. For instance, in **Chapter 7** we encountered selection of cancer patients that we did not anticipate. In this study, we included cancer patients with at least one cognitive assessment before cancer diagnosis and – as an extension of **Chapter 5** – at least one cognitive assessment after diagnosis. We had to exclude 64.3% of the participants with cancer because they had no cognitive assessment after diagnosis. These cancer patients were older and had more aggressive cancer types than included patients, indicating that we selected the healthiest cancer patients. Almost half of the excluded cancer patients had died within five years after their last cognitive assessment. Due to the selection of the studied population, the effect estimates may have been underestimated. Because of the long interval between assessments, these patients would probably not have been missed by clinical studies (**Figure 3**).

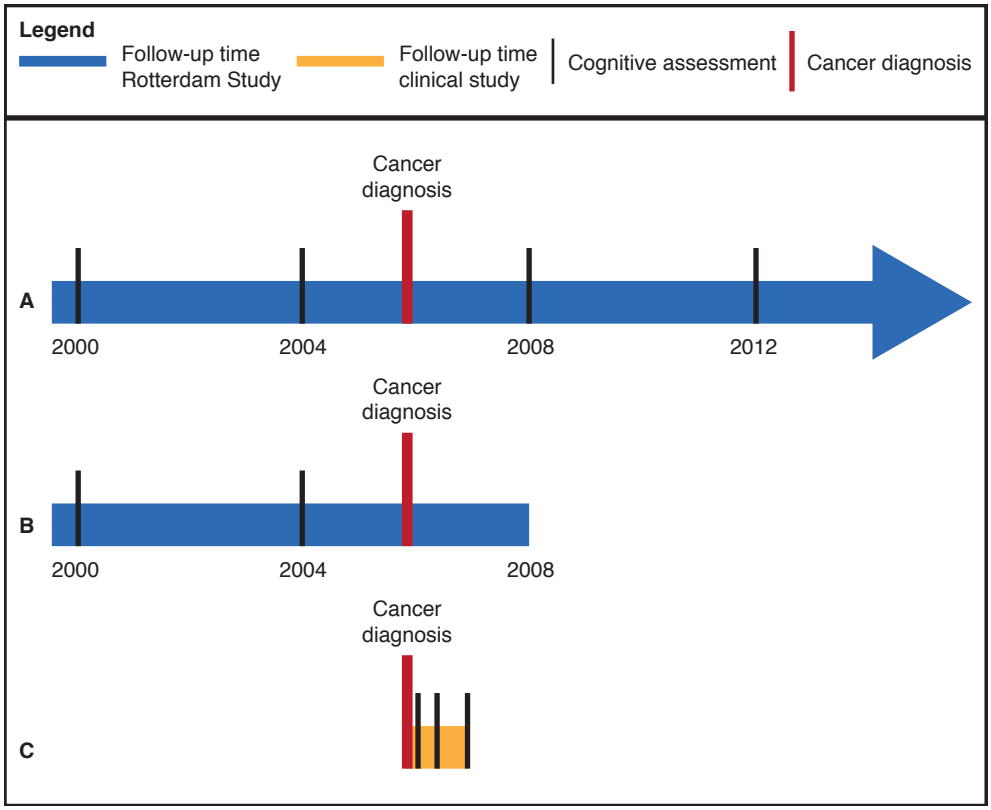


Figure 3 Study design of the Rotterdam Study versus that of clinical studies.
A) Participants in the Rotterdam Study undergo a cognitive assessment every three to six years. During follow-up, some participants are diagnosed with cancer. Therefore, we can study cognitive assessments before and after cancer diagnosis. B) Some participants who are diagnosed with cancer die or are lost to follow-up before their first cognitive assessment after cancer diagnosis. These participants could not have been included in our study on cognitive function after cancer diagnosis, which may have resulted in selection bias. C) In clinical studies, cancer patients are assessed multiple times shortly after cancer diagnosis. Therefore, patients who die within two years after cancer diagnosis may still have participated in a clinical study.

Secondly, we were interested in the change in cognitive function from before to after cancer diagnosis. The population-based setting allowed us to study purely the effects of cancer itself and shared risk factors on cognitive function before cancer diagnosis by limiting the effects of psychological factors. Although our findings suggest that cognitive function is not affected before cancer diagnosis, the median time between the date of the last cognitive assessment before cancer diagnosis and the date of diagnosis ranged between 2.4 and 3.7 years. Therefore, we cannot rule out that cognitive function is also not affected directly preceding cancer diagnosis. After cancer diagnosis, the median time between the date of diagnosis and the date of first cognitive assessment ranged between 2.7 and 3.8 years. These

findings provide insight in the overall trajectory of cognitive function from before to after cancer diagnosis in the general population of cancer patients, but are not directly comparable to clinical studies that have assessed cognitive function in a subgroup of cancer patients multiple times shortly after cancer diagnosis and treatment.

Thirdly, we aimed to study the change in cognitive function in a large population of cancer patients with different cancer types and treatments to understand cognitive change in the general population of cancer patients. Although the number of included patients in clinical studies has been growing over the last years, the largest study – which has a follow-up duration shorter than one year – comprises 580 breast cancer patients.[112] We included more cancer patients in our studies on cognitive function, but these cancer patients represented a heterogeneous group of cancer patients. This enabled us to draw conclusions about cancer patients in general, but not about subgroups of cancer patients and survivors.

Ascertainment of cancer

Table 1 shows the total number of patients per cancer type for different age categories in the Rotterdam Study and in the Netherlands. The distribution of different cancer types in the Rotterdam Study is comparable to that in the Netherlands. In the Rotterdam Study, cancers are registered based on medical discharge letters and the general practitioner's status in addition to linkage with the national hospital discharge registry, pathology databases, and the Netherlands Cancer Registry. Using these different sources, we can collect information about both pathology-confirmed and non-pathology-confirmed cancer diagnoses. To limit false positive findings, we included only pathology-confirmed cancers in our primary analyses and performed sensitivity analyses – results were not always shown – by including non-pathology-confirmed cancers. Neglecting non-pathology-confirmed cancers might result in information bias, i.e., bias that occurs as a result of misclassification of the exposure or outcome. Information bias can be differential if misclassification of the disease is related to the exposure.[113] For instance, persons with cognitive impairment or dementia might be less likely to undergo pathological confirmation of the tumour (surveillance bias). This can result in either an under- or an overestimation of the association.

Another important aspect in the diagnosis of cancer is the date of diagnosis. The date of cancer diagnosis is primarily based on the date of pathological confirmation of the cancer. In absence of pathological confirmation, we used the date of hospital admission or hospital discharge letter. We showed in **Chapter 2** that the date of cancer diagnosis in the Rotterdam Study was often inaccurate (11.8% of the cancers). We have updated the date of diagnosis by evaluating the original medical files. Accuracy of the date of diagnosis is in particular important when studying exposures before cancer diagnosis. For instance, when studying

Table 1 Overview of number of patients for each cancer type per age category in the Rotterdam Study and in the Netherlands in the period from 1990-2014.

Cancer type	Rotterdam Study*			The Netherlands†		
	Age 45-59 years	Age 59-74 years	Age ≥75 years	Age 45-59 years	Age 59-74 years	Age ≥75 years
Head and neck	9 (5.8)	61 (3.3)	43 (2.1)	23 707 (6.1)	31 625 (4.2)	15 445 (3.0)
Oesophagus and gastric	3 (1.9)	95 (5.1)	160 (7.6)	15 918 (4.1)	36 650 (4.9)	31 594 (6.2)
Colorectal	18 (11.6)	267 (14.4)	374 (17.9)	41 616 (10.6)	109 841 (14.7)	96 427 (18.8)
Hepato-pancreato-biliary	6 (3.9)	91 (4.9)	102 (4.9)	11 069 (2.8)	28 943 (3.9)	24 697 (4.8)
Lung and mesothelioma	21 (13.5)	320 (17.3)	362 (17.3)	52 200 (13.3)	130 971 (17.5)	72 784 (14.2)
Bone and soft tissue	1 (0.6)	21 (1.1)	15 (0.7)	4756 (1.2)	5396 (0.7)	4012 (0.8)
Breast	40 (25.8)	260 (14.0)	224 (10.7)	101 743 (26.0)	96 229 (12.9)	55 517 (10.8)
Female genital organs	10 (6.5)	73 (3.9)	97 (4.6)	25 796 (6.6)	37 409 (5.0)	25 284 (4.9)
Male genital organs	17 (11.0)	291 (15.7)	203 (9.7)	23 573 (6.0)	116 167 (15.5)	68 789 (13.4)
Urinary tract	7 (4.5)	131 (7.1)	155 (7.4)	16 026 (4.1)	38 310 (5.1)	30 554 (6.0)
Haematological	12 (7.7)	135 (7.3)	202 (9.6)	28 843 (7.4)	52 847 (7.1)	39 921 (7.8)
Melanoma	6 (3.9)	39 (2.1)	44 (2.1)	24 336 (6.2)	22 630 (3.0)	12 029 (2.3)
Other	2 (1.3)	12 (0.6)	32 (1.5)	5790 (1.5)	8833 (1.2)	6258 (1.2)
Unknown primary origin	0 (0.0)	37 (2.0)	75 (3.6)	9027 (2.3)	22 923 (3.1)	25 269 (4.9)
Central nervous system	3 (1.9)	18 (1.0)	6 (0.3)	7209 (1.8)	9226 (1.2)	3871 (0.8)
Total	155	1851	2094	391 609	748 000	512 451

**Only pathology-confirmed cancers are included, because most cancers in the Netherlands Cancer Registry are based on pathology. † Numbers are obtained from the Netherlands Cancer Registry.*

cognitive function before cancer diagnosis, we would ideally have assessed cognition before start of the diagnostic work-up of cancer to fully exclude the effects of psychological factors such as stress. Although the date of cancer diagnosis marks a new phase in a person's life, it does not reflect the origin of the cancer. The incipient phase of cancer varies between five to forty years for solid tumours. Against this background, it is challenging to study the causal effect of certain factors such as plasma amyloid-β (**Chapter 10**) and atherosclerosis (**Chapter 15**) on cancer. This is furthermore complicated by the fact that latency periods – i.e., the period between biological initiation of cancer and cancer diagnosis – differ per cancer type. Unfortunately, data on latency periods is limited. Estimates of these periods can be obtained using statistical models, but – to reduce the effects of potential biases – these models can only be used for cancer types with high mortality rates and limited availability of effective treatment options to allow the disease to follow its natural course.[114] Also, the obtained estimates can differ per statistical model and are probably not truly exact. For instance, the latency period for pancreatic cancer was estimated at 8.6 years using statistical models, whereas a biological study estimated its latency period between 18.5 and 21.2 years.[115] Despite these limitations, the estimates obtained from statistical models may help to distinguish between cancers with short and long latency periods. The following latency periods have been estimated: a relatively short latency period for hepatic cancer (10.8 years), pancreatic cancer (8.6 years), and lung cancer (13.6 years), and a long latency period for acute myeloid leukaemia (25.8 years), stomach cancer (22.9 years), and brain cancer (21.9 years).[114] Given these long latency periods, any association found in **Chapter 10** and **15** may therefore also reflect reverse causation, i.e., cancer itself may cause higher levels of plasma amyloid-β and atherosclerosis.

Lastly, we wanted to highlight the availability of detailed information about cancer stage and treatment. The Netherlands Cancer Registry provides information about the TNM Classification of Malignant Tumours, but this type of staging differs per cancer type. In addition, often one aspect of the TNM stage was missing. Due to these reasons, we were only able to classify patients into non-metastatic and metastatic disease at moment of cancer diagnosis. In addition, the Netherlands Cancer Registry collects information about cancer treatment, but only about the first-line therapy. Nowadays patients often receive second- or third-line therapies. To capture also these lines of therapy, we collected information about cancer treatment by evaluating the original medical files of the participants that were collected by the Rotterdam Study. This was not feasible for cancer stage, since this information was often missing in the medical files.

Ascertainment of cognitive function

Cognitive function in participants of the Rotterdam Study is assessed using a neuropsychological battery. Participants undergo cognitive screening during home interviews using the Mini-Mental State Examination. From 1997 onwards, participants are assessed at the research centre using the Word Fluency Test, Letter-Digit Substitution Test, and Stroop Test. The test battery was further expanded with the Purdue Pegboard Test in 1999 and the Word Learning Test in 2002. Lastly, in 2006 the Design Organisation Test was added to the study protocol and in 2009 gait assessment. We did not include the Design Organisation Test and gait assessment in the studies on cancer and cognition due to the limited number of participants who completed these assessments before the end of complete cancer follow-up. Besides these objective measures of cognitive function, participants received three questions about memory complaints that may relate to subjective cognitive function. During home interviews, participants were asked the following three questions: (i) 'Do you have more problems remembering things than before?'; (ii) 'Has there been an increase in the times that you forgot what you were up to?'; and (iii) 'Do you have more word-finding problems than before?'.

Together, the objective cognitive tests and measures of self-reported memory complaints can provide good insight in a person's cognitive function. Although it has been shown that objective and subjective cognitive function correlate poorly,[116] our findings on mild cognitive impairment and the risk of cancer might indicate that the combination of objective and subjective cognitive function is important. In this section on the ascertainment of cognitive function, I will elaborate on two concerns: (i) practice effects; and (ii) use of the general cognitive factor.

Practice effects

Repeated exposure to the same cognitive test can result in test-retest effects, including practice effects.[117] Persons become more familiar with the tests and can develop strategies to deal with certain tests, such as clustering words during the Word Learning Test. Failure of accounting for practice effects can result in artificial improvement in cognitive function over time. Different studies have quantified the magnitude of practice effects, but most studies have determined these effects in a relatively short time period (ranging between six days to one year).[117-121]

In **Chapter 4**, we write the following limitation in the Discussion section: '*Second, repetitive administering of cognitive tests can lead to learning effects, which could have led to overestimating performance with increasing age. However, these effects are expected to be limited, since the median test interval was 5.1 years for cognitive assessments and 5.4 years for motor assessments.*'. In the first sentence we acknowledged that practice effects might have influenced our findings. However, in the second sentence, we attenuated this

acknowledgement by speculating that the impact of practice effects is limited because of the long time period between assessments. The number of studies that has investigated practice effects over a long – i.e., multiple years – interval is very limited. One study has revealed that – depending on the administered cognitive test – up to seven to 13 years had to elapse before the advantage of the prior cognitive assessment was eliminated.[122] This study, however, has investigated practice effects in persons between ages 18 and 58 years and may therefore not be generalisable to the population of the Rotterdam Study. Hence, I briefly explored if there were any indications of practice effects in the Rotterdam Study.

I focused on the following four cognitive tests: Word Fluency Test, Letter-Digit Substitution Test, Stroop Test: Interference, and the Word Learning Test: Delayed recall. Next, I calculated the mean test score of participants who were aged 65 years at time of their first assessment, of those who were aged 65 years at their second assessment, and of participants who were aged 65 years at their third assessment, see **Figure 4**. The median (interquartile range) time between assessments was 5 years (4 to 6) for those with two assessments and 2 years (2 to 2) for those with three assessments. Most participants who underwent three assessments participated in an additional examination round – the Rotterdam Scan Study – which took place between the original examination rounds. Therefore, their median time between assessments is lower than that of participants with only two assessments. I repeated this for the age of 70 years (**Figure 4**). Interestingly, persons who had undergone at least one cognitive test previously scored higher on that cognitive test than persons of the same age who were exposed to that cognitive test for the first time.

These practice effects need to be further explored, including the impact of sex and education on these effects. Also, it has been shown that the magnitude of practice effects may differ between cognitively intact and cognitively impaired persons. For instance, in persons with amnestic mild cognitive impairment, loss of short-term practice effects was related to worse cognitive outcomes after one year.[123] Furthermore, patients with dementia did not show practice effects.[124] Against this background, it would be interesting to investigate if cancer patients and survivors show the same practice effects as cognitively intact persons without a history of cancer.

General cognitive function

The studies in this thesis primarily focused on individual cognitive tests. To evaluate general cognitive function, we additionally studied the general cognitive factor in the **Chapters 5**, **6**, **13**, and **16**. The general cognitive factor can be used as proxy of intelligence among young persons. In older persons, it is more related to general cognitive function as it decreases with advancing age,[32] whereas intelligence remains stable. It accounts for around 40 to 50% of

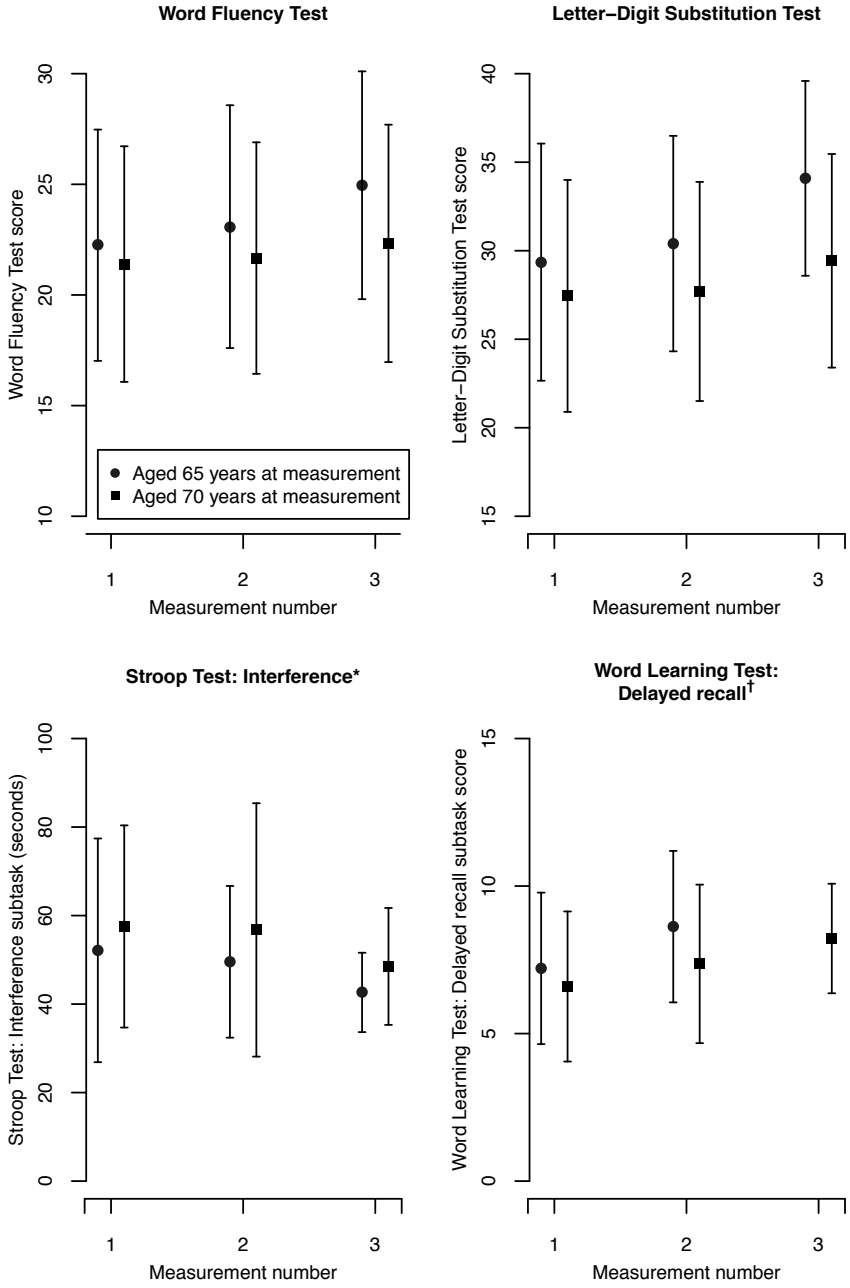


Figure 4 Practice effects in the Rotterdam Study.
*Mean test scores and corresponding standard deviations are plotted for participants aged 65 or 70 years at their first, second, and third assessment. * Higher score indicates worse performance. † Only one participant was aged 65 years at time of the third assessment. Therefore, the standard deviation could not be calculated.*

the shared variance between cognitive tests.[125] In other words, it explains that persons who are good at one cognitive test tend to be good at other types of cognitive tests. Part of the remaining variance is attributable to tests within a specific cognitive domain – persons who are good in a test related to a specific domain tend to be good in other tests related to that same domain – or to specific cognitive skills associated with individual cognitive tests. It has been shown that the general cognitive factor is independent from the cognitive tests used within the neuropsychological battery and may therefore be used to compare results with other studies.[32]

The general cognitive factor can be calculated using principal component analysis. In this thesis, we included the following five individual cognitive tests to calculate the general cognitive factor: Word Fluency Test, Letter-Digit Substitution Test, Stroop Test: Interference, Purdue Pegboard Test, and Word Learning Test: Delayed recall. If a cognitive test consisted of multiple subtasks we included only one subtask to prevent distortion of the factor loadings due to correlation between subtasks. The general cognitive factor was identified as the first unrotated component of the principal component analysis and explained 53.4% of the variance in cognitive tests in the first assessment round in which all tests were administered (i.e., fourth assessment round of the first subcohort, second round of the second subcohort, and first round of the third subcohort).

Despite multiple advantages such as reflecting general cognitive function, reducing the amount of data – and thereby the number of comparisons – and being comparable to other studies, it has also an important disadvantage. All individual cognitive tests have to be completed in order to calculate this factor. This results not only in a smaller, but also in a selected study population. Some of the included cognitive tests were included in the Rotterdam Study from 2002 onwards. Therefore, the general cognitive factor could only be calculated from 2002 onwards. **Table 2** illustrates the problem of this selection. Participants who had completed all five cognitive tests were on average younger, had a higher educational level, and had higher cognitive test scores than participants with at least one, but not more than four of the cognitive test results. It is therefore important to keep this selection in mind when interpreting the results of the general cognitive factor.

Ascertainment of dementia

Whereas cognitive assessments – except for the Mini-Mental State Examination – take place in the research centre of the Rotterdam Study, potentially resulting in missing assessments after the diagnosis of cancer, information on a dementia diagnosis is continuously obtained through linkage with medical records from general practitioners and the regional institute for outpatient mental health care.[126] In addition to this linkage, participants are screened for dementia during

the home interview with the Mini-Mental State Examination and the Geriatric Mental Schedule organic level. Using this combined approach, we aim to capture the majority of the dementia diagnoses. Some studies on cancer and dementia have relied on administrative claims data to identify dementia diagnoses, for instance through records of the health insurance program Medicare[63,72,127-130] or through International Classification of Diseases codes.[131] This approach is much less accurate and more sensitive to information bias due to potential misclassification.

Similar to cancer, dementia has a long incipient phase. Although many studies have tried to characterise this incipient phase by studying dynamic biomarkers such as amyloid-β, tau, and brain tissue volumes, it still is unknown how many years the pathophysiological process of dementia starts before clinical manifestation of the disease.[132] Therefore, we cannot rule

Table 2 Overview of characteristics and cognitive test scores of participants who had complete or incomplete cognitive test scores during the first assessment round in which all tests were administered.

Characteristic	Participants with complete cognitive test scores (N=7413)*	Participants with incomplete cognitive test scores (N=1433)†
Age at cognitive assessment, years, mean (SD)	65.5 years (10.1)	67.9 years (10.8)
Women, No. (%)	4274 (57.7)	793 (55.3)
Educational level, No. (%)		
Primary	747 (10.1)	219 (15.3)
Lower	2980 (40.2)	574 (40.1)
Intermediate	2183 (29.4)	384 (26.8)
Higher	1430 (19.3)	240 (16.7)
Cognitive test score		
Word Fluency Test, mean (SD)	22.2 (5.8)	20.5 (6.6)
Letter-Digit Substitution Test, mean (SD)	29.2 (7.3)	26.6 (7.8)
Stroop Test: Interference, seconds, median (IQR)	46.9 (38.7 to 59.7)	55.9 (43.5 to 78.9)
Purdue Pegboard Test, mean (SD)	35.2 (5.4)	33.7 (6.1)
Word Learning Test: Delayed recall, mean (SD)	7.2 (2.9)	6.1 (3)

*Missing values are not imputed and therefore numbers do not always sum up to 100%. * Cognitive assessments took place at the fourth visit of the first subcohort, the second visit of the second subcohort, and the first visit of the third subcohort. Those with complete cognitive test scores had completed the Word Fluency Test, Letter-Digit Substitution Test, Stroop test: Interference subtask, Purdue Pegboard Test, and Word Learning Test: Delayed recall. Those with incomplete cognitive test scores had at least one of these tests complete. † 472 (32.9%) had missing Word Fluency Test score, 252 participants (17.6%) had missing Letter-Digit Substitution Test score, 834 (58.2%) had missing Stroop Test: Interference, 440 (30.7%) had missing Purdue Pegboard Test score, and 823 (57.4%) had missing Word Learning Test: Delayed recall score.*
IQR = interquartile range, SD = standard deviation.

out reverse causation when studying an exposure, for instance cancer (**Chapter 12**) or inflammation (**Chapter 14**), in relation to the risk of dementia.

Statistical approach

Throughout this thesis, we used different statistical approaches to study our research questions. Regarding longitudinal data, we used the linear mixed model, Cox proportional hazards model, and joint model. The choice of these models primarily depended on the research question, but we could have used different models to analyse our data. For instance, we could have used joint models – which jointly model a time-to-event outcome with the longitudinal response – to determine the change in cognitive function in relation to the risk of cancer. We chose however the nested case-control setting with linear mixed models to fully control for age, and – if there was a difference in change in cognitive function between cancer patients and cancer-free controls – to determine the rate of cognitive decline towards cancer diagnosis. In this last part of Methodological considerations, I will briefly touch upon the shortcomings and merits of the used statistical models, methods to deal with the competing risk of death, and confounding.

Statistical models to deal with longitudinal data

Longitudinal data poses several challenges.[133] Measurements that are repeated over time within the same person are correlated. Furthermore, different persons are measured at different moments in time and measurements within persons are often missing, resulting in unbalanced data. For these reasons, simple statistical methods such as linear regression models are not optimal for analysing longitudinal data. We therefore used linear mixed models to analyse repeated measurements of cognitive function in **Chapter 4**, **5** and **7**. To illustrate the unbalanced data, I have included the individual, raw trajectories of cognitive function in the plots of **Chapter 7** in **Figure 5**. Each person has a unique trajectory, i.e., each person has a different intercept and slope of this trajectory. We assume that persons are randomly selected from the population. Therefore, the corresponding regression coefficients are sampled from a population of regression coefficients. Under this assumption, different persons share the same random effects, which accounts for the correlation between repeated measurements within one person. In addition, the linear mixed model can handle unbalanced data, enabling us to also include persons with only one measurement.

In **Chapter 6**, **9**, **10**, **11**, **12**, and **15** we studied either cancer or dementia as an outcome. To deal with the time until the outcome of interest – i.e., the time that a person was at risk – we used Cox proportional hazards models. Simple statistical methods cannot be used to analyse such data because of censoring, i.e., persons who do not experience the outcome of interest are censored. I will elaborate on two types of censoring – informative and non-informative

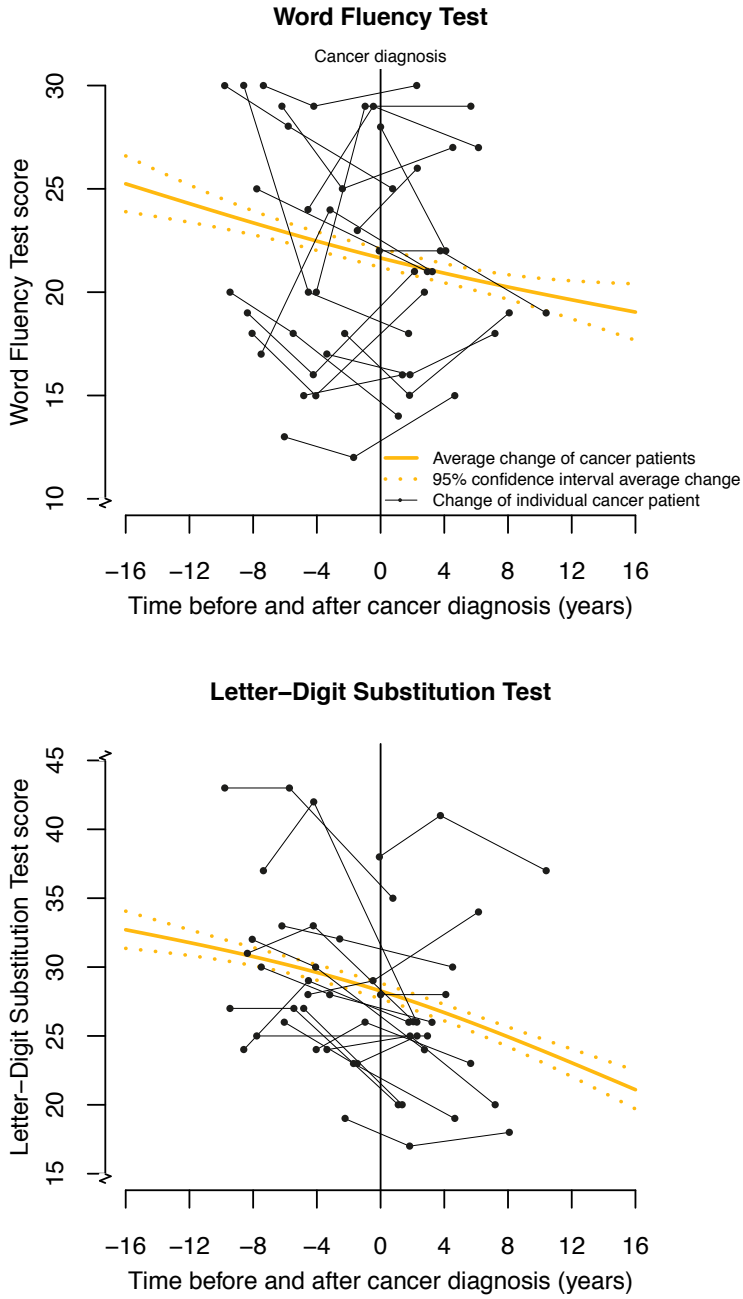


Figure 5 Trajectories of cognitive test scores in cancer patients from before to after diagnosis. *The trajectories estimated for the total population of cancer patients are shown in yellow. The trajectories of randomly selected individual cancer patients are shown in black. The black dots represent the individual measurements.*

Box 1 Immortal time.

The date of cancer diagnosis usually differs from the date of study entry. Participants with a longer follow-up are more likely to be diagnosed with cancer during study follow-up than those with a shorter follow-up. Therefore, a cancer diagnosis is only observed when a participant survives up to the date of the potential cancer diagnosis. The time between study entry and cancer diagnosis is then referred to as immortal time, i.e., the follow-up time during which the outcome cannot occur. Note that immortal time bias differs from survival bias.[134,135] Misclassification of immortal time can result in information bias, whereas exclusion of immortal time can result in selection bias.[136]

There are different ways to deal with immortal time of which three are presented in **Figure 6**.[137] The first solution uses the time-dependent Cox proportional hazards model in which the time before cancer diagnosis is categorised as unexposed and the time after cancer diagnosis is categorised as exposed. The second solution uses inverse probability weights to account for the time until cancer diagnosis. The third solution emulates a trial by cloning each participant to a 'cancer arm' and a 'non-cancer arm'. These arms are then identical at baseline. A participant with cancer will then be censored in the 'non-cancer arm' at time of cancer diagnosis, whereas a participant without cancer will be censored in the 'cancer arm' after a specified time period. Subsequently, inverse probability weighting can be used to account for this informative censoring.

censoring – in the Competing risk of death section. We further extended the traditional Cox proportional hazards model by studying dementia (**Chapter 9**) and cancer (**Chapter 12**) as time-dependent variable. This approach can account for immortal time (**Box 1**). We could not use this extended model to study the change in inflammatory ratios and the risk of dementia in **Chapter 14**, because the extended Cox proportional hazards model assumes that the trajectories of continuous, time-dependent variables are fully specified and measured without error. Inflammatory ratios are endogenous variables that were measured only during the research centre visits. Therefore, the complete trajectory of these variables is often unobserved, resulting in an unrealistic, step-wise trajectory. Furthermore, endogenous variables are often measured with error. To deal with endogenous, time-dependent variables, we used the joint model.

Joint models link the survival model with repeated measurements of an endogenous variable. The linear mixed model can estimate the complete trajectory of the variable and can account for the measurement error or variability. Using joint models, we could estimate the difference in inflammatory ratios in relation to dementia by using all repeated measurements

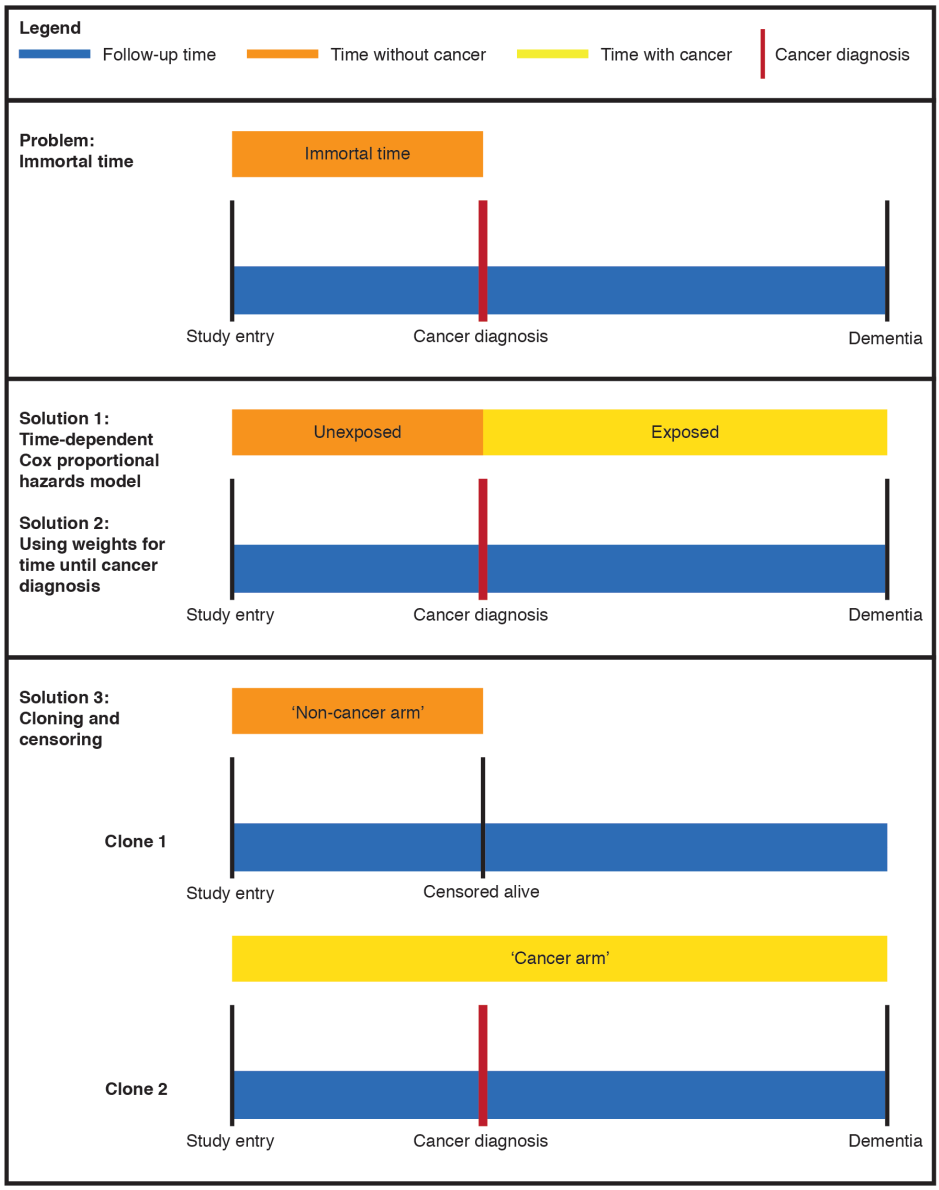


Figure 6 Illustration of immortal time.
The time between study entry and cancer diagnosis is referred to as immortal time, because death cannot have occurred before the cancer diagnosis. Immortal time can be solved by (1) using time-dependent Cox proportional hazards models in which immortal time is classified as time not exposed to cancer, (2) using inverse probability weights for the time until cancer diagnosis, or (3) using an emulated trial design. In this design, participants are cloned to a 'non-cancer arm' and a 'cancer arm'. Participants who are diagnosed with cancer during follow-up are censored at the date of cancer diagnosis in the 'non-cancer arm'. This figure has been adapted from Maringe et al. (2020).[137]

of the inflammatory ratios while accounting for measurement error or variability of these ratios.

Competing risk of death

We minimised the effects of surveillance and survival bias by studying the preclinical stages of cancer and dementia. Nevertheless, death can preclude occurrence of the outcome of interest in participants who are at risk for the outcome. We tried to take the competing risk of death into account by using cause-specific Cox proportional hazards models. These models are often preferred when studying aetiological research questions.[138,139] For aetiological associations, we are interested in the risk of the outcome of interest in participants who have not developed this outcome at certain time *t* (risk set at time *t*). When they experience the competing event of death, participants can be removed from the risk set at time *t*. Another approach to deal with competing risk is by using the subdistribution hazards model of Fine and Gray.[140] This model is primarily used for predictive and prognostic research questions and calculates the absolute risk of the outcome by keeping persons in the risk set even after experiencing the competing risk of death. If the risk of death is different between participants with and without exposure, the risk set is artificially inflated in the group with the highest mortality rate. This results by definition in a lower subdistribution hazard. In predictive and prognostic studies, estimates can be influenced by having a reduced number of participants remaining at risk for the outcome due to an increased number of deaths. Therefore, it is more appropriate to keep the participants in the risk set after the competing event has occurred. Since the subdistribution hazard can provide information about the distribution of death between different groups of exposure, it can be used in combination with cause-specific Cox proportional hazards models to get insight in the problem of the competing risk of death in aetiological research questions. Note that the subdistribution hazards model of Fine and Gray can only be used for time-independent exposures.[141]

One of the assumptions of the cause-specific Cox proportional hazards model however is that censoring is non-informative.[142] Censoring at any time during the follow-up must be independent of changing values of prognostic factors during follow-up.[143] This assumption is unlikely to be met if competing events are defined as censoring events. Because cancer and dementia are strongly related to death, censoring for death is not non-informative. Therefore, censoring for death as competing event in the relation between cancer and dementia might result in biased hazard ratios. We have accounted for this informative censoring in **Chapter 12** by using inverse probability weights for loss to follow-up and death.

Confounding

Confounders are variables that are related to both the exposure of interest and the outcome of interest. Confounding is discussed in more detail in **Box 2**. In most studies in this thesis, we corrected and stratified for covariates that are associated with both cancer and cognitive impairment, i.e., age (either as covariate or as time scale), sex, educational level, body mass index, smoking status, and alcohol use. In few studies we also included the following covariates: psycholeptic drug use, Centre for Epidemiologic Studies Depression scale sum-score, diabetes mellitus, hypercholesterolaemia, blood pressure, and hypertension. We selected these confounders based on existing knowledge. Another often used approach is to test whether a covariate is a confounder in the used dataset. This approach is not adequate, because statistics alone cannot make determinations about the temporal order. Also, statistical analyses cannot distinguish between confounders and mediators.[144] It remains however challenging to select the optimal set of confounders when studying all cancer types, because the relation between a confounder and cancer differs per cancer type. In addition, we were not able to correct for other potential confounders due to missing or incomplete data, which might have resulted in residual confounding. Potential confounders are diet, physical activity, frailty, anxiety, and fatigue. For instance, it has been shown that before systemic cancer treatment, differences in cognitive function and white matter integrity between breast cancer patients and cancer-free controls were no longer statistically significant after correcting for fatigue, emphasising the importance of taking fatigue into account.[145]

IMPLICATIONS

The number of patients with cancer is increasing worldwide.[146] About 20% to 30% of all non-CNS cancer patients have cognitive problems, which can last up to more than twenty years after cessation of treatment in a subgroup of cancer survivors.[16,81] Therefore, more insight in cognitive problems and the risk of dementia in cancer patients is necessary to inform patients and clinicians, and to develop prevention and intervention strategies. I will first discuss implications of our findings on cancer registration for cancer registries. Subsequently, I will focus on clinical implications. Implications for future research will be discussed separately in the Directions for future research section.

Box 2 Confounding.

Confounding is a distortion that occurs when the exposure of interest is mixed together with the effect of another variable that is associated with the outcome.[113] Let me illustrate confounding with the following example: the relation between carrying a cigarette lighter and the risk of lung cancer (**Figure 7**).[147] Persons who carry a cigarette lighter have a higher risk of lung cancer than persons who do not carry a lighter. The cigarette lighter itself does not cause cancer. Carrying a cigarette lighter is mixed up with smoking, i.e., the relation between carrying a cigarette lighter and the risk of lung cancer is confounded by smoking. This results in a spurious association between carrying a cigarette lighter and the risk of lung cancer.

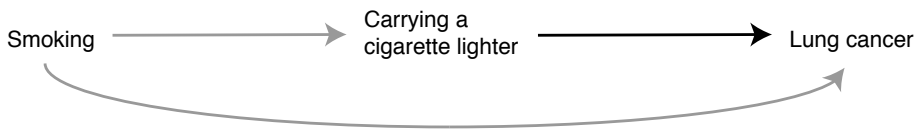


Figure 7 Directed acyclic graph to illustrate confounding.

A variable is a confounder if it is a common cause of both the exposure and outcome.[148] Note that carrying a lighter itself is not a confounder in the relation between smoking and the risk of lung cancer, because carrying a lighter is the result of smoking, i.e., carrying a lighter does not cause smoking.[149]

There are several methods to deal with confounding. The following three methods can deal with confounding on the level of the study design. Firstly, you can prevent confounding by randomisation. The main advantage is that randomisation also controls for unknown confounders. Secondly, you can restrict the studied population, for instance by selecting only non-smokers. This method limits the generalisability. Thirdly, you can match two groups based on the confounding factor. On the level of data analysis, you can control for confounding by stratification, multi-variable modelling, or by using an instrumental variable. This last method also controls for unknown confounders.

Cancer registries

Cancers that are not confirmed by pathology were often missed by the Netherlands Cancer Registry. Of all registered cancers in the Rotterdam Study, 11.7% was non-pathology-confirmed. Importantly, pathological confirmation was associated with tumour and patient characteristics, and survival. This underlines the necessity to continuously improve the quality of cancer registration and to combine multiple sources of cancer registration, for instance by standardised linkages between a population-based study and the national cancer registry.

A second implication – or recommendation – for cancer registries would be to collect follow-up data of cancer patients. The Netherlands Cancer Registry registers the first-line cancer treatment, but not the subsequent lines of treatment. This information is insufficient to address research questions after cancer diagnosis. Cancer registries could for instance update treatment-related information every six months.[150,151] Given that not all cancers in the Rotterdam Study are registered by the Netherlands Cancer Registry, we should also consider to document details on cancer treatment in the Rotterdam Study.

Patients and clinicians

At a population-level, cognitive function in cancer patients changes similarly to that in cancer-free persons. Furthermore, we found no evidence that cancer patients have a higher risk of dementia. These findings may provide some reassurance to cancer patients, in particular those with favourable cancer types who received only local treatment. Patients treated with systemic treatments such as chemotherapy might have different cognitive trajectories. In addition, findings on a group-level are not always generalisable to the individual-level.[152]

We therefore suggest using a personalised approach, rather than screening all cancer patients for cognitive problems. Patients and clinicians should be educated about cognitive problems in cancer patients in order to signal cognitive problems in an early stage. Patients can subsequently be referred to a neuropsychologist to undergo cognitive assessment and – if necessary – cognitive rehabilitation.[153] This can result in improved quality of life and daily life functioning of cancer patients and survivors.

Importantly, among the growing population of cancer patients and survivors the percentage of elderly patients and survivors will further increase,[154] which poses several challenges for physicians, caregivers, and healthcare systems. Firstly, given that cancer treatment may accelerate ageing processes, elderly patients in particular may be more vulnerable to the effects of cancer treatment on the brain.[155] Vulnerable elderly patients and survivors may be identified by comprehensive geriatric assessments before and following cancer treatment in order to start interventions such as cognitive rehabilitation in an early stage.[156] Use of such geriatric assessments has also been associated with improved survival and physical state.[157]

Secondly, elderly patient care is often complex and requires involvement of caregivers.[158] Patients with comorbid cancer and dementia receive less often cancer treatment and have poorer survival than patients without dementia.[159-164] In addition, comorbid cancer and dementia has been associated with agitation, depression, and sleep disturbances, but patients are often not able to report such symptoms.[165] Therefore, care for patients with comorbid cancer and cognitive impairment or dementia should be further improved and healthcare professionals should pay close attention to this group of patients.

DIRECTIONS FOR FUTURE RESEARCH

The studies presented in this thesis have contributed to the understanding of the change in cognitive function and the risk of dementia in cancer patients. Many knowledge gaps in this field are still not filled. Furthermore, our work has generated several new questions that need to be answered. In this section, I will discuss some of these remaining knowledge gaps and new questions with regard to risk factors of cognitive problems in cancer patients, mechanisms underpinning these cognitive problems, and approaches to deal with the relation between cancer and dementia. During this discussion, I will also highlight some of our ongoing studies.

Identifying cancer patients at high risk of cognitive problems

At a population-level, we found that cognitive function in cancer patients declined similarly to that in persons without a history of cancer. In addition, previous clinical studies have shown that cancer patients with specific types of cancer or treatment have more often cognitive problems than cancer-free persons.[16-28] This suggests that certain subgroups of cancer patients and survivors – that could not have been captured in our population-based study – might be vulnerable to develop cognitive problems. Identification of these high risk patients is necessary in order to minimise the negative impact of cognitive problems on quality of life.

Several studies have focused on genetics to identify high risk patients.[166] The most frequently studied genes are apolipoprotein E (*APOE*) and catechol-O-methyltransferase (*COMT*), which are associated with cognitive function and the risk of dementia irrespective of cancer status.[167,168] Some studies have suggested that cancer patients who carry at least one *APOE* ε4 allele have more often cognitive impairment than non-carriers.[19,169-171] This has also been supported by a preclinical study showing that mice who carried the *APOE* ε4 allele and were treated with chemotherapy performed worse than (i) mice with the *APOE* ε3 allele and (ii) untreated mice with the *APOE* ε4 allele.[172] In addition, it has been shown that breast

cancer patients who were *COMT-Val* carriers had worse cognitive function than (i) breast cancer patients who were non-carriers and (ii) cancer-free controls who were also *COMT-Val* carriers.[173] Nevertheless, findings across different studies are inconsistent.[166]

We have recently directed our attention to two other genes that are well known in the oncology field: *BRCA1* and *BRCA2*. *BRCA1* and *BRCA2* are tumour suppressor genes that are involved in DNA double-strand break repair by homologous recombination.[174,175] Persons with a germline *BRCA1* or *BRCA2* mutation have a strongly increased risk of developing cancer.[176-178] Interestingly, recent evidence has highlighted the potential role of *BRCA1* in impaired cognitive function and dementia.[179-181] We have previously investigated cognitive function in breast cancer survivors with a germline *BRCA1* mutation and found a higher prevalence of cognitive impairment in these survivors than in breast cancer survivors without such a mutation (21% cognitive impairment in patients without a mutation versus 36% in patients with a mutation, unpublished data). However, breast cancer survivors with a *BRCA1* mutation had received more intensive cancer treatment than patients without a mutation, which by itself can affect cognitive function.[182,183] To study purely the effect of *BRCA1* and *BRCA2* on cognitive function, we are currently studying prevalence of cognitive problems and dementia in cancer-naive men with a germline *BRCA1* or *BRCA2* mutation. The risk of cancer in men with such a mutation is low compared to the risk of cancer in women, as well as the performance of risk-reducing surgeries, which may also affect cognitive health.

Other interesting genes may be those linked to Lynch syndrome or Li-Fraumeni syndrome. In addition, epigenetic modifications including DNA methylation, histone modification, and microRNA regulation, may explain some of the shared aetiology between cancer and dementia and are worthwhile investigating.[51]

Apart from genetic factors, it has been suggested that characteristics such as age and diabetes mellitus are associated with cognitive impairment in cancer patients.[19] When starting this thesis, we aimed to identify risk factor profiles for cognitive decline in cancer patients. We realised however that part of the effect of risk factors on cognitive function is mediated by cancer. For instance, if we want to estimate the effect of smoking on cognitive decline in cancer patients, we need to estimate the direct effect of smoking on cognition and the indirect effect that is mediated through cancer (**Figure 8**). If we do not take this mediated effect into account and condition on cancer, we create a selection bias, resulting in a distorted association between smoking and cognitive decline in cancer patients.[184] This effect could be taken into account by performing a mediation analysis. An alternative option is to apply existing prediction models for cognitive impairment or dementia to cancer patients,[185] assuming that the effects of these risk factors on cognitive function and dementia in cancer patients are similar to the effects in cancer-free persons.

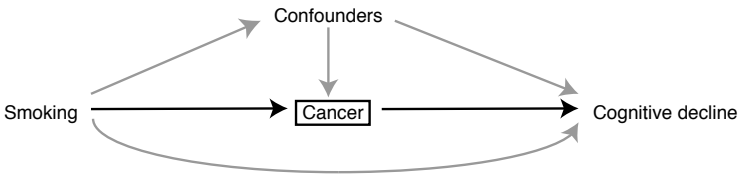


Figure 8 Directed acyclic graph to illustrate selection bias when conditioning on a collider.
When estimating the effect of smoking on cognitive function in cancer patients, we need to take the direct effect (effect of smoking on cognitive decline) and the indirect effect (effect of smoking on cognitive decline through cancer) into account. When conditioning on cancer, for instance by stratification, we open the path between confounders and smoking. This will result in selection bias.

Certain cancer treatments such as chemotherapy are probably more harmful to cognitive function than local cancer treatments. Our study on cognitive function before and after cancer diagnosis included mainly cancer patients without treatment or who were treated with surgery or radiotherapy. In order to investigate effects of specific treatments, I would recommend researchers to include a large, homogenous population of cancer patients. For instance, combining multiple population-based cohort studies may result in a large number of cancer patients treated with systemic treatment and may therefore shed further light on the cognitive trajectory after systemic treatment. We will also further contribute to the understanding of the course of cognitive function after systemic treatment by inviting the breast cancer survivors in **Chapter 13** and **16** for a follow-up study more than ten years after the original study. Importantly, many patients currently receive multiple years of different lines of cancer treatment to improve their survival. The potential synergistic or cumulative effects of such treatments on cognitive function have not been well established yet. Future longitudinal studies should therefore closely monitor cognitive effects of concurrent and sequential treatments.

Unravelling underlying mechanisms

We found that inflammation and changes in cerebral blood flow may underlie cognitive problems and possibly dementia in cancer patients. The effects of anti-inflammatory drugs on cognitive function have been investigated in tumour-bearing mice, showing that low-dose aspirin can result in improved memory function.[91] It would be of great interest to further explore the effects of anti-inflammatory drugs and cardiovascular risk management in cancer patients and survivors using clinical trials. Also, given that our findings suggest that an imbalance of the immune system towards the innate immunity may underlie late cognitive problems in cancer patients, future studies should further investigate the effects of immunotherapy on cognitive function.[186]

Inflammation and vascular factors are probably not the only mechanisms by which cancer

or cancer treatment can result in changes in cognitive function. Since it has been proposed that in particular cancer treatment leads to acceleration of ageing processes, other ageing pathways may also be related to impaired cognitive function in cancer patients.[187,188] For instance, chemotherapy has been associated with shortened telomeres.[189] In breast cancer survivors, lower telomerase – resulting in shorter telomeres – was associated with worse cognitive functioning, but longitudinal studies that include pre-treatment assessments are needed to assess if telomere length can be used as biomarker for cognitive decline in cancer patients and to determine causality of the relation.[190] Aspirin has been shown to increase telomerase activity and might therefore maintain telomere length.[191] If causally related, treatment with aspirin might prevent cognitive problems in cancer patients. Other ageing-related markers that may be of interest are markers of cellular senescence or DNA damage, including senescence-associated cytokines such as interleukin-6 and interleukin-8, and expression of *INK4a/ARF* transcripts.[187] Trajectories of such markers could be determined in large longitudinal studies that also incorporate assessments of cognitive function and neuroimaging.

In addition to ageing-related processes, several studies have proposed a role for the gut microbiota in relation to cognitive impairment in cancer patients.[192,193] The gut microbiota is an important modulator of the immune system that can influence brain function and behaviour.[194] Disruption has been related to hepatic encephalopathy and psychiatric diseases. Chemotherapy and radiotherapy can induce changes in the composition of gut microbiota.[195] The impact of the gut microbiota on cognitive function could be investigated by studying the change in composition of the gut microbiota from pre- to post cancer treatment in relation to changes in cognitive function. If related to impaired cognitive function in cancer patients, the gut microbiota could be explored as therapeutic target through restoration of the gut microbiota.

Lastly, recent evidence has shown that cancer might not only affect the brain, but that the brain might also influence cancer initiation and progression. Several studies have reported a higher risk of cancer in patients with depression.[196-199] We are currently investigating the relation between psychosocial factors including depression, anxiety, and grief in the PSYchosocial factors and Cancer (PSY-CA) consortium that is composed of 18 cohort studies. Also after clinical manifestation of cancer, psychosocial conditions might affect tumour biology.[200] For instance, stress-induced activation of the sympathetic nervous system may stimulate tumour angiogenesis – and therefore tumour growth – via the release of noradrenaline.[201] Unravelling the exact involved mechanisms and the physiological effects may help to further elucidate the link between cancer and the brain.

Studying the relation between cancer and dementia

We investigated the relation between cancer and dementia by studying preclinical stages of both diseases and using advanced statistical methods. Our results do not support an inverse association between these two diseases and – if anything – might point towards a positive, biological link. Nevertheless, each year several studies have been published that show an inverse link between cancer and dementia without using appropriate statistical methods to account for the competing risk of death. Moreover, several studies have extensively discussed the potential biological mechanisms underlying this inverse link, aiming to identify prevention and intervention targets for both diseases.[68,69] Given our findings, I would challenge future studies to use different approaches in order to limit the effects of surveillance and survival bias on the relation between cancer and dementia. In line with our studies, it would be interesting to study trajectories of plasma amyloid-β levels in relation to the risk of cancer or to use amyloid positron emission tomography neuroimaging in cancer patients. In addition, one can investigate the trajectories of carcinoembryonic antigen levels in relation to the risk of dementia, or determine the risk of dementia in patients with carcinoma in situ. Regarding innovative statistical approaches, simulation studies might provide additional insight in the potential magnitude of bias in the link between cancer and dementia. Data could then be generated such that patients with cancer have a higher risk of dementia. Also causal inference frameworks may be used to clearly specify the research question and to estimate the causal relation between cancer and dementia.

Improving cognitive assessments

Lastly, I want to highlight an innovating strategy in the assessment of cognitive function. We have recently developed the Amsterdam Cognition Scan, which is an online cognitive assessment tool.[202,203] Using this tool, participants can be assessed from home, which is time- and cost-efficient compared to traditional cognitive tests. Computer experience can easily be corrected for by using a measure of self-reported computer use per week.[204] This strategy might be of particular interest in population-based studies, because we observed that many cancer patients did not have a cognitive assessment after cancer diagnosis. It is likely that also participants with other disabling diseases skip visits to the research centre after the diagnosis. Using an online tool, participants can be assessed more frequently and do not have to visit the research centre. In addition, data in the Amsterdam Cognition Scan is stored per mouse click, allowing to perform more sophisticated analyses that may capture more subtle changes in cognitive function by disentangle different cognitive subprocesses.[205] This could provide more insight in the change in cognitive function during ageing in the general population and in cancer patients.

CONCLUDING REMARKS

The link between cancer and the brain remains complex. In general, cognitive function in cancer patients does not change differently than cognitive function in persons without a history of cancer. In addition, we found that – at a population-level – cancer patients are not at increased risk of dementia, but there might be a positive, biological link between cancer and dementia. I challenge future studies to provide further insight in the link between these two diseases. Specific cancer types or systemic treatments may be related to accelerated cognitive decline, but single population-based studies are not the optimal setting to investigate those subgroups of patients and survivors. In such a subgroup of breast cancer survivors, we found that inflammation and cerebral blood flow may be related to late impaired cognitive function. These mechanisms could be considered as targets for prevention and intervention strategies.

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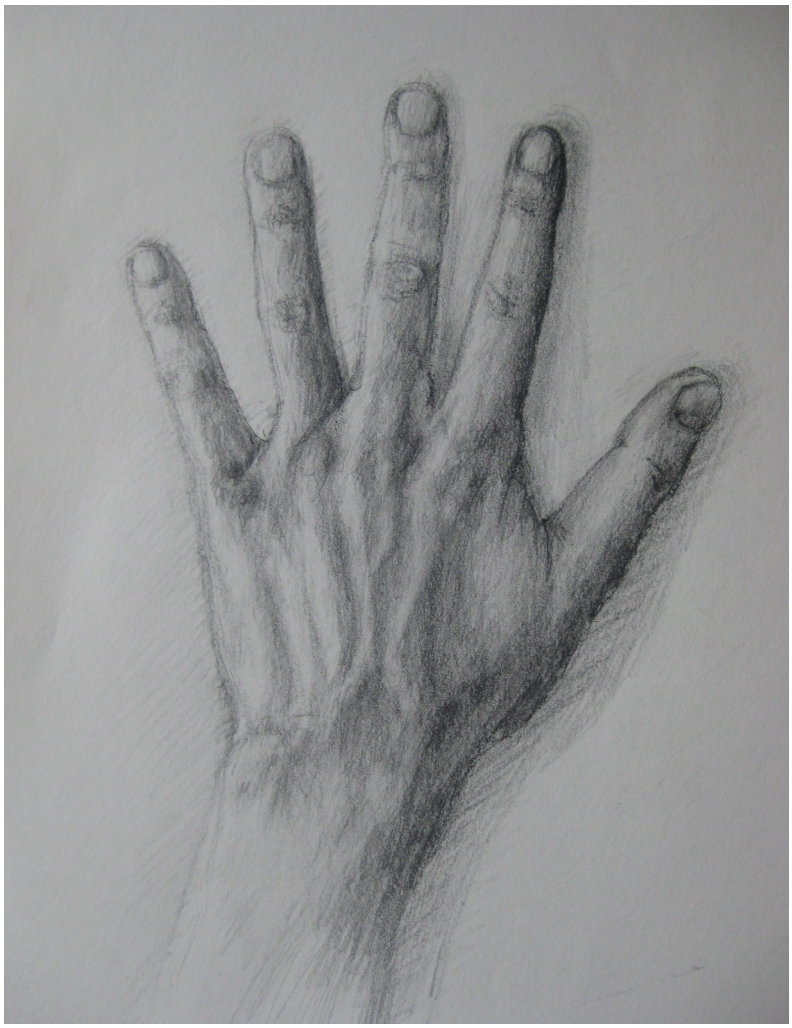
Summary



SUMMARY

Great improvements in cancer screening and treatment have ensured prolonged survival of many cancer patients. In turn, this has resulted in a growing number of patients and survivors who have to deal with long-term and late side effects of the disease itself and its treatment. Cognitive problems are among the most frequently reported side effects that can negatively affect daily functioning and quality of life. Current studies have primarily studied subgroups of cancer patients and may therefore not be generalisable to other populations of cancer patients. Also, the origin of cognitive problems, the course of cognitive function, and the risk of dementia in non-central nervous system (CNS) cancer patients remain poorly understood. Such knowledge is of great importance to develop prevention and intervention strategies for cognitive problems in non-CNS cancer patients. This thesis focused on cognitive function from before to after cancer diagnosis in the general population of non-CNS cancer patients. In addition, this thesis investigated the association between cancer and dementia, and focused on potential mechanisms underlying cognitive problems and dementia in non-CNS cancer patients. The studies presented in this thesis were embedded in the Rotterdam Study, a large prospective population-based cohort study in the Netherlands.

Part I discusses the quality of cancer registration. We first compared completeness and accuracy of cancer registration by the Rotterdam Study with that by the Netherlands Cancer Registry in **Chapter 2**. We found high completeness for pathology-confirmed cancers, but cancers without pathological confirmation were often not registered by the Netherlands Cancer Registry. In the Rotterdam Study, the date of cancer diagnosis was often inaccurately registered. In **Chapter 3**, we took a closer look at the non-pathology-confirmed cancers and found that these cancers were more present in older patients than in younger patients. Also, patients with non-pathology-confirmed cancer had a lower overall survival than patients with pathology-confirmed cancer. These findings indicate that combining multiple sources of cancer registration could improve the quality of cancer registration and that more effort should be put into capturing non-pathology-confirmed cancers.

Next, **Part II** focused on the change in cognitive function. We provided a standard of trajectories of cognitive and motor function during ageing in the general population in **Chapter 4**. We subsequently studied in **Chapter 5** the change in cognitive function in cancer patients prior to their cancer diagnosis. We found that cognitive function in persons who will be diagnosed with cancer changes similarly to that in persons who will remain free of cancer. Since changes in cognitive function correlate moderately with changes in brain structure, we investigated brain structure before cancer diagnosis in **Chapter 6**. We found no relation

between different measurements of brain structure and the risk of cancer, indicating that cancer patients do not have more brain abnormalities before clinical manifestation of cancer. Lastly, in **Chapter 7**, we extended our study on cognitive function prior to cancer diagnosis and visualised the trajectories of cognitive function after diagnosis. Also after diagnosis, cognitive function in cancer patients changed similarly to that in cancer-free persons. These findings indicate that in general, cognitive function in cancer patients changes similarly to that in cancer-free persons, thereby providing some reassurance to cancer patients. This suggests that the effect of cancer itself on cognitive function is limited. Larger numbers are needed to assess cognitive change in patients with specific types of cancer and systemic treatment, and to identify high risk patients.

Part III was dedicated to the relation between cancer and dementia. Previous studies have shown that cancer patients have a lower risk of dementia and vice versa, but the direction of this relation may be influenced by methodological bias. We shed light on the biological relation between these two diseases using alternative approaches to minimise effects of surveillance and survival bias. We first showed in **Chapter 8** that Alzheimer's disease, the most common type of dementia, followed a multistep process similar to the process of cancer. In **Chapter 9**, **10**, and **11**, we studied the preclinical stage of either cancer or dementia and linked it to the other disease. We showed that patients with dementia have a lower risk of cancer, whereas persons with mild cognitive impairment, a preclinical stage of dementia, have a higher risk of cancer. Also, persons with higher levels of plasma amyloid-β – one of the earliest changes in preclinical dementia – had a higher risk of cancer. Lastly, we found that higher levels of the tumour marker carcinoembryonic antigen were related to a higher risk of dementia. In **Chapter 12**, we used alternative methods to account for selection bias to further study the risk of dementia in patients with cancer and found that they have a similar risk of dementia to that of persons without a history of cancer. Our findings indicate that patients with cancer are not at decreased risk of dementia. From a biological perspective, cancer and dementia may be positively related.

Part IV investigated mechanisms underpinning cognitive problems and dementia in cancer patients. In **Chapter 13** and **14**, we focused on inflammatory ratios that can broadly capture the relation between innate and adaptive immunity. We found that breast cancer survivors – who were treated with chemotherapy on average twenty years ago – had higher inflammatory ratios than women without a history of cancer. Also, higher inflammatory ratios were associated with lower general cognitive function in breast cancer survivors and in cancer-free women, but the association was more pronounced in the breast cancer survivors. In addition, we found that higher levels of the same inflammatory ratios were related to a higher risk of dementia in the general population. These findings suggest that an imbalance in

the immune system towards innate immunity may underlie cognitive problems and potentially dementia in cancer patients. In **Chapter 15** and **16** we determined the role of vascular factors. We showed that atherosclerosis can be present before clinical manifestation of cancer. After cancer, breast cancer survivors had lower cerebral perfusion than women without a history of cancer. Together, the findings from this Part suggest that inflammation and vascular factors may underlie cognitive problems – and potentially dementia – in cancer patients.

Lastly, in **Part V**, I reviewed our findings in a broader perspective and discussed several methodological considerations, including the difference between population-based settings and clinical settings, the potential impact of practice effects, and methods to deal with competing risk of death. In addition, I discussed the implications of our research for cancer registries and for patients and clinicians. Lastly, I provided directions for future research to identify cancer patients at high risk of cognitive problems, to further unravel underlying mechanisms, to study the biological relation between cancer and dementia, and to improve cognitive assessments.

SAMENVATTING

Verbeteringen in kankerscreening en behandeling hebben geleid tot een betere overleving van vele patiënten met kanker. Echter heeft dit ook tot gevolg dat veel kankerpatiënten en overlevers te maken hebben met lange termijn en late bijwerkingen. Deze bijwerkingen kunnen het gevolg zijn van de kanker zelf of van de kankerbehandeling. Een van de meest gerapporteerde bijwerkingen zijn cognitieve klachten. Cognitieve klachten kunnen het dagelijks functioneren en de kwaliteit van leven negatief beïnvloeden. Eerdere studies naar het cognitief functioneren van kankerpatiënten hebben zich voornamelijk gericht op subgroepen van kankerpatiënten, waardoor de resultaten mogelijk niet generaliseerbaar zijn naar patiënten met andere soorten kanker of kankerbehandelingen. Daarnaast zijn de oorzaken van cognitieve problemen, het beloop van het cognitief functioneren en het risico op dementie bij patiënten met niet-centraal zenuwstelsel (CZS) kanker onduidelijk. Meer kennis over het ontstaan en het beloop van cognitieve problemen is nodig voor het ontwikkelen van preventie en interventie strategieën voor cognitieve problemen bij niet-CZS kankerpatiënten. Dit proefschrift is gericht op het beloop van het cognitief functioneren in de algemene populatie van kankerpatiënten van voor tot na de kankerdiagnose. Daarnaast bestudeert dit proefschrift de relatie tussen kanker en dementie, alsmede verschillende mechanismen die mogelijk ten grondslag liggen aan cognitieve problemen en dementie bij niet-CZS kankerpatiënten. De studies in dit proefschrift zijn gebaseerd op het Rotterdamse ERGO-onderzoek (Erasmus Rotterdam Gezondheid en Ouderen), een groot prospectief populatieonderzoek in Nederland.

Deel I onderzocht de kwaliteit van kankerregistratie. In **Hoofdstuk 2** hebben we de compleetheid en accuraatheid van kankerregistratie door het ERGO-onderzoek vergeleken met dat door de Nederlandse Kankerregistratie. We constateerden dat meer dan 95% van alle pathologisch bevestigde kankers was geregistreerd door beide registraties. Het aantal geregistreerde niet-pathologisch bevestigde kankers was daarentegen veel lager in de Nederlandse Kankerregistratie dan in het ERGO-onderzoek. Daarnaast vonden we dat de datum van kankerdiagnose vaak niet juist was geregistreerd in het ERGO-onderzoek. Vervolgens hebben we ons in **Hoofdstuk 3** gericht op de niet-pathologisch bevestigde kankers. We vonden dat oude patiënten vaker een niet-pathologisch bevestigde kanker hadden dan jonge patiënten. Bovendien hadden patiënten met een niet-pathologisch bevestigde kanker een slechtere overleving dan patiënten met een pathologisch bevestigde kanker. Op basis van deze bevindingen hebben we geconcludeerd dat het belangrijk is om verschillende bronnen van kankerregistratie te combineren om de kwaliteit van registratie te waarborgen. Daarnaast is het belangrijk dat er meer inspanningen worden verricht om ook de

niet-pathologisch bevestigde kankers te registreren.

Vervolgens richtte **Deel II** zich op de verandering van het cognitief functioneren over de tijd. In **Hoofdstuk 4** hebben we een standaard gepresenteerd waarin het beloop van cognitieve en motorische functies in de algemene bevolking tijdens het ouder worden is weergegeven. Vervolgens hebben we in **Hoofdstuk 5** het beloop van het cognitief functioneren bij kankerpatiënten voor de kankerdiagnose bestudeerd. We vonden dat het beloop van het cognitief functioneren van personen die in de toekomst met kanker worden gediagnosticeerd hetzelfde is als dat van personen die niet met kanker worden gediagnosticeerd. Gezien veranderingen in het cognitief functioneren niet een op een samenhangen met veranderingen in de structuur van de hersenen, hebben we in **Hoofdstuk 6** de hersenstructuur onderzocht van personen die in de toekomst met kanker worden gediagnosticeerd. We vonden geen relatie tussen verschillende maten van de hersenstructuur en het risico op kanker, wat betekent dat kankerpatiënten geen veranderingen hebben in de hersenstructuur voordat zij worden gediagnosticeerd met kanker. Tot slot hebben we in **Hoofdstuk 8** onze studie voor kankerdiagnose uitgebreid door tevens het beloop van het cognitief functioneren na kankerdiagnose in kaart te brengen. Ook na kankerdiagnose was het beloop van het cognitief functioneren bij kankerpatiënten hetzelfde als bij personen zonder kanker. Onze bevindingen geven aan dat in het algemeen het cognitief functioneren van kankerpatiënten hetzelfde verandert als dat van personen zonder kanker, wat enigszins geruststellend is voor kankerpatiënten. Dit betekent dat het effect van kanker zelf op de hersenen beperkt is. Grotere aantallen patiënten zijn nodig om de verandering van het cognitief functioneren te onderzoeken in patiënten met specifieke kankersoorten en na systemische kankerbehandeling. Daarnaast is het belangrijk om patiënten te identificeren die mogelijk wel een verhoogd risico hebben op het ontwikkelen van cognitieve problemen.

Deel III was gewijd aan de relatie tussen kanker en dementie. Eerdere studies hebben laten zien dat kankerpatiënten een verlaagd risico hebben op dementie en dat patiënten met dementie een verlaagd risico hebben op kanker. De inverse richting van deze relatie kan echter beïnvloed worden door methodologische bias. We hebben in dit Deel verschillende alternatieve methoden toegepast om de effecten van methodologische bias te beperken. Hiermee hebben we geprobeerd ons inzicht in de biologische relatie tussen kanker en dementie te vergroten. Eerst hebben we in **Hoofdstuk 8** laten zien dat de ziekte van Alzheimer, de meest voorkomende vorm van dementie, een vergelijkbaar stappen proces volgt als kanker. In **Hoofdstuk 9**, **10** en **11** hebben we het preklinische stadium van zowel kanker als dementie onderzocht en vervolgens gerelateerd aan de andere, klinisch gemanifesteerde ziekte. We hebben geconstateerd dat patiënten met dementie inderdaad een lager risico op kanker hebben, terwijl personen met een geringe cognitieve stoornis, een

preklinisch stadium van dementie, een hoger risico op kanker hebben. Bovendien hadden personen met hoge waarden van plasma amyloïd-β – een van de eerste veranderingen in preklinische dementie – een hoger risico op kanker. Als laatst vonden we dat hogere waarden van de tumormarker carcino-embryonaal antigeen gerelateerd waren aan een hoger risico op dementie. In **Hoofdstuk 12** hebben we alternatieve methoden gebruikt om de gevolgen van selectie bias op de relatie tussen kanker en dementie te verminderen. We vonden dat het risico op dementie bij patiënten met kanker hetzelfde is als dat van personen zonder kanker. Dit betekent dat kankerpatiënten geen verlaagd risico hebben op dementie. Vanuit een biologisch perspectief zijn kanker en dementie mogelijk juist positief gerelateerd.

Deel IV onderzocht de mechanismen die mogelijk ten grondslag liggen aan cognitieve problemen en dementie bij kankerpatiënten. In **Hoofdstuk 13** en **14** hebben we ons gericht op inflammatoire ratio's die de verhouding tussen het aangeboren en het adaptief immuunsysteem weergeven. We vonden dat borstkanker overlevers – wie twintig jaar geleden behandeld waren met chemotherapie – hogere inflammatoire ratio's hadden dan vrouwen zonder voorgeschiedenis van kanker. Daarnaast waren hogere inflammatoire ratio's geassocieerd met een lager algemeen cognitief functioneren in zowel borstkanker overlevers als in vrouwen zonder kanker. Echter was deze associatie meer uitgesproken in de borstkanker overlevers. Verder vonden we dat hogere inflammatoire ratio's in de algemene populatie waren gerelateerd aan een hoger risico op dementie. Deze bevindingen suggereren dat een disbalans van het immuunsysteem richting het aangeboren systeem mogelijk een rol kan spelen in de pathogenese van cognitieve problemen en mogelijk dementie bij kankerpatiënten. In **Hoofdstuk 15** en **16** hebben we de rol van vasculaire factoren onderzocht. We hebben laten zien dat atherosclerose al aanwezig kan zijn voor klinische manifestatie van kanker. Na kankerdiagnose hadden overlevers van borstkanker een lagere doorbloeding van de hersenen dan vrouwen zonder kanker in de voorgeschiedenis. De resultaten van dit Deel suggereren dat inflammatie en vasculaire factoren mogelijk ten grondslag liggen aan cognitieve problemen – en mogelijk dementie – bij kankerpatiënten.

Ten slotte heb ik in **Deel V** onze bevindingen in een breder perspectief geplaatst en heb ik verschillende methodologische overwegingen besproken, onder andere het verschil tussen populatieonderzoek en klinisch onderzoek, de mogelijke impact van leereffecten en methoden voor het omgaan met overlijden als concurrerend risico. Daarnaast heb ik de relevantie van dit onderzoek voor kankerregistraties, patiënten en clinici beschreven. Als laatste heb ik aanbevelingen gedaan voor toekomstig onderzoek zoals het identificeren van kankerpatiënten met een verhoogd risico op het ontwikkelen van cognitieve problemen, het verder onderzoeken van de onderliggende mechanismen, het bestuderen van de biologische relatie tussen kanker en dementie en het verbeteren van cognitieve onderzoeken.

Epilogue

DANKWOORD

Na vier jaar mag ik dan eindelijk beginnen met het schrijven van mijn dankwoord. Een van de leukste en meest gelezen hoofdstukken van elk boekje. Ik heb vaak naar dit moment uitgekeken. Tijdens het schrijven reflecteer ik op een bijzondere, mooie en bovenal leerzame periode. Vele mensen hebben aan deze periode en dit boekje bijgedragen, van wie ik een aantal in het bijzonder wil bedanken.

Allereerst mijn twee promotoren, **Prof.dr. Ikram** en **Prof.dr. Schagen**, van wie ik van beiden een prachtige oratie heb mogen meemaken. **Prof.dr. Ikram**, beste Arfan, de eerste keer dat we elkaar spraken, gaf je al aan dat ik welkom was om onderzoek te doen op de afdeling epidemiologie, ook als het project met het Nederlands Kanker Instituut niet zou lukken. Het voelde fijn dat je toen al vertrouwen in me had. Je vertelde dat je de zandbak en enkele benodigdheden (emmer, schep) zou geven en dat je als promovendus er zelf iets van moet maken. Die vrijheid was erg fijn en zorgt ervoor dat je je onderwerp geheel eigen maakt. Arfan, ik bewonder je kennis en kunde, en je passie voor het vak. Dank voor alles wat je me de afgelopen jaren hebt geleerd. **Prof.dr. Schagen**, beste Sanne, het Nederlands Kanker Instituut voelde al snel vertrouwd door onze wekelijkse meeting op dinsdag. Je was er niet alleen voor onderzoekszaken, maar was ook erg betrokken op persoonlijk vlak. Niet alleen bij mij, maar bij alle promovendi in onze cognitie groep. Het voelt erg speciaal dat ik onder leiding van een van de meest vooraanstaande onderzoekers op het gebied van kanker en cognitie mijn onderzoek heb mogen uitvoeren. Verder heb ik genoten van alle congres bezoeken! Dank voor alle begeleiding, inspiratie en adviezen. Inmiddels mis ik onze wekelijkse meetings, evenals die met de cognitie groep, maar ik hoop dat daar later weer verandering in komt.

Verder wil ik graag de leden van mij promotiecommissie bedanken en in het bijzonder de leden van de leescommissie. **Prof.dr. Breteler**, ik kijk er naar uit om u – als promotor van mijn promotor – tijdens mijn promotie te ontmoeten. Het voelt erg bijzonder dat u ook in mijn promotiecommissie plaatsneemt. **Prof.dr. Ikram**, beste Kamran, als hoofd van de Neuro-epidemiologie hebben we elkaar regelmatig gezien en gesproken. Als ik ergens mee zat of advies nodig had, kon ik ook altijd bij jou terecht. Jouw brede interesses in wiskunde en natuurkunde gecombineerd met enthousiasme geven een extra dimensie aan de epidemiologie. **Prof.dr. Stricker**, beste Bruno, als een van de weinige promovendi die binnen ERGO de oncologie dataset gebruikten, heb ik ook regelmatig onder uw begeleiding gewerkt. Veel dank voor al uw kritische adviezen en uw goede gevoel voor humor.

Daarnaast wil ik alle co-auteurs bedanken voor de samenwerkingen. In het bijzonder allereerst **Prof.dr. Hauptmann** en **dr. Jóźwiak**. Beste Michael en Kasia, als epidemioloog heb ik enorm veel van jullie geleerd. Dit heeft mij laten inzien dat de combinatie van epidemiologen en statistici leidt tot een beter onderzoek. Michael, jouw commentaar en suggesties, ook als ik voor de zoveelste keer een manuscript naar je opstuurde, zijn altijd erg waardevol geweest. Het was een voorrecht om met jullie samen te werken. **Dr. Compter**, beste Annette, dank voor al je hulp en adviezen. Het was heel erg fijn om met jou samen te werken. **Prof.dr. van Leeuwen**, dank voor alle begeleiding, inspiratie en stimulatie. Ik ben erg blij dat u plaatsneemt in mijn grote commissie. **Dr. Ruiter**, beste Rikje, dank voor al je begeleiding binnen de ERGO. Mede door jou hebben we de kwaliteit van de oncologie database kunnen verbeteren. **Prof. dr. Vernooij**, beste Meike, dank voor je begeleiding. Je bent een inspiratie voor velen. **Dr. Koppelmans**, beste Vincent, mijn voorganger, wat vond ik het leuk om jou in het echt te ontmoeten nadat ik al meerdere keren jouw boekje grondig had doorgenomen! We hebben samen nog twee hele leuke projecten gedaan waarbij ik tijdens de Skype meetings meerdere keren prachtige natuurfoto's van Utah ontving.

Verder wil ik ook graag de mensen van het ERGO team bedanken en in het bijzonder **Nano**, **Frank**, en **Jolande**. Nano, dank voor al het oplossen van mijn computer problemen. Frank, bedankt voor alle datasets en voor alle hulp bij het koppelen met de Nederlandse Kankerregistratie. Jolande, als ik iets niet wist of niet kon vinden, dan wist jij het altijd. Jullie zijn onmisbaar voor ERGO.

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Collega's van de Neuro-epi, ik ben erg blij dat ik deel uit heb mogen maken van deze groep. Mede door jullie heb ik het de afgelopen vier jaar erg naar mijn zin gehad. Ik mocht al snel in de 'Drie Epi musketiers'-app groep van **Pauline**, **Eline** en **Pınar**. Pauline, jij was de eerste echte dr. van de nieuwe lichting. Met jouw enthousiasme en lach vrolijkte je altijd de afdeling op! Eline, jij was er altijd voor me op R- én persoonlijk gebied. Ik hoop dat we nog vaak (met Rutger erbij) gaan afspreken. P**ı**nar, wij waren kamergenootjes vanaf dag 1 en hebben een prachtig paper samen. Ik wens je alle succes toe en hoop dat je snel richting de USA kan. **Silvan**, ook wij hebben een aantal mooie papers samen, we vulden elkaar goed aan. Zonder jou had dit boekje er anders uit gezien. **Thom**, inmiddels ben jij al gepromoveerd en hoop ik

dat je je droom bij de Neuro waar gaat maken! **Alis** en **Noor**, allebei al AIOS Neurologie, jullie hebben mijn laatste loodjes van dichtbij meegemaakt en hadden altijd een luisterend oor. Hopelijk kom ik jullie later tegen als (Interne Geneeskunde) collega in het EMC! Liefste MIJK, **Maria**, **Isabelle**, en **Janine**, wat een eer om met jullie het geweldige Neuro-epi Efteling uitje te organiseren. Wat een werk, maar vooral ook veel lol hebben we gehad! Ik hoop dat we in de toekomst nog een keer met elkaar naar de Efteling gaan! Janine, mocht er ooit nog een Whitestar 2 komen dan moeten we wel weer samen gaan. **Joyce**, ook mijn kamergenootje, ondanks dat jouw project vele tegenslagen heeft gekend, ben je altijd doorgegaan, keep going! **Paloma**, you are forming the bridge between Neuro-epi and causal inference, and I am lucky to have the opportunity to work with you on a great project! **Sander**, **Jendé**, **Lisanne**, **Rowina**, **Amber**, **Tian**, **Sanne**, **Cevdet** en **Tosca**, jullie wens ik nog heel veel succes met (voor sommigen ook al de laatste loodjes) jullie promotietraject. Postdocs **Frank**, **Gena**, **Jeremy** en **Rebecca**, dank voor jullie bereidheid om altijd te helpen. **Daniel**, jouw wil ik het in het bijzonder nog danken voor je mega snelle antwoorden op al mijn random vragen (avond, weekend, maakt niet uit!). **Gabriëlle** en **Erica**, dank voor al jullie hulp, geduld en geregel!

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Annet, van Junior Med School naar dezelfde studiegroep in jaar 1 van Geneeskunde tot promotieonderzoek op dezelfde afdeling in het EMC. We hebben een lange weg afgelegd samen. Ik wens je heel veel succes met het afronden van je onderzoek en uiteindelijk met je opleiding tot oogarts. In de toekomst moeten we ons geannuleerde reisje naar München nog eens inhalen!

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PHD PORTFOLIO

Name:	Kimberly Dieudonnee van der Willik
Research school:	Netherlands Institute for Health Sciences (NIHES)
Erasmus Medical Centre department:	Epidemiology
Netherlands Cancer Institute department:	Psychosocial Research and Epidemiology
PhD period:	September 2016 – September 2020
Promotors:	Prof.dr. M.A. Ikram and Prof.dr. S.B. Schagen

1. PhD training	**Year**	**ECTS***
Research skills		
Master of Science in Clinical Epidemiology (NIHES)	2016-2018	70
General academic skills		
Workshop on Adobe InDesign CC 2019	2020	0.15
Biomedical English Writing and Communication	2019-2020	3.0
Follow-up Workshop on Adobe Photoshop and Illustrator CC 2019	2019	0.3
Workshop on Adobe Photoshop and Illustrator CC 2019	2019	0.3
Workshop on Presenting skills	2019	1.0
Scientific Integrity	2018	0.3
Basic course on 'R'	2016	2.0
In-depth courses		
Donders Neuroimaging Toolkit	2019	2.0
Introduction into clinical and fundamental oncology Dutch Society for Oncology (NVvO)	2017	2.0
Conferences		
International Neuropsychological Society, Denver, United States *Oral presentation: Trajectories of cognitive and motor function between ages 45 and 90 years: a population-based study*	2020	1.0
International Cognition and Cancer Task Force, Denver, United States *Oral presentation: Brain structure prior to non-central nervous system cancer diagnosis: a population-based cohort study* *Poster presentation: Cognitive trajectories before and after cancer diagnosis: a population-based study.* *Poster presentation: Long-term effects of adjuvant treatment for breast cancer on carotid plaques and cerebral perfusion.*	2020	1.0
Alzheimer's Association International Conference, Los Angeles, United States *Oral presentation: Trajectories of cognitive and motor function between ages 45 and 90 years: a population-based study* *Poster presentation: Brain structure prior to non-central nervous system cancer diagnosis: a population-based cohort study*	2019	1.2
International Cognition and Cancer Task Force, Sydney, Australia *Oral presentation: Inflammation markers and cognitive performance in breast cancer survivors 20 years after completion of chemotherapy: a cohort study*	2018	1.0

International Neuropsychological Society Washington, United States *Oral presentation: Mild cognitive impairment and dementia show contrasting associations with risk of cancer*	2018	1.2
Alzheimer's Association International Conference, London, United Kingdom *Poster presentation: Mild cognitive impairment and dementia show contrasting associations with risk of cancer*	2017	1.2
Vascular Behavioural and Cognitive Disorders, Amsterdam, the Netherlands *Attendance*	2016	0.3
Seminars and symposia		
Departmental research seminars	2016-2020	4.0
Oncology Graduate School (OOA) Retreat *Oral presentation: Brain structure prior to non-central nervous system cancer diagnosis: a population-based cohort study*	2019	2.0
Dutch Neuro-Oncology Society (LWNO) symposium *Oral presentation: Brain structure prior to non-central nervous system cancer diagnosis: a population-based cohort study*	2019	0.5
Oncology Graduate School (OOA) Retreat *Oral presentation: Mild cognitive impairment and dementia show contrasting associations with risk of cancer*	2017	2.0
2. Teaching activities	**Year**	**ECTS***
Supervising master thesis		
E.E.D van de Velde *Cognitive function in cancer patients before and after cancer diagnosis: the population-based Rotterdam Study*	2019-2020	4.0
Teaching practicals		
Practicals in epidemiology at Faculty of Medicine, Erasmus MC, Rotterdam *Clinical trials and Diagnostic tests*	2017	0.4
3. Other activities	**Year**	**ECTS***
Peer-review	2017-2020	2.0

** 1 ECTS (European Credit Transfer System) equals a workload of 28 hours*

LIST OF PUBLICATIONS

van Tuijl LA, Voogd AC, de Graeff A, Hoogendoorn AW, Ranchor AV, Pan K-Y, Basten M, Lamers F, Geerlings MI, Abell JG, Awadalla P, Bakker MF, Beekman ATF, Bjerkeset O, Boyd A, Cui Y, Galenkamp H, Garssen B, Hellingman S, Huisman M, Huss A, Keats MR, Kok AAL, Luik AI, Noisel N, Onland-Moret NC, Payette Y, Penninx BJWH, Portengen L, Rissanen I, Roest AM, Rosmalen JGM, Ruiter R, Schoevers RA, Soave DM, Spaan M, Steptoe A, Stronks K, Sund ER, Sweeney E, Teyhan A, Vaartjes I, **van der Willik KD**, van Leeuwen FE, van Petersen R, Verschuren WMM, Visseren FLJ, Vermeulen R, Dekker J. Psychosocial Factors and Cancer Incidence (PSY-CA): protocol for individual participant data meta-analyses. Submitted.

Heshmatollah A, **van der Willik KD**, Koudstaal PJ, Aribas E, Kavousi M, Ikram MA, Ikram MK. Transient neurological attacks: impact on life expectancy and years lived with stroke and cardiovascular disease. Submitted.

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SB, Ikram MK, Luik AI, Ikram MA. Trajectories of cognitive and motor function between ages 45 and 90 years: a population-based study. Journal of Gerontology: Medical Sciences. 2020; in press.

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ABOUT THE AUTHOR

Kimberly Dieudonnee van der Willik was born on December 28th, 1992 at the Ikazia Hospital in Rotterdam, the Netherlands. She attended secondary school at the Dalton Lyceum in Barendrecht and was admitted to the Junior Med School program of the Erasmus Medical Centre in Rotterdam in 2008. In 2009, she completed the Junior Med School program and won the Gerrit Jan Mulder Prize together with Marjolein Nanninga for their work on eye movements in prematurely aged mice at the Department of Neuroscience (principal investigator Prof.dr. Maarten A. Frens, supervision by dr. Marcella Spoor). In 2010, she graduated from secondary school (Gymnasium, cum laude) and started with her medical training at the Erasmus Medical Centre. During her medical training, she participated in the Erasmus Medical Centre Honours Class and in the Netherlands-Asia Honours Summer School.

During her master, she studied hormonal therapy resistance in breast cancer patients at the Department of Medical Oncology and Cancer Genomic Netherlands of the Erasmus Medical Centre Cancer Institute. She received the Gerrit Jan Mulder prize and the KNAW Van Walree grant for her master thesis (principal investigator Prof.dr. Els M.J.J. Berns, supervision by dr. Maurice P.H.M. Jansen). In 2016, she completed her medical training cum laude for which the Batavian Society for Experimental Philosophy awarded her the Steven Hoogendijk prize.

From September 2016 onwards, she conducted the work described in this thesis at the Department of Epidemiology of the Erasmus Medical Centre (principal investigator Prof.dr. M. Arfan Ikram) and at the Department of Psychosocial Research and Epidemiology of the Netherlands Cancer Institute in Amsterdam (principal investigator Prof.dr. Sanne B. Schagen). As part of her research training, she obtained her master degree in Clinical Epidemiology from the Netherlands Institute for Health Sciences. In 2017 and in 2019, she won the Young Investigator Award at the International Cognition and Cancer Task Force conference (Sydney, Australia and Denver, United States).

As of October 2020, Kimberly started working as a resident not in training at the Department of Internal Medicine of the Ikazia Hospital in Rotterdam. Her aim is to start her residency training in internal medicine, followed by a training in medical oncology. She wants to combine her clinical work with research.

Toeval bestaat niet...