Identification and treatment of patients at high cardiovascular risk

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IDENTIFICATION AND TREATMENT OF PATIENTS AT HIGH CARDIOVASCULAR RISK

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THESIS OUTLINE

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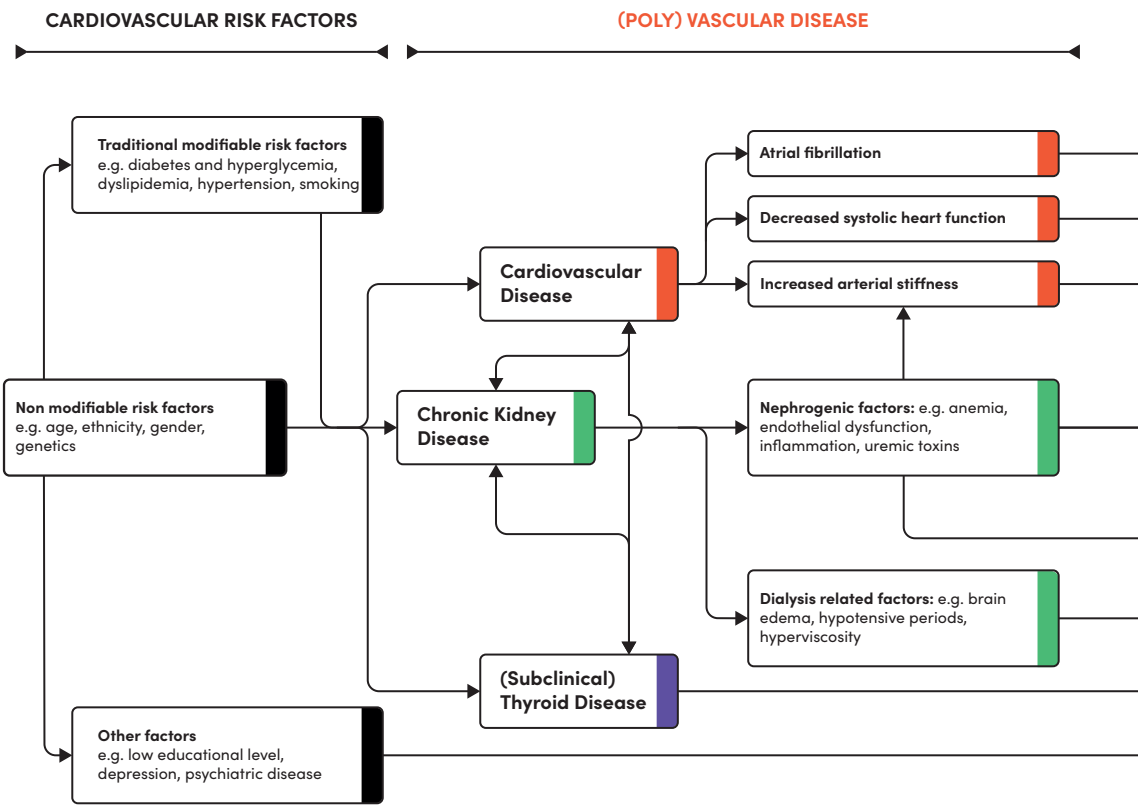
CHAPTER 1.

GENERAL INTRODUCTION AND OUTLINE

GENERAL INTRODUCTION

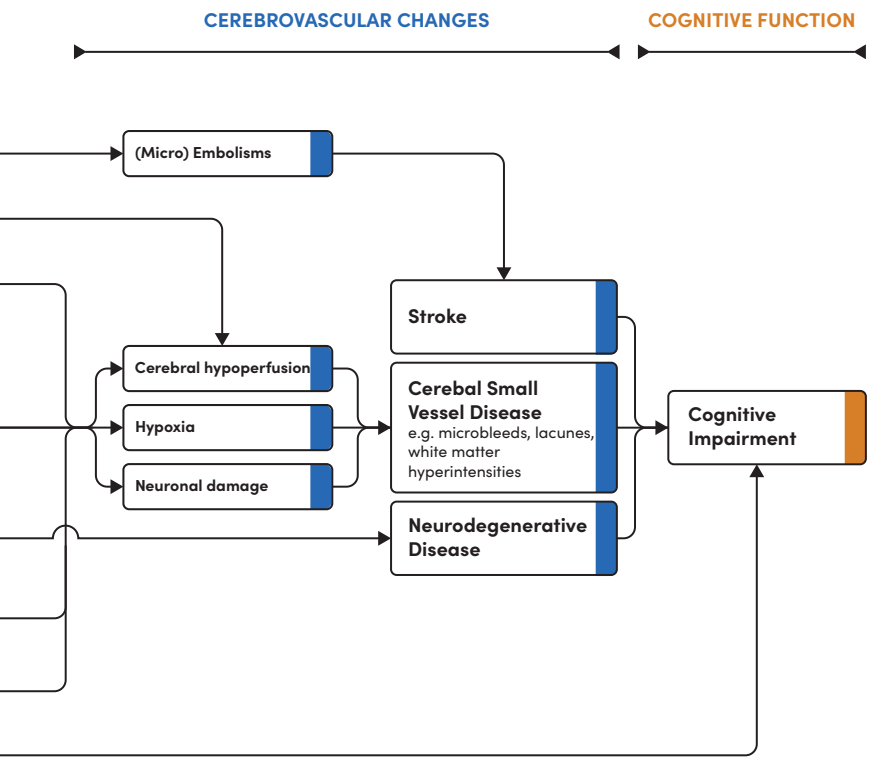
Worldwide the population is aging, leading to an absolute increase in age-related diseases, such as cardiovascular disease and cognitive impairment. The growing number and proportion of older individuals results in the expectation that the population >60 years is growing from 900 million in 2015 to 2 billion by 2050 worldwide (World Health Organisation). As age-related diseases are increasingly prevalent, there is a growing need for knowledge regarding the interplay between different (co)morbidities, the relation with the various underlying pathophysiological mechanisms and ultimately prevention and treatment options of these (co)morbidities. Figure 1 shows a flowchart where we hypothesize how cardiovascular risk factors can lead to different kind of intertwined vascular diseases and ultimately to cerebrovascular changes and cognitive dysfunction in old age.

FIGURE 1. FLOWCHART THESIS



CARDIOVASCULAR RISK FACTORS LEADING TO (POLY) VASCULAR DISEASE

Cardiovascular risk factors can be divided in non-modifiable and modifiable risk factors. These risk factors, such as diabetes and hypertension, can lead to both micro- and macrovascular disease in various or multiple simultaneous organs. These organs include the heart, kidney and thyroid, resulting in cardiovascular disease, chronic kidney disease and (subclinical) thyroid disease, respectively. Next to organ-specific risk factors, cardiovascular disease, chronic kidney disease and (subclinical) thyroid disease in part share a common cause due to similar hemodynamic determinants. The heart, kidney and thyroid are all low resistance end organs exposed to high-volume blood flow and therefore predisposed for vascular damage.



INTERPLAY BETWEEN (POLY) VASCULAR DISEASES

As explained above, cardiovascular disease, chronic kidney disease and (subclinical) thyroid disease often coexist due to shared vascular risk factors. However, also other hypotheses regarding the interplay between these organs exist, including the association between cardiovascular disease and chronic kidney disease, cardiovascular disease and thyroid disease, and chronic kidney disease and thyroid disease, respectively, which are discussed separately below.

First, the interaction between cardiovascular disease and chronic kidney disease can not only be explained by similar vascular risk factors, but also by the contribution of other factors, such as inflammatory processes, anemia and endothelial dysfunction. These factors potentially work both ways, meaning that chronic kidney disease is accompanied by a substantial cardiovascular risk and cardiovascular disease can lead to an increased kidney disease risk (1).

Second, (subclinical) thyroid diseases can possibly lead to cardiovascular disease. Subclinical hyperthyroidism has been linked to both atrial fibrillation and coronary heart diseases, whereas subclinical hypothyroidism has been associated with hypercholesterolemia and atherosclerosis (2).

Third, associations of (subclinical) thyroid diseases and chronic kidney disease have also been described, linking both lower or higher thyroid function to a lower estimated glomerular filtration rate (eGFR) and increased prevalence of chronic kidney disease. Thyroid hormone changes may predispose to kidney dysfunction, due to possible alterations in kidney hemodynamics and structure, both direct and indirect via the cardiovascular system (3). Furthermore, kidney dysfunction may predispose to thyroid hormone changes, via influence on the metabolism of thyroid hormones or due to chronic illness (such as chronic kidney disease) leading to nonthyroidal illness as potential underlying pathophysiological mechanisms (4, 5).

ASSOCIATION OF (POLY) VASCULAR DISEASE WITH CEREBROVASCULAR CHANGES AND COGNITIVE IMPAIRMENT.

Cardiovascular disease, chronic kidney disease and (subclinical) thyroid disease have all been identified as potential independent risk factors for the development of structural cerebrovascular changes and cognitive impairment. The role of these three organs in cerebrovascular changes and cognitive impairment are discussed separately below.

First, the association between cardiovascular disease via atrial fibrillation leading to (micro) embolisms and stroke is widely known (6). Furthermore, the association between cardiovascular structure and function with both cerebrovascular changes and cognitive impairment can be divided into two possible mechanisms, namely increased arterial stiffness and impaired systolic heart function. Arterial stiffness can cause microvascular damage in the brain due to an increased impact of pulsatility on the microvasculature (7) and impaired systolic heart function could cause cerebral ischemia because of hypoperfusion in the brain due to decreased cardiac output (8, 9).

Second, just as cardiovascular disease, chronic kidney disease, and especially end-stage renal disease, also has been identified as independent risk factor for structural cerebrovascular changes and cognitive impairment (10). Although the underlying pathophysiological mechanisms of cognitive dysfunction in chronic kidney disease remain largely unknown, several candidate mechanisms have been suggested apart from cardiovascular risk factors (11). These mechanisms include nephrogenic factors as anemia, endothelial dysfunction, inflammation and uremic toxins leading to microvascular damage and cerebral small vessel disease (12). Furthermore, in end-stage renal disease determinants related to dialysis, such as intradialytic hypotension or cerebral oedema can lead to cerebral hypoperfusion or neuronal damage (13).

Third, also both subclinical hyper- and hypothyroidism have been implicated as risk factors for cerebrovascular changes and cognitive impairment, although results of epidemiological studies have been conflicting (14, 15). For instance,

associations have been found between increasing thyroid stimulating hormone (TSH) levels and increased risk of stroke (16). However, underlying pathophysiological mechanisms remain largely unknown, and main candidate mechanisms seem to act via cardiovascular disease.

AIMS OF THIS THESIS

IDENTIFICATION OF HIGH-RISK PATIENTS

As described above, the interplay between various organs is complex and it is known that patients with multiple comorbidities can be at high cardiovascular risk. The identification of these patients and better understanding of the interplay and underlying mechanisms of these diseases can be the first step towards prevention of not only cognitive impairment, but also major cardiovascular events and even death. The prevalence and magnitude of the potential interactions, as described in Figure 1, remain subject of debate. Therefore, in part 1 of this thesis we aim to investigate different interactions as hypothesized in Figure 1 to identify patients at high cardiovascular risk.

OPTIMIZING TREATMENT FOR HIGH-RISK PATIENTS

Treating patients at high risk of cardiovascular disease can be a therapeutic challenge, as for instance relatively frail patients can be more sensitive to side effects of treatment. Finding treatment strategies with high benefit but low risk of adverse events is therefore essential. Therefore, in part 2 of this thesis we aim to study the optimization of treatment in patients with these high-risk factors as identified in part 1.

OUTLINE OF THIS THESIS

This thesis is divided into two parts, namely identification (part 1) and optimizing treatment of high-risk patients (part 2).

PART 1: IDENTIFICATION OF HIGH-RISK PATIENTS

Chapter 2 is a review of the literature focussed on the diagnostic and therapeutic challenge of patients with possible coronary microvascular disease. The association of (poly) vascular risk factors and diseases with cerebrovascular changes and cognitive impairment in older patients is discussed in chapter 3 till 6. We start in chapter 3 to evaluate the role of genetically influenced inflammation via complement receptor 1 gene polymorphisms and cognitive function in patients at risk of cardiovascular disease. In chapter 4 the association of cardiovascular structure and function with cerebrovascular changes and cognitive function in patients with end-stage renal disease is studied, where in chapter 5 the association of kidney function with cognitive function in patients at risk of cardiovascular disease is studied. Finally, chapter 6 assesses whether kidney function modifies the association of thyroid hormones and the risk of cardiovascular outcomes in patients with subclinical thyroid dysfunction.

PART 2: OPTIMIZING TREATMENT FOR HIGH-RISK PATIENTS

Chapter 7 and 8 both describe studies with treatment of the PCSK9 (proprotein convertase subtilisin–kexin type 9) inhibitor alirocumab, where chapter 7 investigates patients with polyvascular disease (coronary, peripheral and cerebral artery disease) to prevent major adverse cardiovascular events (MACE) and death, and chapter 8 patients with coronary artery disease to prevent stroke. Chapter 9 further elaborates on the efficacy and safety of treatment with another PCSK9 inhibitor inclisiran in patients with chronic kidney disease. We summarize the potential uses of PCSK9 inhibition in chapter 10, where we describe the treatment of easily identifiable high-risk patients, as identified in the first part of this thesis. In chapter 11, the effect of thyroid hormone therapy on cardiovascular outcomes is discussed for older patients with subclinical hypothyroidism.

Finally, in chapter 12 the main conclusions and their clinical implications are provided including a proposal for future perspectives.

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PART 1.

IDENTIFICATION OF HIGH-RISK CARDIOVASCULAR PATIENTS

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CHAPTER 2.

MICROVASCULAR DISEASE IN WOMEN AND MEN

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Based on: Chest pain in the absence of obstructive coronary artery disease: A critical review of current concepts focusing on sex specificity, microcirculatory function, and clinical implications. Int J Cardiol. 2019;280:19-28.

ABSTRACT

Patients presenting with chest pain suggestive of coronary artery disease (CAD) who at coronary arteriography appear to be free of obstructive disease have presented a diagnostic and therapeutic challenge since the 1970's. Studies in female patient populations have suggested that this is predominantly a women's syndrome usually caused by microvascular endothelial dependent and independent dysfunction. A critical review of the literature focusing on studies including both women and men revealed that apart from a higher incidence of this syndrome in women there are no clinical relevant differences between both sexes. In women a lower coronary flow reserve has been reported but this appears to be mainly due to a higher basal flow. Important questions with regard to the clinical implications of microvascular dysfunction have yet to be resolved in studies involving women as well as men in which a distinction is made between patients with normal coronary arteries and those with nonobstructive disease.

INTRODUCTION

Towards the end of the 20th century several health studies in women were started. In some studies, such as the Women's Health Initiative and the Nurses' Health Studies , the long underrated subject of cardiovascular diseases in women were part of a broader spectrum of female health issues . Other studies were specifically designed to gain more insight in gender related aspects of cardiovascular disease in women, such as the Heart and Estrogen/progestin Replacement Study (HERS) [1] and the Women's Ischemia Syndrome Evaluation Study (WISE) [2]. These studies have generated a host of data that have greatly enhanced our understanding of cardiovascular disease in women, however, since these studies included no men they allow no conclusions about the influence of sex in topics that are not inherently of a female nature.

A subject that has practical consequences concerns the large group of female patients with chest pain and normal or near normal coronary arteriogram (CAG). This was recognized in the WISE study which included as one of three major objectives: "to explore mechanisms for symptoms and myocardial ischemia in the absence of epicardial coronary artery stenoses" [2]. The results of WISE have contributed to the currently widespread perception that women with chest pain and angiographically normal or near normal coronary arteries, in contrast with men, frequently have microvascular or endothelial dysfunction. However, without questioning the existence of these disorders, it may be doubted whether they are predominantly 'women's diseases' and a frequent cause of chest pain associated with adverse prognostic consequences. There is a risk that women with chest pain in the absence of coronary artery obstructions are too easily supposed to have microvascular dysfunction. This could falsely confirm their fear of having a serious cardiac condition which may cause anxiety and lead to repeated hospitalizations and catheterizations.

To contribute to a balanced assessment of the clinical implications of this syndrome we critically reviewed the literature, focusing on the reported higher incidence and more serious consequences in women compared to men.

EVOLUTION OF INSIGHTS INTO THE SYNDROME OF CHEST PAIN IN THE ABSENCE OF OBSTRUCTIVE CORONARY ARTERY DISEASE

Soon after the introduction of coronary arteriography in the early 1960's [3] it became apparent that a considerable proportion of patients with typical or atypical angina pectoris or other evidence or cardiac ischemia had angiographically normal or near normal coronary arteries [4, 5]. Because of its unknown origin this 'syndrome' became known as 'syndrome X'. Patients with proven spasm of the epicardial coronary arteries or myocardial bridging and patients with aortic stenosis were generally excluded but some investigators used the term 'syndrome X' to classify all other patients with chest pain in the absence of obstructive coronary artery disease (CAD) whereas others included only patients with typical angina pectoris plus objective evidence of myocardial ischemia. Later most investigators required in addition a positive exercise test and various exclusion criteria, such as the presence of diabetes mellitus or renal failure, were used by different investigators [6].

Several causes of syndrome X have been postulated but eventually it has become generally accepted that the symptoms, if they are due to cardiac ischemia, must be related to impairment of the coronary microcirculation. Therefore, in lieu of 'syndrome X' Cannon and Epstein in 1988 introduced the term 'microvascular angina' [7]. At present many physicians use the latter term although the definitions still vary and it has not been proven that all cases have the same underlying mechanism [8, 9].

PHYSIOLOGY AND DIAGNOSIS OF MICROVASCULAR AND ENDOTHELIAL DYSFUNCTION

In the last decade several excellent reviews on the coronary microcirculation and the role of the endothelium have been published [8, 10-14]. In this paragraph we briefly review mechanisms involved in microvascular dysfunction and commonly used diagnostic parameters to allow a better understanding of the merits and limitations of published studies.

To adjust for changes in perfusion pressure and to comply with changing metabolic demands the coronary flow must be regulated. Since the epicardial coronary arteries are mainly conduits with very low resistance, the microcirculation is responsible for regulating coronary flow which is achieved by constriction and dilatation of 'resistance vessels' consisting of pre-arterioles ('small resistance arteries') and arterioles. The large and medium sized arterioles mainly respond to flow and pressure, a process that is in part endothelium dependent. The small arterioles are modulated by metabolic demand [12]. Therefore, to achieve maximal coronary flow in clinical investigations administration of a vasodilator of endothelium dependent vessels (such as nitroglycerin) and a vasodilator of endothelium independent vessels (such as adenosine or dipyramidole) is required.

Since the diameter of the vessels of the microcirculation is <400 μm the microcirculation cannot be imaged and its integrity can only be assessed by examining its function [15]. A frequently used functional parameter is the coronary flow reserve (CFR) which is the quotient of maximal (hyperemic) coronary flow divided by basal coronary flow. To account for inter-individual differences in metabolic demand basal flow is often corrected for the rate-pressure product [16, 17]. At catheterization CFR can be assessed by either volumetric determination of coronary flow with thermodilution or flow velocity measurement using a Doppler catheter [16-20]. Flow velocity measurements have also been made noninvasively by transthoracic echo-Doppler of the left anterior descending artery [18].

A significant limitation of CFR is that it does not reveal whether a low CFR, usually defined as <2.5, is due to a low hyperemic flow or a high basal flow. Furthermore, CFR is in part dependent on the perfusion (aortic) pressure pressure.

The index of microcirculatory resistance (IMR) introduced by Fearon et al. does account for perfusion pressure and is defined as the mean distal coronary pressure (Pd) minus venous pressure divided by flow [19, 20]. The index reportedly has superior reproducibility and is less influenced by hemodynamic perturbations than CFR in the evaluation of microvascular angina [21-24].

Coronary flow may non-invasively be determined by positron emission tomography (PET). This appears to be the most accurate method to quantitate coronary flow volumes per unit of mass [10, 25, 26] but this modality is not widely available and entails a significant amount of radiation. Cardiac resonance imaging is a non-invasive technique that appears to have promise in the diagnosis of microvascular coronary disease and additionally has the potential to distinguish sub-endocardial from sub-epicardial perfusion [27, 28].

To assess coronary endothelial function at catheterization at present mostly intracoronary infusion of acetylcholine (Ach) is used. Ach releases NO from an intact endothelium which causes vasodilation but it also has a vasoconstrictor effect on the smooth muscle cells of the vessel wall. In the presence of an intact endothelium the net effect is vasodilation of epicardial vessels as well as endothelium dependent vessels of the microcirculation whereas in the presence of a dysfunctional endothelium no dilatation or vasoconstriction results. The effect is measurable by hemodynamic parameters but also the occurrence of angina pectoris and ischemic ECG shifts in the absence of spasm of the epicardial arteries has been considered evidence of microvascular involvement [29].

ETIOLOGY OF MICROVASCULAR AND ENDOTHELIAL DYSFUNCTION

Microvascular dysfunction may be caused by structural anatomical changes of the arterioles, formerly called 'small vessel disease', or endothelial dysfunction. It has also been suggested that microvascular spasm and not just reduced vasodilator capacity may cause microvascular angina pectoris [30, 31] which also explains the occurrence of signs of ischemia at Ach tests [29].

Probably the most frequent cause of structural changes is systemic hypertension which for a long time has been shown to induce thickening of the media of coronary arterioles and to reduce the microcirculatory vasodilator capacity [32-34]. Another common cause that is held responsible for structural and functional changes of the coronary microcirculation is diabetes mellitus, but it has been argued that the reduced CFR which is often present in these patients is mainly due to an increased basal flow and not to a reduced maximal flow [35]. Structural changes of the microcirculation may also be associated with various less common disorders such as systemic immune diseases, hypertrophic cardiomyopathy and fibromuscular dysplasia [10, 12, 36, 37] and may constitute a major problem in heart transplant patients [38].

Ingestion of toxic substances may affect the coronary microcirculation [39] and the microcirculatory toxicity of agents used in the treatment of cancer is being recognized as a serious risk factor to be considered in the choice of therapeutic options and cardioprotective measures [40].

Furthermore, iatrogenic damage to the microcirculation may occur during and after percutaneous and surgical interventions due to coronary artery embolization [41, 42] and in patients undergoing revascularization procedures for acute coronary syndromes the occurrence of 'no reflow' and 'reperfusion injury' is in part related to obstruction of the microcirculation [43].

Endothelial dependent dysfunction of the microcirculation may be associated with structural changes but is usually associated with cardiovascular risk factors such as smoking and advanced age and often occurs in the course of developing coronary atherosclerosis [11, 13].

THE ANGIOGRAPHIC DIAGNOSIS OF NORMAL CORONARY ARTERIES AND NON-OBSTRUCTIVE DISEASE

To obtain more accurate and objective assessments of coronary arteriograms than achievable by visual interpretation in an early stage measuring devices were introduced, first hand held calipers and later also electronic caliper based systems [3] as were used in the WISE study [2, 44]. The most objective and accurate assessments, however, may be obtained by computer assisted systems with automated edge detection, so-called 'quantitative coronary angiography (QCA)', which has become the standard in current clinical investigations [45].

Compared with state of the art QCA systems visual assessments and caliper measurements tend to overestimate lesions causing <70% lumen diameter reduction [46-48]. Since usually a cutoff of 50% diameter reduction is used to separate obstructive from non-obstructive lesions it is unlikely that visual or caliper assessments have led to a significant number of false classifications as non-obstructive disease. Furthermore, vessel wall irregularities causing < 20% narrowing may be better perceived in motion studies than in still images as are employed in quantitative assessments because in motion studies random noise, such as quantum noise and graininess of cine film, is to a large extent smoothened [15] and in digital recordings the limiting size of pixels is less disturbing. This may in part explain why in the analyses in the WISE study vessel lumen reductions < 20% were regularly observed in still images of otherwise normal looking arteries [49].

Comparing the outcome of visual interpretation with QCA in the PROMISE (Prospective Multicenter Imaging Study for the Evaluation of Chest Pain) trial Shah et al. recently found that patients with lumen reductions < 50% according to QCA but > 50% according to visual assessment had a poorer prognosis than patients in whom both methods agreed on the absence of >50% diameter reductions (1 year event rate 3.1 vs 0.9%) [48]. This suggests that 50% lumen diameter reduction per QCA may not be an optimal cutoff to predict clinical outcome. Furthermore, significant disagreement between angiographic and physiologic assessment of stenosis severity has been demonstrated [50].

Considering all limitations, it appears that the angiographic diagnosis of no or non-obstructive CAD is not as straightforward as it may seem. Nonetheless, at present expert visual or caliper aided interpretation, including motion studies and using 50% diameter reduction as cutoff, may still be considered a reasonable method to separate obstructive from non-obstructive CAD. In future studies, however, it may be advantageous to use more often state of the art QCA (with newly defined cutoff levels) in combination with functional parameters.

INCIDENCE OF TYPICAL OR ATYPICAL ANGINA PECTORIS IN THE ABSENCE OF OBSTRUCTIVE CAD

The incidence of angina pectoris or angina pectoris like chest pains in the absence of obstructive CAD has always been substantial, particularly in women. In 1986 Kemp et al. reported that in the CASS registry of 21,483 patients 4,304 patients (20%) had normal or near normal coronary arteries [51]. In 2012 Jespersen et al. reported that of 11,223 patients referred for CAG because of stable angina pectoris 65% of the women and 32% of the men had no obstructive CAD [52]. In 2014 Patel et al. reported that of 661,063 patients undergoing elective CAG, mainly for atypical chest pain or stable angina pectoris, 58.4% had either normal coronary arteries or non-obstructive (< 50% diameter obstruction) disease [53]. Young age, female sex and atypical symptoms were among the predictors of non-obstructive disease but noninvasive test findings had minimal incremental value. Similar results had been reported earlier in investigations based on other large data registries [54, 55].

INCIDENCE OF MICROVASCULAR OR ENDOTHELIAL DYSFUNCTION IN THE ABSENCE OF OBSTRUCTIVE CAD

Judging by the literature there are very few centers in which testing for microvascular or endothelial dysfunction is standard procedure. Data on the incidence in patients with chest pain in the absence of obstructive CAD are therefore scarce and subject to considerable selection bias. Furthermore, differences in methodology to assess and criteria to define dysfunction inhibit reliable comparisons between published studies. In this study we reviewed the literature to examine the differences in microvascular function between women and men, focusing on stable patients with symptoms of ischemic heart disease in the absence of obstructive CAD. For details of our search strategy see Supplement.

Table 1 summarizes studies that compare female and male patients with angiographically proven absence of obstructive CAD[21, 29, 56-59]. In the vast majority of the patients microvascular and endothelial function was assessed to reveal a potential alternative cause of angina pectoris or similar symptoms. In 5 of the 6 studies adenosine or dipyridamole was used to determine endothelium independent vasodilation, in 2 of these in combination with Ach to assess endothelium dependent vasomotion. In the 2 studies with PET [56, 58] women had a higher basal and hyperemic blood flow than men and in multivariate analysis Danad et al. found that male gender, together with age and BMI, had a negative impact on hyperemic blood flow. These findings are in agreement with a study of Sara et al. who found in a series in a series of 1,439 patients a higher baseline as well as peak flow velocity in women than in men [59]. With adenosine and Ach tests there was some sort of microvascular dysfunction in two thirds of their patients but age, and not sex, was in multivariate analysis the only variable that independently predicted abnormal microvascular function. In contrast, Aziz et al., extending a study previously published by Ong et al., observed with Ach provocation in patients with <50% luminal diameter reduction clinical evidence of what was considered

Table 1. Microvascular dysfunction in women versus men without angiographically obstructive coronary artery disease

Year, first author	Demographic details	Coronary artery disease	Microvascular dysfunction
1994, Rosen [56]	29 patients with 'syndrome X' and 20 matched controls 17 W: age 56 ± 6.6 12 M: age 51.6 ± 10.3	CAG without even minimal luminal irregularities	H2(15)O-PET (dipyridamole)
2008, Han [57]	142 patients 'referred for CAG' 89 W, 53 M: age 49.3 ± 11.7	CAG <30% stenosis	Intracoronary Doppler (adenosine, acetylcholine)
2011, Danad [58]	128 patients 89% with chest pain 78 W: age 55 ± 10 50 M: age 52 ± 10	CTCA <50% stenosis CAG <30% stenosis or FFR > 0.8	H2(15)O-PET/CT (adenosine)
2015, Sara [59]	1,439 patients angina pectoris, vasospasm (27.9%), history of m.i. (14.9%) 937 W, 502 M: age 51.1 (17-81)	CAG < 40% stenosis	Intracoronary Doppler (adenosine, acetylcholine)
2015, Kobayashi [21]	157 patients with angina 117 W: age 53.5 ± 13.5 40 M: age 53.7 ± 11.3	CAG <50% stenosis	Intracoronary thermodilution (adenosine)
2017, Aziz [29]	1,379 patients with angina 806 W: 63.9 ± 11.1 573 M: 59.0 ± 11.6	CAG <50% stenosis	CAG (acetylcholine) Symptoms and ischemic ECG changes

Abbreviations: CAG, coronary angiography; CFR, coronary flow reserve; CMD, coronary microvascular dysfunction; CTCA, computed tomography coronary angiography; FFR, fractional flow reserve; IMR, index of microcirculatory resistance; M, men; MBF = myocardial blood flow; NS = not significant (p> 0.05); OR = odds ratio; PET = positron emission tomography; RPP = rate pressure product; Tmn = mean transit time; W = women.

	CFR	MBF	Other
		Baseline W > M (p<0.001) 1.18+/-0.20 vs 0.88+/- 0.19 Corrected for RPP W > M (p = 0.01) 1.35+/-0.32 vs 1.02+/-0.25 Stress W > M (p=NS)	W = M (p=NS) coronary vasodilator reserve No difference between patients and controls
	W < M (p<0.001) 2.80 (2.40-3.20) vs 3.30 (2.90-4.00)		W < M diffuse epicardial endothelial dysfunction
		Baseline W > M (p< 0.01) 1.09 +/- 0.30 vs 0.91+/- 0.34 Corrected for RPP W = M (p=NS) Stress W > M (p<0.001) 3.78± 1.27 vs 2.90±0.85 also in multivariate analysis	
			Peak flow velocity Baseline W > M (p<0.001) 26.0 (21.0 – 33.0) vs 22.0 (17.0-27.0) Maximal flow W > M (p<0.001) 49.0 (36.0-66.0) vs 45.0 (30.0-63.0)
	W < M (p=0.004) 3.8+/-1.6 vs 4.8+/-1.9 Baseline Tmn W < M (p = 0.005) 1.05+/-0.46 vs 1.31+/-0.51 Stress Tmn W = M (p=NS)		IMR W = M (p = NS)
			W > M abnormal coronary vasomotion 70.2% vs 43.1% (p<0.001) W > M CMD 42.4% vs 20.2% (p<0.001) In multivariate analysis OR 4.3 (3.1 – 5.5) (p<0.001)

microvascular spasm (occurrence of symptoms and ischemic ECG changes) in 42.4% of the women versus 20.2% of the men [29, 60]. Remarkably, in 22.6% of women and 28.2% of the men the test result was classified as 'unspecific reaction'. Unfortunately, neither in the study by Aziz et al. nor in other studies were the patients stratified according to the presence or absence of non-obstructive CAD but in the previous study by Ong et al. (63) patients with <20% narrowing were selected who showed similar reactions compared to patients with <50% narrowing.

Table 2 summarizes the results of studies in which no angiographic data are available but obstructive CAD was considered unlikely on clinical grounds [16, 17, 61-65]. Four of the 7 studies were executed with PET in healthy volunteers with mean ages ranging from 29 [64] to 65 [16] years. In the volunteers basal coronary flow correlated positively with age [16] and in two studies with volunteers [17, 62] as well as in hyperlipidemic patients [61] basal flow was significantly higher in women than in men. In another study [64] baseline and maximal flow were non-significantly higher in women than in men but the resulting lower CFR in women reached borderline statistical significance.

Insofar as a comparison between the patients of table 1 and the patients of table 2 is meaningful it appears that in patients as well as healthy volunteers women have on average a higher basal coronary flow than men and consequently with identical maximal flow a lower CFR.

PROGNOSIS BASED ON ANGIOGRAPHIC FINDINGS

In 1973 Bruschke et al. in a minimal 5-year follow-up study were the first to report that the prognosis of patients examined for chest pain who appeared to have angiographically normal coronary arteries was excellent but that the prognosis of patients with vessel wall irregularities causing ≤ 50% diameter reduction, lesions generally considered 'non-obstructive', was significantly less favorable [66]. The prognostic difference between normal arteries and non-obstructive disease has been confirmed in almost all studies based on invasive coronary angiography (ICA) as summarized in table 3 [5, 18, 51, 52,

66-73] and more recently in studies based on computed tomography coronary angiography (CTCA) [74, 75]. In a comprehensive meta-analysis including CAG and CTCA results Wang et al. calculated for patients with stable angina the following annualized hard cardiac event rates: patients with normal coronary arteries 0.3% (95% CI 0.1%-0.4%), patients with non-obstructive (1-≤50%) disease 0.7% (95% CI 0.5-1.0%), patients with obstructive disease 2.7% (95% CI 1.7%-3.7%) [75]. Thus the prognosis in patients with non-obstructive disease is still markedly better than in patients with obstructive CAD.

In part due to the small numbers of adverse events only a few CAG studies have tried to subdivide the prognosis in non-obstructive CAD according to extent and severity of the arterial involvement. A large retrospective cohort study of US veterans comprising 8,391 patients with normal coronary arteries and 8,384 patients with non-obstructive CAD was reported by Maddox et al. [76]. The results are difficult to compare with the results of other studies because the definition of non-obstructive disease is slightly different from commonly used definitions and 95.5% of the patients were men. Nonetheless it is interesting that an increasing 1-year myocardial infarction rate was noted in patients with 1 to 3 vessel non-obstructive CAD.

In 2013 Sharaf et al. reported on adverse outcomes in women without obstructive coronary artery disease who participated in the WISE study [49]. The CAG revealed normal coronary arteries in 339 patients and non-obstructive CAD in 228 patients. Cardiovascular death or myocardial infarction at 10 years occurred in 6.7% of the women with normal arteries and in 12.8% of the women with non-obstructive disease. It should be noted that the patients with non-obstructive disease as compared with their 'normal' counterparts were older and more often postmenopausal, had in a higher percentage prior myocardial infarction and prior PTCA, and were more often current smokers or had diabetes. The WISE investigators suggested that their findings specifically related to women [77], however, as shown in table 3 CAG studies including both sexes contradict this suggestion.

Table 2. Microvascular dysfunction in women versus men without clinical evidence of obstructive coronary artery disease

Year, first author	Demographic details	Microvascular dysfunction
1993, Czernin [16]	40 healthy volunteers, women only postmenopausal 12 W: age 62 ± 9 28 M: age 66 ± 9	(13)N ammonia PET (dipyridamole)
1999, Duvernoy [61]	30 hyperlipidemic patients 15 W: age 53 ± 4 15 M: age 50 ± 8	H2(15)O-PET (adenosine)
2001, Chareonthaitawee [62]	169 healthy volunteers 38 W, 131 M age 46 ± 12	H2(15)O-PET (dipyridamole)
2010, Cortigiani [63]	1,660 patients with chest pain syndrome, no wall motion abnormality on echocardiogram at rest and stress 906 W: age 65 ± 11 754 M: age 61 ± 12	Echo Doppler LAD (dipyridamole)
2011, Sdringola [64]	125 healthy volunteers 30 W, 95 M age 29 ± 5	Rb-82 PET (dipyridamole)
2014, Murthy [65]	1,218 patients 813 W: age 62.3 (54.1-71.6) 405 M: age 61.2 (52.8-69.8) Subgroup with no CAC on CT: 307 W, 97 M	Rest/stress PET CT no visual evidence of CAD
2016, Range [17]	26 healthy volunteers 16 W: age 34 ± 7 10 M: age 34 ± 3	H2(15)O-PET (adenosine, cold-pressor)

Abbreviations: CAC; coronary artery calcium, CAD; coronary artery disease, CFR; coronary flow reserve, M; men, MBF; myocardial blood flow, NS; not significant (p>0.05), PET; positron emission tomography, RPP; rate pressure product, W; women.

	CFR	MBF	Other
	< 50 years 4.08 ≥ 50 years 3.01	W = M (p=NS) Baseline 0.94±0.25 vs 0.91±0.25 Stress 2.61±0.54 vs 2.73±0.63	
		W > M (p = 0.001) Baseline 94 ± 22 vs 72 ± 23 Stress 296 ± 56 vs 226 ± 47 in multivariate analysis (p=0.02)	
		Corrected for RPP Baseline W > M (p<0.001) - septum 1.52±0.31 vs 1.28±0.32 - anterior 1.74±0.41 vs 1.36±0.38 - lateral 1.72±0.43 vs 1.31±0.27 - inferior 1.40±0.28 vs 1.18±0.32 Stress W = M	
	W < M (p=0.04) 2.52±0.62 vs 2.57±0.66		
	W < M (p=0.049) 3.85±0.59 vs 4.08±0.90	Baseline W = M (p=NS) 0.76±0.15 vs 0.69±0.15 Stress W = M (p=NS) 2.83±0.4 vs 2.72±0.61	
	PET CT (dipyridamole, adenosine, regadenoson, dobutamine)	W = M (p=NS)	Baseline W > M (p<0.0001) 1.2 (0.95–1.53) vs 0.92 (0.75–1.17) Stress W > M (p<0.0001) 2.38 (1.82–3.14) vs 1.85 (1.3–2.51)
	W: 3.07+/- 1.12 M: 3.44 +/- 0.92 W = M (p=NS)	Baseline W > M (p=0.003) 1.10 +/- 0.18 vs 0.85 +/- 0.20 Baseline corrected for RPP W > M 1.41 +/- 0.33 vs 1.16 +/- 0.19 ml/min/ml; p = 0.024). Cold-pressor W > M (p=0.026) 1.39 +/- 0.38 vs 1.06 +/- 0.28 Adenosine W = M (p=NS)	Coronary vascular resistance Baseline W < M 81 +/- 14 vs 107 +/- 22 (p=0.006) Cold-pressor W < M 71 +/- 17 vs 91 +/- 20 (p=0.013) Adenosine W = M (p=NS)

Table 3. Invasive coronary angiography studies comparing MACE in women versus men					
Year, first author	**Number of patients**		**Endpoints**	**Difference in event rate (W vs M)**	
	Women	**Men**			
1973, Bruschke [66]	196	304	Myocardial infarction Cardiac death	W > M	>5 year follow-up; extended by Proudfit et al. to 10 year follow-up [73]
1973, Kemp [5]	99	101	All cause death	W = M	Difference W vs M extracted from data
1986, Kemp [51]	2,102	1,949	All cause death Cardiac death	W = M	Data from Coronary Artery Surgery (CASS) registry
1986, Papanicolaou [68]	865	626	Myocardial infarction Cardiac death	W = M	Difference W vs M extracted from data
1994, Sullivan [69]	83	55	Myocardial infarction All cause death Readmissions	W = M	
1995, Lichtlen [70]	61	115	Myocardial infarction Cardiac death	W = M	
2009, Sicari [18]	223	394	Myocardial infarction Death	W = M	
2012, Jespersen [52]	3,073	2,110	Cardiovascular death Myocardial infarction Heart failure Stroke	W = M	
2013, Sedlak [67]	1,757	1,330	All cause death Myocardial infarction Coronary revascularization Stroke	W > M	W with nonobstructive CAD at higher risk of cardiac event only in first year of follow-up
2015, Johnston [71]	6,610	4,517	All cause death Myocardial infarction Coronary revascularization Stroke	W < M	Concerns group with stable chest pain
2016, Sinning [72]	158	212	All cause death Myocardial infarction	W = M	Only patients with 30-50% luminal diameter reduction included

Abbreviations: CAD; coronary artery disease, M; men, MACE; major adverse cardiovascular events, W; women.

CTCA studies are comparable with invasive CAG studies in that both methods depict the anatomical status of the epicardial coronary arteries. However, the resolution of CT scanners, particularly of the older <64-slice scanners is lower than what is obtainable by invasive CAG with motion studies. The PICTURE study demonstrated that current 64-row CTCA compared with QCA as reference has a high sensitivity but still somewhat lower specificity (88.9%) in classifying CAD [78]. Nonetheless the results of CTCA are interesting among others because the use of this technique rapidly increases and in a growing number of cases is used to replace ICA.

In 2012 Abdulla et al. assessed the prognostic value of 64-slice CTCA in a meta-analysis including 2,045 patients with normal CTA and 2,068 patients with non-obstructive CAD. The cumulative major adverse cardiovascular event (MACE) rate over 21 months was 0.5% in the normal and 3.5% in the non-obstructive group [79]. In 2014 Jiang et al. published a meta-analysis of CTCA studies and concluded that the event rate for a normal CTCA was comparable with the event rate among healthy low-risk individuals and that the presence of non-obstructive CAD and obstructive CAD incrementally increased the risk of adverse events [74]. In these prognostic aspects the results of CTCA studies corroborate the findings in invasive CAG studies. Furthermore, several CTCA studies have demonstrated an increasing occurrence of MACE with increasing extent of non-obstructive disease [80-83].

Only a few CTCA studies have assessed the significance of sex but two CONFIRM (Coronary CT angiography for clinical outcomes) studies by respectively Leipsic et al. [81] and Schulman-Marcus et al. [83] have specifically addressed this issue. Leipsic et al. did a propensity analysis comprising 5,731 women and 5,731 men and after matching found that women and men experienced identical annualized rates of myocardial infarction, death, and MACE. In multivariate analysis non-obstructive CAD was associated with similarly increased MACE, for both women and men [81]. Schulman-Marcus et al., using the long-term CONFIRM registry, followed 5,632 patients for 5 years and found that there were no distinct sex-specific differences in the risk of MACE defined as death or myocardial infarction [83].

Unfortunately, in spite of the relatively benign prognosis with regard to the occurrence of cardiovascular events, symptoms may persist for many years [68, 70, 84] which may lead to repeated hospitalizations and recatheterizations which seems to occur more often in women than in men.

PROGNOSTIC SIGNIFICANCE OF MICROVASCULAR OR ENDOTHELIAL DYSFUNCTION

The role of endothelial dysfunction in atherogenesis is well established [11, 13] but only a few studies have assessed its prognostic significance in patients with angina pectoris or angina-like chest pain in the absence of obstructive CAD. Table 4 lists prognostic studies that included both women and men with normal coronary arteries or non-obstructive disease demonstrated by ICA in whom microvascular and/or endothelial dysfunction was examined by direct investigation of the coronary circulation [18, 85-88]. In three studies Ach was used to determine endothelial function epicardial coronary arteries and endothelial dependent microvascular vessels [86-88]. These studies demonstrated that endothelial dysfunction of epicardial as well as of

Table 4. Prognostic significance of endothelial and microvascular function in women versus men without obstructive coronary artery disease

Year, first author	Number of patients		Main parameter	Median/mean follow-up (months)	Difference in event rate (W vs M)
	Women	Men			
2000, Al Suwaidi [86]	104	53	ΔCBF (acetylcholine, adenosine)	28	W = M
2002, Halcox [87]	42	134	ΔCVR (acetylcholine, adenosine)	46	W < M, but in multivariate analysis W = M
2003, Schindler [85]	39	91	ΔLA (cold pressor test)	45	W = M
2009, Sicari [18]	223	171	CFR (echo-Doppler of LAD)	51	W = M
2016, Reriani [88]	320	150	ΔCBF (acetylcholine, nitroglycerin)	116	Not reported

Abbreviations: CBF; coronary blood flow, CFR; coronary flow reserve, CVR; coronary vascular resistance, LA; luminal area, LAD; left anterior descending artery, M; men, W; women.

microvascular vessels is associated with more cardiovascular events. Studies by Schindler et al. [85] and Sicari et al. [18] were restricted to the effect of Ach on epicardial coronary arteries and both studies concluded that abnormal vasomotion was associated with increased cardiovascular risk. Halcox et al. reported that endothelium independent responses were not predictive of outcome [87] but the other studies allow no conclusions in this regard.

Except Reriani [88], who did not report on differences between sexes, all investigators reported similar results in women and men. Likewise Schäginger et al. found that sex was not associated with different outcome in patients with endothelial dysfunction but they did not clearly differentiate between patients with and those without coronary atherosclerosis [89]. Taqueti et al., however, found in a median 3-year follow-up study of 329 patients, 43% of whom were female, that only women with severely impaired CFR (<1.6) had a significant increased cardiovascular risk but it is unclear to what extent this concerned the 77 patients with no or non-obstructive coronary atherosclerosis [90] (according to the definition of the investigators including patients with 50-74% obstruction in one artery [91].

Studies restricted to women have confirmed the prognostic significance of microvascular or endothelial dysfunction. In a 48 months (median) follow-up of 163 women of the WISE study, 75% of whom had normal coronary arteries or non-obstructive disease, von Mering et al. found that the response of epicardial coronary arteries to Ach, characterized by percentage change of cross-sectional area, and degree of CAD in multivariate regression analysis were the only variables that predicted cardiovascular events [92].

In 2010 Pepine et al. published the results of a WISE substudy designed to investigate whether microvascular dysfunction predicts major adverse outcomes among women with signs and symptoms of ischemia [93]. The study included 189 women, 152 of whom were free of obstructive CAD. Endothelium-independent function of the microcirculation was assessed by determining CFR with adenosine and using a cutoff of 2.32 because this appeared the value best predictive of MACE. The investigators concluded

that coronary microvascular reactivity to adenosine significantly improves prediction of MACE over angiographic CAD severity and CAD risk factors. It must be noted, however, that of the 25 first major events 8 were cases of stroke, which makes a direct causal effect of cardiac microvascular dysfunction unlikely, 6 events were cases of congestive heart failure which is unusual in this category of patients, and that cause of death (n=8) in most cases could not be ascertained.

Bugiardini et al. focused on endothelial function in relation to duration of symptoms and future development of coronary atherosclerosis [94]. They selected 42 women with de novo angina pectoris and angiographically normal coronary arteries without irregularities. With intracoronary Ach vasoconstriction was seen in 22 patients, 13 of them still had angina pectoris at the end of follow-up. Vasodilation was seen in 20 patients who all experienced complete resolution of chest pain. At the end of a ≥ 10 year follow-up coronary angiography was repeated in 37 patients which showed variable degrees of coronary lumen stenosis in all symptomatic patients and normal-appearing coronary arteries in the others. The authors conclude that endothelial dysfunction in a setting of normal appearing coronary arteries is often associated with persistence of chest pain and development of coronary atherosclerosis.

DISCUSSION

In spite of the development of sophisticated noninvasive tests that are frequently used in the selection of patients for ICA the percentage of patients undergoing ICA for chest pain who appear to be free of obstructive CAD has not diminished since the publication of the Coronary Artery Surgery Study (CASS) results in 1986 [51, 53]. In publications this 'syndrome' is consistently more prevalent in women than in men but the reported women/men prevalence ratios vary considerably. The difference between sexes may in part be due to a selection bias. Women more often than men may have atypical symptoms [95] which may contribute to more noninvasive tests being performed in women and in cases of positive test results to more referrals for ICA. In a meta-analysis of studies using different noninvasive myocardial perfusion modalities Takx et al. concluded that PET, CT, and MRI can accurately rule out hemodynamically significant CAD but that SPECT and echocardiography are clearly less accurate for this purpose [96]. Patel et al. found that the results of noninvasive tests, in most cases SPECT, only weakly correlated with the likelihood of obstructive CAD [53]. These studies indicate that reliance on functional tests in the selection of patients for ICA may contribute to a high percentage of patients who are found to be free of obstructive CAD, which could affect more women than men.

It has been suggested that women more often than men have microvascular dysfunction that may cause symptoms of ischemic heart disease [77, 93]. However, this concept is not substantiated by studies that include both sexes. It is true that in some studies women have lower CFR than men but quantitative PET studies in patients as well as healthy volunteers have demonstrated that the difference is mainly due to a higher basal coronary flow in women and not to a lower maximal flow. A common cause of microvascular disease is hypertension which may lead to symptoms of cardiac ischemia by a combination of factors, that is: maximal coronary flow is reduced whereas a higher workload increases oxygen demand and myocardial vascular rarefaction may occur because the vasculature does not develop in proportion with the increasing myocardial mass associated with left ventricular hypertrophy. [32, 33]. In animal models and in patients it has been demonstrated that reverse

coronary microvascular remodeling may be achieved by antihypertensive treatment but in this respect not all classes of drugs appear to be equally effective [97, 98].

The role of endothelium dependent microvascular vasodilation and microvascular spasm is still uncertain and merits further investigations. Provocation tests with intracoronary Ach, especially in high doses [29], have generated evidence of epicardial and microvascular endothelial dysfunction in a considerable proportion of patients which in one study concerned more women than men (70% vs 43%) [29]. However, it remains to be proven that in physiologic conditions coronary epicardial or endothelium dependent microvascular dysfunction is a common cause of chest pains considered compatible with cardiac ischemia. Furthermore, since endothelial dysfunction is closely associated with the initiation and progression of atherosclerosis [11, 13] it is difficult to ascertain which of these two processes is responsible for the prognostic consequences. The link with atherosclerosis especially in the presence of hypertension may also explain the relatively high incidence of non-cardiac vascular events such as stroke in patients with endothelial dysfunction.

Regrettably, in the literature rarely a separation is made between patients with normal coronary arteries and patients with nonobstructive disease. There is overwhelming evidence that patients with suspicious chest pain and normal CAG irrespective of sex have a cardiovascular prognosis that is as least as good as the prognosis in the population at large. Therefore, if these patients frequently have endothelium dependent or endothelium independent microvascular dysfunction that either is a benign condition or occurs just as often in the population at large. In patients with nonobstructive disease, which may be interpreted as an early stage of coronary atherosclerosis, the prognosis is less favorable, albeit still better than in patients with obstructive disease, and it is conceivable that in these patients endothelium dependent and endothelium independent microvascular dysfunction play a greater role. Hopefully, in future studies and in clinical practice this aspect will be given more consideration and we recommend that undiscriminative terms like ‘no significant CAD’ be avoided.

CONCLUDING REMARKS

Apart from a higher prevalence in women we found in the literature no convincing evidence of essential differences between women and men referred for ICA because of stable symptoms compatible with ischemic heart disease who are found to be free of obstructive CAD.

In the past the significance of microvascular and endothelial dysfunction probably has been underestimated but the full clinical significance of these disorders has yet to be clarified. In the meantime there is no justification to assume without objective proof that particularly in female patients angina-like symptoms in the absence of obstructive CAD are probably due to microvascular dysfunction. The Coronary Vasomotion Disorders International Study Group (COVADIS) recently published a scheme for standardization of diagnostic criteria for microvascular angina [99]. According to this a diagnosis of 'definitive microvascular angina' requires evidence of impaired coronary microvascular function which at present can only reliably be obtained by invasive investigations or PET. Obviously there is a need for widely available harmless non-invasive methods to assess microvascular function. In this respect studies with MR stress tests have shown promising results[27, 28].

Given the favorable prognosis in patients, women as well as men, with normal CAG additional tests to assess microvascular or endothelial function may not always be required but these tests may provide useful information about the cause of the patient's symptoms. However, even with proven microvascular or endothelial dysfunction it may remain uncertain if there is a causal relationship with the patient's symptoms. Physicians should therefore be careful not to cause unnecessary anxiety when they inform patients about these disorders that may be mainly risk factors; it also should not withhold them to search for alternative causes of angina or angina-like chest pains such as aortic stenosis, myocardial bridging [22] and non-cardiac causes.

Remaining questions, particularly about the role of the microvasculature should be addressed in future studies that include men as well as women and

if there are sex related differences we should try to analyze these and thus enhance our understanding of the mechanisms involved in the syndrome of angina pectoris in the absence of obstructive CAD. Perhaps also more emphasis should be placed on gender, defined as non-biological aspects of being male or female (e.g. social roles, personality traits), than on sex alone [100].

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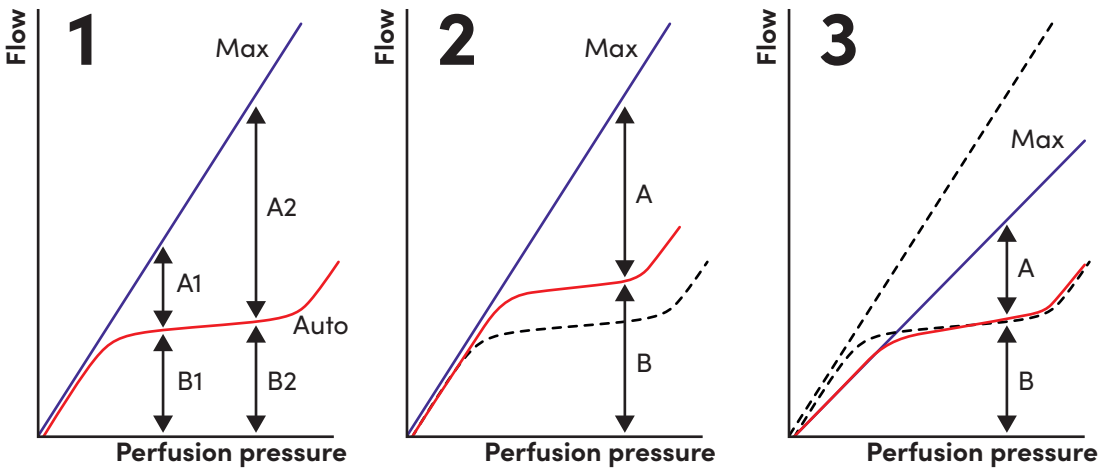
SUPPLEMENTAL MATERIAL

SUPPLEMENTAL FIGURE

CFR, defined as maximal hyperemic flow (A+B) divided by basal autoregulated flow (B), increases with increasing perfusion pressure (panel 1) and decreases with elevated basal flow (panel 2) or reduced maximal flow due to microcirculatory dysfunction (panel 3).

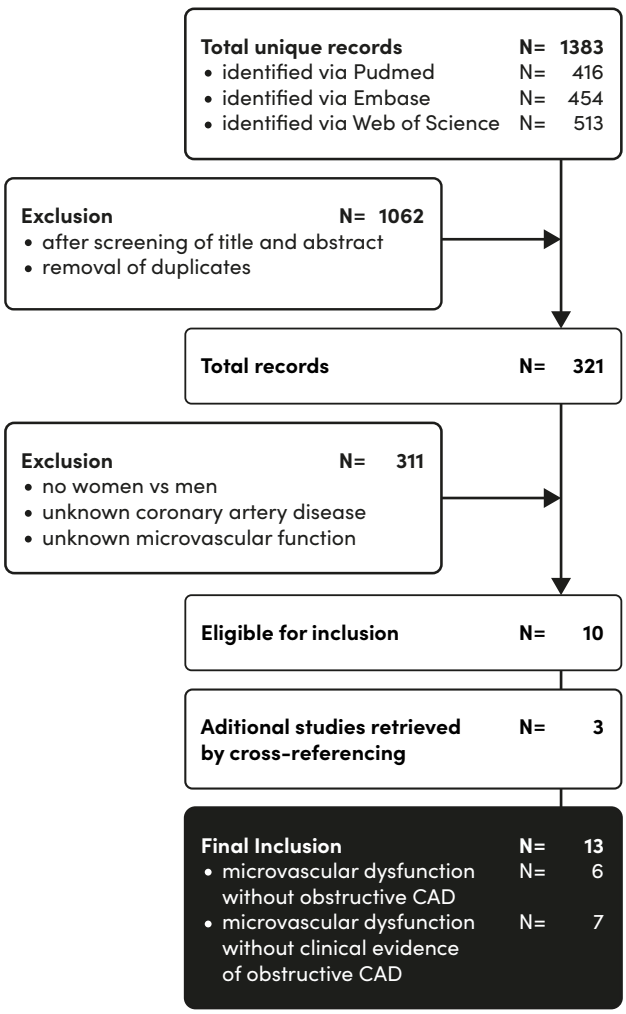


METHODS FULL LITERATURE SEARCH

We searched PubMed with no date restriction and also EMBASE and Web of Science. The last search was performed on the 30th of August 2017. All titles and abstracts of all retrieved literature was screened for relevance. When considered relevant, full-text reports were assessed for eligibility for inclusion. To identify additional relevant studies all reference lists from the identified literature were manually screened. Studies were considered eligible for this study if they met all three of the following criteria. First, it had to be a direct comparison between women and men. Second, only patients without obstructive coronary arteries were included. Third, microvascular function had to be known.

The database searches identified 1,383 unique citations. After the screening by title and abstract, 321 articles were considered potentially relevant for our review. After full text review 13 articles were included, see also the supplemented flow-chart. Seven articles met all criteria and seven articles missed angiographic data about CAD but concerned mostly healthy volunteers.

FLOWCHART LITERATURE SEARCH



FULL SEARCH STRATEGY PUBMED

("near-normal coronary angiography" [ti] OR "non-obstructive" [ti] OR "nonobstructive" [ti] OR "wall irregularities" [ti] OR "atherosclerosis" [ti] OR "Endothelium, Vascular"[majr] OR "endothelium"[ti] OR "endothelial"[ti] OR "myocardial blood flow"[ti] OR "coronary blood flow"[ti] OR "coronary flow velocity"[ti] OR "coronary flow reserve"[ti] OR "coronary vascular resistance"[ti] OR "coronary vascular reserve"[ti] OR "microcirculatory resistance"[ti] OR "Blood Flow Velocity"[majr] OR "Blood Flow Velocity"[ti] OR "Vascular Resistance"[majr] OR "Vascular Resistance"[ti] OR "Fractional Flow Reserve, Myocardial"[majr] OR "Fractional Flow Reserve"[ti] OR "Microvascular Angina"[majr] OR "syndrome X"[ti] OR "coronary microcirculatory dysfunction"[ti] OR "microvascular"[ti] OR "coronary microcirculation"[ti]) AND ("Sex"[majr] OR "Sex"[ti] OR "sex-specific"[ti] OR "gender"[ti] OR "gender-specific"[ti] OR (("Men"[majr] OR "Men"[ti] OR "male"[ti]) AND ("female"[ti] OR "Women"[majr] OR "Women"[ti]))) AND ("coronary"[tw] OR "cardiac"[tw] OR "cardiovascular"[tw] OR "heart"[tw] OR "cardiology"[tw] OR "cardiologic"[tw]) NOT ("metabolic"[ti] OR "Metabolic Syndrome X"[Mesh]) NOT "pulmonary vascular resistance"[tw] NOT "Male Urogenital Diseases"[Mesh] NOT "Female Urogenital Diseases"[Mesh]

FULL SEARCH STRATEGY EMBASE

("near-normal coronary angiography".ti. OR "non-obstructive".ti. OR "nonobstructive".ti. OR "wall irregularities".ti. OR "atherosclerosis".ti. OR exp *vascular endothelium/ OR "endothelium".ti. OR "endothelial".ti. OR "myocardial blood flow".ti. OR "coronary blood flow".ti. OR "coronary flow velocity".ti. OR "coronary flow reserve".ti. OR "coronary vascular resistance".ti. OR "coronary vascular reserve".ti. OR "microcirculatory resistance".ti. OR exp *blood flow velocity/ OR "Blood Flow Velocity".ti. OR exp *vascular resistance/ OR "Vascular Resistance".ti. OR exp *fractional flow reserve/ OR "Fractional Flow Reserve".ti. OR exp *syndrome X/ OR "Microvascular Angina".ti. OR "syndrome X".ti. OR "coronary microcirculatory dysfunction".ti. OR "microvascular".ti. OR "coronary microcirculation".ti.) AND (exp *"Sex"/ OR "Sex".ti. OR "sex-specific". ti. OR exp *gender/ OR "gender".ti. OR "gender-specific".ti. OR ((*male/ OR

"Men".ti. OR "male".ti.) AND ("female".ti. OR *female/ OR "Women".ti.))) AND ("coronary".mp. OR "cardiac".mp. OR "cardiovascular".mp. OR "heart".mp. OR "cardiology".mp. OR "cardiologic".mp.) NOT ("metabolic".ti. OR exp Metabolic Syndrome X/) NOT (exp lung vascular resistance/ OR "pulmonary vascular resistance".mp.) NOT exp urogenital tract disease/ NOT (conference OR conference abstract OR conference paper OR "conference review").pt.

FULL SEARCH STRATEGY WEB OF SCIENCE

TI=("near-normal coronary angiography" OR "non-obstructive" OR "nonobstructive" OR "wall irregularities" OR "atherosclerosis" OR "endothelium" OR "endothelial" OR "myocardial blood flow" OR "coronary blood flow" OR "coronary flow velocity" OR "coronary flow reserve" OR "coronary vascular resistance" OR "coronary vascular reserve" OR "microcirculatory resistance" OR "Blood Flow Velocity" OR "Vascular Resistance" OR "Fractional Flow Reserve" OR "Microvascular Angina" OR "syndrome X" OR "coronary microcirculatory dysfunction" OR "microvascular" OR "coronary microcirculation") AND TI=("Sex" OR "sex-specific" OR "gender" OR "gender-specific" OR (("Men" OR "male") AND ("female" OR "Women"))) AND TS=("coronary" OR "cardiac" OR "cardiovascular" OR "heart" OR "cardiology" OR "cardiologic") NOT TI=("metabolic") NOT TS=("Metabolic Syndrome X") NOT TS=("pulmonary vascular resistance" OR "Male Urogenital" OR "Female Urogenital")

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CHAPTER 3.

CR1 POLYMORPHISM AND COGNITION IN OLDER PATIENTS WITH VASCULAR DISEASE

Laurien E. Zijlstra, J. Wouter Jukema, Simon P. Mooijaart, Marijke A. de Vries, David J. Stott, Manuel Castro Cabezas, Stella Trompet

Based on: Association of complement receptor 1 gene polymorphisms with cognitive function. Physiological genomics. 50 (2018) 102-103.

ABSTRACT

Introduction: Previous evidence suggest involvement of the complement receptor 1 (CR1) in development of Alzheimer's disease. We investigated the association of CR1 gene polymorphisms with cognitive function in older subjects.

Methods: Single nucleotide polymorphisms (SNPs) within the CR1 region on chromosome 1 (n=73) were assessed in 5244 participants in the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER). In total, 51.9% were female and mean age was 75.3 years). Linear regression, adjusted for age, sex, country and use of pravastatin, was used to assess the association between the SNPs and cognitive function.

Results: All 73 SNPs within the genomic region of the CR1 gene on chromosome 1 were extracted. Eighteen were independent, using a relatively stringent R2 threshold of >0.8 with LDlink. Twelve of the 18 investigated CR1 SNPs were significantly associated with a decline in cognitive function (all p <0.05).

Conclusion: These data indicate that genetic variation within the CR1 gene is not only associated with Alzheimer's disease, but also with general cognitive function during late-life.

INTRODUCTION

Previous evidence suggest that the complement receptor 1 (CR1) may be involved in the development of multiple diseases. For instance, as previously reported, genetic variation within the CR1 gene is associated with inflammation and the risk of incident coronary artery disease [1]. Also, in multiple genome-wide association studies (GWAS) concerning Alzheimer's disease the CR1 locus has been identified as a candidate gene [2].

CR1 is a receptor for the complement proteins C3b and C4b. The complement system is involved in the innate immune system and functions as a first line defense mechanism against infection. The complement system can be activated via different pathways, ultimately leading to activation of the protein C3, which is splitted in C3b and C3a. This leads to the facilitation of opsonization and phagocytosis of pathogens. Via the CR1 receptor, circulating erythrocytes can carry immune complexes and opsonized micro-organisms to these phagocytic cells.

As described in a review by Alexander et al. previous studies have illustrated that the complement system not only is involved in regulation of the innate immunity, but also plays a role in the nervous system and therefore neurological disorders [3]. As mentioned above, the CR1 gene has been associated to Alzheimer's disease, a neurogenerative disease that is the most common cause of dementia in older patients. There is also evidence that variation within the CR1 gene is associated with general loss of cognitive function, which is the first clinical presentation of Alzheimer's disease. For instance, Chibnik et al found an association between variation in CR1 (rs6656401) with cognitive aging in the general population [4].

Therefore, we investigated the association of CR1 gene polymorphisms with cognitive function in older patients.

METHODS

PHENOTYPE

We investigated the association of CR1 gene polymorphisms with cognitive function decline. Detailed description of how cognitive function was measured has been published previously [5].

At baseline cognitive dysfunction was screened using the Mini-Mental State Examination (MMSE). We used the generally used cut-off point of 24 (of 30) points. Participants with a score below this cut-off at baseline, were excluded from PROspective Study of Pravastatin in the Elderly at Risk (PROSPER).

Outcome variables were derived from three other widely used cognitive function tests. First, selective attention was assessed using the Stroop Colour Word Test, which consist of three parts, namely color names, colored patches and color names printed in incongruously colored ink. The time required to read the names or to identify colors is recorded. We used an abbreviated version of the test with 40 elements. Second, the speed of processing of general information was assessed using the Letter-Digit Coding Test, which is a modification of the procedurally identical Symbol-Digits Modalities Test. Third, verbal learning was assessed using the Picture- Word Learning Test, which is derived from the Groningen Fifteen Words Test. Outcomes are measured in three different trials and divided in recall and delayed recall after 20 minutes.

COHORT DETAILS

All data come from PROSPER, a study that investigated the relationship between statin treatment and the risk of coronary disease (n=5804). Measurement of cognitive decline was a prespecified endpoint. In summary, older participants were enrolled in Ireland, Scotland and The Netherlands. Patients were included if they had a history of, or an increased risk for, vascular disease and a baseline cholesterol between 4.0 – 9.0 mmol/l. Of the

5804 participants, 5244 subjects were included for genetic analysis and 51.9% was female with a mean age of 75.3 +/- 3.4 years. Mean follow-up was 3.2 years. As mentioned above, one of the exclusion criteria was a poor cognitive function at baseline (MMSE <24). Detailed description, including all other in- and exclusion criteria, has been published previously [5].

TYPE OF STUDY

Candidate gene.

DETAILS OF DE SNPS STUDIED

From the GWAS study, performed in PROSPER, all 73 SNPs within the genomic region of the CR1 gene on chromosome 1 were selected with PLINK software. Using LDlink, 18 SNPs were independent, taking a relatively stringent R2 threshold of >0.8, see Table 1. Details are described previously.

Table 1. Independent SNPs within the CR1 gene	
1.	rs9429944
2.	rs1831150
3.	rs1986158
4.	rs4844597
5.	rs1887632
6.	rs9429945
7.	rs4844599
8.	rs11117956
9.	rs6656401
10.	rs12729446
11.	rs3886100
12.	rs12038575
13.	rs10127904
14.	rs12144461
15.	rs17259045
16.	rs4844609
17.	rs12734030
18.	rs6661764

Abbreviations: CR1, complement receptor 1; SNP, single nucleotide polymorphism;

ANALYSIS MODEL

Allele frequencies were estimated and pairwise linkage disequilibrium (LD) between the investigated SNPs was estimated and plotted with the program Haploview. Associations between the CR1 SNPs and cognitive function were assessed with linear regression adjusted for sex, age, country and treatment with pravastatin. PLINK statistical software was used for performance of all statistical analyses.

RESULTS

All SNPs were in Hardy Weinberg equilibrium ($p>0.05$). The results of the association between the 18 CR1 SNPs and four cognitive performance tests showed significant associations for 12 out of the 18 SNPs (all $p<0.05$). A negative association with the Stroop Colour Word test indicates an increase in cognitive function, which is in line with a positive association for the other three tests. For 9 SNPs the minor allele frequency was associated with an increase in cognitive function, whereas for 3 SNPs the minor allele frequency was associated with a decrease. Furthermore, 4 SNPs (rs9429944, rs4844599, rs11117956, rs12734030) showed statistical significant association with all 4 cognitive performance tests. Details of these SNPs, including scores for all four tests, are described in Table 2.

INTERPRETATION

Twelve of the 18 investigated CR1 SNPs were significantly associated with cognitive function. Therefore, these data indicate that genetic variation within the CR1 gene is not only associated with Alzheimer's disease, but also with general cognitive function decline during late-life. Furthermore, 3 of these 12 SNPs that were associated with an improved cognitive function, were associated with lower levels of C-reactive protein (CRP) [1]. These data further strengthen previous evidence of Alexander et al for the role of the complement system in cognitive function [3]. However, future studies investigating the functionality of these CR1 SNPs are necessary.

Table 2. Assocation between the CR1 SNPs and cognitive fun ction

rsID	Locu s name	Chr	Position	EA/ALT	Trait	EAF	β (SE)/Z-score	P	N
rs9429944	CR1	1	205736930	T/A	Stroop	0.2342	-1.63(0.638)/-2.554	0.01067	4822
					LDT		0.5938(0.1833)/3.239	0.001206	
					PLTi		0.08935(0.04249)/2.103	0.03553	
					PLTd		0.1324(0.06159)/2.149	0.03166	
rs1831150	CR1	1	205737061	G/A	Stroop	0.3316	1.003(0.6235)/1.609	0.1076	4156
					LDT		-0.4181(0.1801)/-2.322	0.0203	
					PLTi		-0.07061(0.04218)/-1.674	0.09422	
					PLTd		-0.0405(0.06089)/-0.6652	0.506	
rs4844597	CR1	1	205737892	C/T	Stroop	0.1806	-0.3644(0.7055)/-0.5165	0.6055	4803
					LDT		0.4398(0.2022)/2.175	0.02966	
					PLTi		0.08023(0.04695)/1.709	0.08753	
					PLTd		0.02314(0.06804)/0.3401	0.7338	
rs9429945	CR1	1	205743391	T/C	Stroop	0.1539	-1.218(0.7806)/-1.56	0.1189	4249
					LDT		0.5347(0.2282)/2.343	0.0192	
					PLTi		0.1291(0.05309)/2.432	0.01504	
					PLTd		0.04581(0.077)/0.5949	0.552	
rs4844599	CR1	1	205745852	G/T	Stroop	0.1234	-2.621(0.8764)/-2.99	0.002804	4086
					LDT		0.7247(0.2498)/2.901	0.003742	
					PLTi		0.1429(0.05873)/2.433	0.01501	
					PLTd		0.2392(0.08515)/2.809	0.004997	
rs11117956	CR1	1	205750982	G/T	Stroop	0.1548	-2.365(0.7877)/-3.002	0.002695	4219
					LDT		0.9442(0.2277)/4.146	3.44E-05	
					PLTi		0.1819(0.05319)/3.421	0.0006308	
					PLTd		0.2474(0.07693)/3.216	0.001308	

rs12729446	CR1	1	205765054	T/C	Stroop	0.02469	-2.385(1.754)/-1.36	0.1738	4767
					LDT		1.047(0.5098)/2.054	0.04002	
					PLTi		0.2818(0.118)/2.388	0.01696	
					PLTd		0.2209(0.1693)/1.305	0.192	
rs3886100	CR1	1	205805750	A/G	Stroop	0.3582	-1.717(0.639)/-2.687	0.007235	3679
					LDT		0.7256(0.1848)/3.926	8.81E-05	
					PLTi		0.1286(0.04266)/3.014	0.002595	
					PLTd		0.09809(0.06189)/1.585	0.1131	
rs12038575	CR1	1	205806895	G/C	Stroop	0.2806	0.7316(0.6051)/1.209	0.2267	4553
					LDT		-0.5014(0.1743)/-2.876	0.004047	
					PLTi		-0.1083(0.04033)/-2.686	0.007252	
					PLTd		-0.1295(0.05842)/-2.218	0.02663	
rs4844609	CR1	1	205849539	A/T	Stroop	0.009695	-8.286(2.868)/-2.89	0.003876	4475
					LDT		3.406(0.8619)/3.952	7.88E-05	
					PLTi		0.04887(0.1929)/0.2533	0.8001	
					PLTd		-0.0762(0.278)/-0.2741	0.784	
rs12734030	CR1	1	205860587	T/C	Stroop	0.1318	-2.398(0.8084)/-2.966	0.003027	4752
					LDT		0.8161(0.2319)/3.519	0.0004376	
					PLTi		0.1245(0.05413)/2.3	0.02148	
					PLTd		0.2316(0.07839)/2.955	0.003148	
rs6661764	CR1	1	205875008	G/C	Stroop	0.2564	0.3203(0.6278)/0.5101	0.61	4636
					LDT		-0.3471(0.1805)/-1.923	0.0546	
					PLTi		-0.1014(0.04199)/-2.415	0.01579	
					PLTd		-0.1503(0.06088)/-2.468	0.01361	

Abbreviations: rsID - dbSNP rsID; locus name. variant annotation; Chr - chromosome; Pos - Build 37 position; EA/ALT - Effect allele and alternative allele; Trait; EAF - Effect allele frequency; β (SE)/Z-score - effect estimate and standard error for a quantitative trait and Z-score for example for a binary trait; *P* - *P*-value for association; N - number of samples analysed;

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SUPPLEMENTAL MATERIAL

Supplemental table. Study genotyping		
Study genotyping / QC	Cohort Name	PROSPER
	Array used and version	Illumina 660K Beadchip
	Genotype calling software	Beadstudio
	Related individuals (yes/no)?	no
	Familial adjustment method (if applicable)	exclusion based on IBS clustering, checked for duplicates. First and second degree relatives
	Population stratification assessment and adjustment	exclusion based on IBS clustering
	Analysis software version	PLINK version 1.07
SNP QC	MAF	NA
	Call Rate	>97.5%
	HWE p-value	>10-6
	# SNPs analysed post-QC	557.192
	Other filtering	No
Imputation information	Number of SNPs used for imputation	557.192
	Imputation software	MACH
	Imputation backbone, if 1000 genomes indicate the release	NA
	Haplotypes used for backbone	NA
	NCBI build	36,2
	Chr X imputed yes/no	No
Sample QC	Call Rate	>95%
	Other QC exclusions (e.g. IBS clustering, heterozygosity, other)	Duplicate and MZ samples, sex mismatch, non-caucasians

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CHAPTER 4.

CARDIOVASCULAR STRUCTURE AND FUNCTION AND CEREBROVASCULAR CHANGES AND COGNITIVE FUNCTION IN OLDER PATIENTS REACHING END-STAGE RENAL DISEASE

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* Van Buren and Mooijaart (shared last authors) contributed equally to this work.

Based on: Association of cardiovascular structure and function with cerebrovascular changes and cognitive function in older patients with end-stage renal disease. Aging (Albany NY). 12 (2020) 1496-1511.

ABSTRACT

The Dutch prospective multicenter cohort study COPE (Cognitive decline in Older Patients with End stage renal disease) aimed to investigate the association of cardiovascular structure and function with cerebrovascular changes and cognitive function in 85 older patients with chronic kidney disease stage 4 and 5, awaiting either dialysis or conservative care. MRI was performed measuring aortic stiffness (pulse wave velocity [PWV]) and cardiac systolic function (ejection fraction and cardiac index). Outcomes were MRI-derived cerebrovascular changes (microbleeds, lacunes and white matter hyperintensities) and cognitive function (memory, executive function and psychomotor speed). Mean age was 76 years and 66% were male. No statistically significant associations were observed between cardiovascular parameters and cerebrovascular changes. Cognitive function was worse in patients with high compared to low PWV in all three cognitive domains. Although there were clinically relevant associations of high PWV with poor cognition in all domains, after adjustment for age, sex and education only the Trail Making Test A remained statistically significant (p=0.030). In conclusion, this study suggests that a higher PWV might be associated with lower cognitive function, suggesting that arterial stiffness may be an underlying mechanism of development of cognitive impairment in older patients with ESRD. Larger studies should replicate and extend these findings.

INTRODUCTION

Cardiovascular diseases and cognitive impairment are frequent and increasingly prevalent, especially in older patients and patients with end-stage renal disease (ESRD) [1-4]. Both chronic kidney disease, especially ESRD, and cardiovascular diseases have been identified as independent risk factors for the development of microvascular damage and cerebral small vessel disease, which can lead to structural cerebrovascular changes and cognitive impairment [5-9]. It is, however, unknown how cardiovascular structure and function associates with brain structure and function in older patients with ESRD.

In ESRD nephrogenic factors as uremic toxins, anaemia and inflammation, are potential underlying mechanisms for the development of cerebrovascular changes and cognitive impairment [9]. Furthermore, cardiovascular risk factors can lead to microvascular damage in both the brain and kidney [9, 10]. The association between cardiovascular structure and function with both cerebrovascular changes and cognitive impairment can be divided into two possible mechanisms in the general population, namely increased arterial stiffness and impaired systolic heart function. Arterial stiffness can cause microvascular damage in the brain due to an increased impact of pulsatility on the microvasculature, which possibly alters brain structure or cognitive functioning [11-13]. Furthermore, impaired systolic heart function could cause cerebral ischemia because of hypoperfusion in the brain due to decreased cardiac output [14-17]. To what extent an altered cardiac structure and function play a role in cerebrovascular changes and cognitive impairment in older patients with ESRD remains unclear.

Figure 1 shows the hypothesis of the current study as well as the potential underlying mechanisms. The aim of this study was to investigate the association of cardiovascular structure and function with cerebrovascular changes and cognitive function in older patients with ESRD.

FIGURE 1. THE HEART-KIDNEY-BRAIN AXIS.

Hypothesis of the current study and the potential underlying pathophysiological mechanisms in the heart-kidney-brain axis.

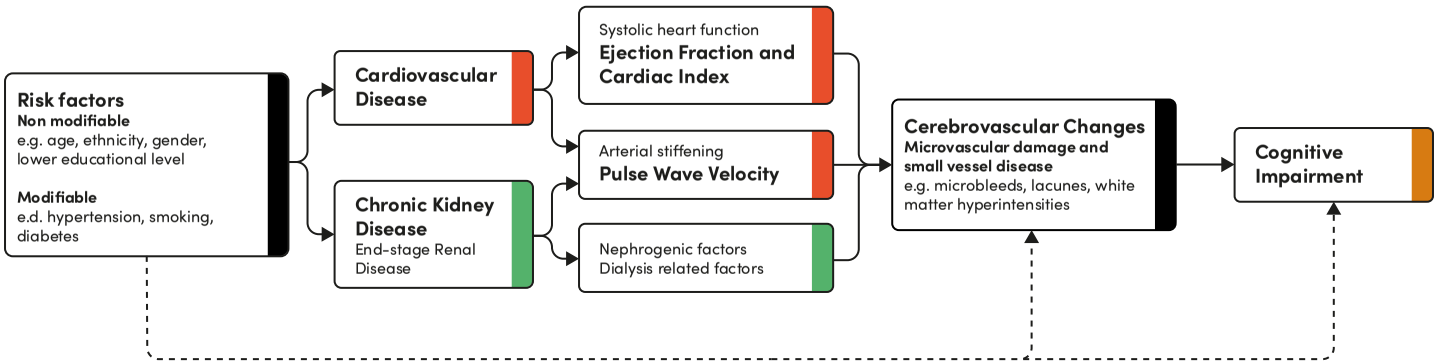
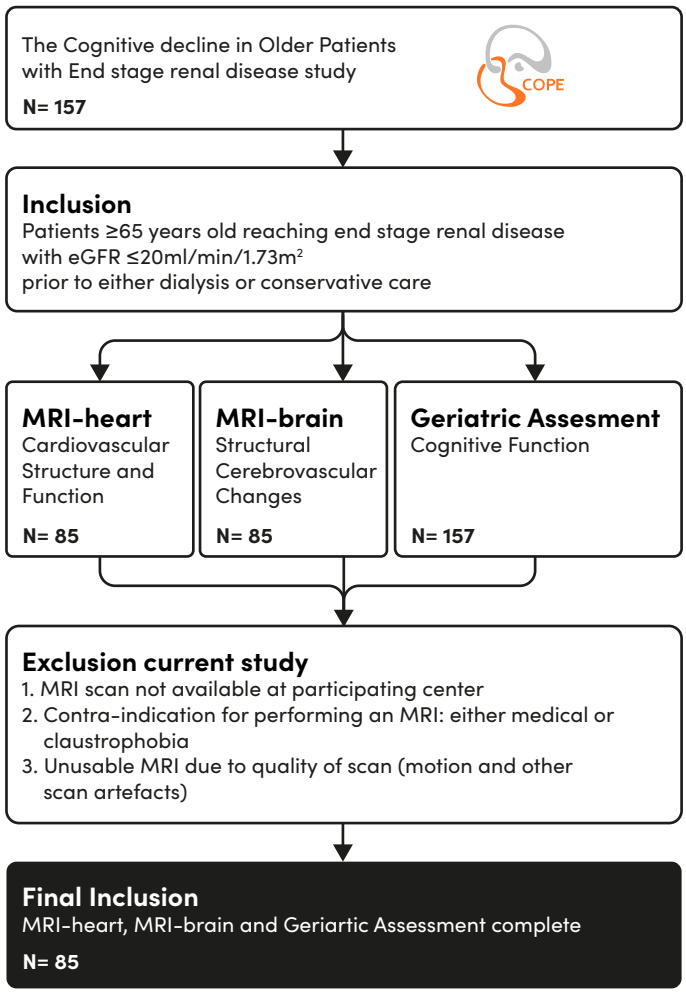


FIGURE 2. FLOWCHART STUDY POPULATION

Inclusion and exclusion criteria of the COPE (The Cognitive decline in Older Patients with End stage renal disease) study.



METHODS

Data of 'The Cognitive decline in Older Patients with End stage renal disease' (COPE) study were used, a Dutch prospective, multicenter cohort study. A detailed description of the rationale and design of COPE, including all in- and exclusion criteria, has been published previously [18]. In summary, patients ≥65 years old reaching ESRD with eGFR ≤20ml/min/1.73m2 (CKD stage 4 and 5) were included, prior to either dialysis or conservative care. The main study objective was to study the association between underlying pathophysiological mechanisms and cognitive decline in patients with ESRD. For this purpose, magnetic resonance imaging (MRI) of the heart and brain and extensive neurocognitive testing were performed. For the current analysis patients without a cardiac MRI were excluded, see flowchart in Figure 2. Written informed consent was obtained from all study participants. The study protocol was approved by the medical ethics committees (METC) of all participating centers (Leiden University Medical Center [LUMC, Leiden], HAGA Hospital [Den Haag], Dialysis Center Zoetermeer [Zoetermeer], Reinier de Graaf Group [Delft] and Jeroen Bosch Hospital [Den Bosch]).

MAGNETIC RESONANCE IMAGING

All MRI scans were made on a 3T Philips Achieva MRI scanner (Philips, Best, The Netherlands). Brain MRI was made with a 32 channel receive coil, heart MRI with an 8-channel receive coil.

Cardiovascular structure and function

The cardiac MRI protocol included flow sensitive imaging by phase contrast MRI for pulse wave velocity (PWV), measuring aortic stiffness [18]. Furthermore, the protocol included TFE (turbo field echo) multi-slice multi-phase cine-imaging of the left ventricle for systolic function, including ejection fraction (EF) and cardiac index (CI). Ejection fraction is the percentage of blood ejected out of the ventricles with each contraction (stroke volume divided by the end diastolic volume in %). Cardiac index is calculated as cardiac output (stroke volume multiplied by heart rate) and corrected for body surface area with use of the Du Bois formula (in l/min/m2) [19]. After exclusion of scans with

poor quality (artifacts mainly caused by movement or distortions) PWV was available for 83 patients, EF for 84 patients and CI for 83 patients.

Cerebrovascular changes

The brain MRI protocol included 3D FLAIR (fluid attenuated inversion recovery) and T2-weighted brain MRI images, which were scored for the presence of markers of small vessel disease, including white matter hyperintensities (WMH) and lacunes. Susceptibility weighted imaging was used to score the presence and distribution of cerebral microbleeds. Cerebrovascular changes were rated as presence of (non) lobar microbleeds, presence of lacunes and grade of WMH according to the Scheltens score [20]. For three patients the brain MRI was of insufficient quality for the rating, due to motion artefacts.

COGNITIVE FUNCTION

Detailed description of the comprehensive geriatric assessment and neuropsychological tests used in COPE has been published previously [18]. Outcome variables were derived from seven widely used neuropsychological tests in five different cognitive domains. First, global cognition was measured by the Mini-Mental State Examination (MMSE), with a general cut-off point of 24 out of 30 points [21]. Visuoconstructible abilities were assessed using the clock drawing test, which is often used as dementia screening test, with scores ranging from 0 to 14 points based on accuracy [22, 23]. Memory was assessed using two tests, namely the 15-Word Verbal Learning Test (15-WVLT), which measures immediate recall (total outcome of five presentations) and delayed recall after 20 minutes [24-26]. Memory was also assessed using the Visual Association Test (VAT) of which the score is based on the number of completed associations reported in two trials [27]. Executive functioning was assessed using the Trail Making Test B (TMTB), which is a switching task. The score of the TMTB is the number of seconds required to complete the task [28, 29]. Furthermore, the Stroop Colour Word Test (SCWT) card III (interference card) was used [30-32]. The SCWT consists of three parts, namely reading colour names (card I), naming coloured patches (card II) and naming colour names printed in incongruously coloured ink (card III). The time in seconds required

to read the names or to identify colours is recorded. We used an abbreviated version of the test with 40 elements [33]. Psychomotor speed was assessed with the Letter Digit Substitution Test (LDST), Trail Making Test A (TMTA) and SCWT card II (naming coloured patches). The LDST is a modification of the procedurally identical Symbol-Digits Modalities Test, with an outcome variable of total number of correct entries completed in 60 seconds [34, 35]. The score of the TMTA, testing visual attention, is the counted the same as the TMTB [28, 29].

STATISTICAL ANALYSIS

Cardiac parameters are dichotomized by clinical cut-off values based on current guidelines [36, 37]. These cut-off values are ≤10m/s and >10m/s for PWV, respectively no aortic stiffness versus aortic stiffness, ≤50% and >50% for EF, and <2.2l/min/m2 and ≥2.2L/min/m2 for CI, both respectively low versus high. All categorical data are presented as numbers with percentages. All continuous data are presented as mean ± standard error or ± standard deviation, or median with interquartile range. Baseline differences between cardiac parameters are assessed using an independent t-test, Mann-Whitney U test or chi-square test. Multivariate linear or logistic regression models are used to assess the associations of cardiac function with cerebrovascular changes and cognitive function. All analyses were adjusted for prespecified confounders, namely age and gender, and also education in case of cognitive function analyses. The data were analyzed using IBM SPSS Statistics version 23. P-values lower than 0.05 were considered statistically significant.

RESULTS

Of the 157 patients included in the COPE study, cardiac magnetic resonance imaging (MRI) scans were available for 85 participants, see the flowchart in Figure 2. Baseline characteristics of all patients are shown in Table 1. Mean±standard deviation (SD) of age was 75.6±6.9 years and 56 (66%) patients were male. Mean±SD eGFR at time of inclusion was 15.8±4.2ml/min/1.73m2. The origin of primary kidney disease was non-vascular in 36% and vascular (mainly diabetes and hypertension) in 64% of all patients. Median [interquartile range (IQR)] pulse wave velocity (PWV) was 9.6m/s [7.8-13.0], ejection fraction (EF) 62% [51-66] and cardiac index (CI) 2.5l/min/m2 [2.1-3.0]. Global cognition in the total population was not impaired, measured by the MMSE with median [IQR] 28 [27-30] out of 30 points and also clock drawing with a median [IQR] of 12 [11-13]. Differences in baseline characteristics for each subgroup of PWV, EF and CI are shown in Supplemental Table 1-3.

CEREBROVASCULAR CHANGES

Table 2 shows the association between cardiac parameters and cerebrovascular changes. No statistically significant associations were observed between cardiac parameters and cerebrovascular changes. Patients with a high PWV, and therefore high aortic stiffness, more often than with low PWV, had more structural cerebrovascular changes, including more microbleeds (both non-lobar and lobar) and lacunes, and a higher mean total white matter hyperintensities (WMH), although differences were of unknown clinical relevance and not statistically significant. Similar non-significant results were seen for patients with a low compared to high EF and a low compared to high CI. A sensitivity analysis based on median PWV, EF and CI and a sensitivity analysis excluding patients with a history of CVA yielded similar results.

Table 1. Baseline Characteristics Total Population (n=85)	
Male gender, n (%)	56 (65.9)
Age, years; mean ± SD	75.6 ± 6.9
Race, Caucasian, n (%)	75 (88.2)
Higher educational level, n (%)	32 (37.6)
Primary kidney disease, n (%)	
Non-vascular cause	30 (35.7)
Vascular cause	54 (64.3)
Comorbidity, n (%)	
Diabetes mellitus	32 (37.6)
Peripheral vascular disease	16 (18.8)
Cerebral vascular accident	23 (27.1)
Heart failure	7 (8.2)
Coronary heart disease	18 (21.2)
Atrial fibrillation	17 (20.5)
Alcohol consumption, n (%)	45 (52.9)
Current smoking, n (%)	14 (16.5)
History of smoking, n (%)	49 (57.6)
Medication use, n (%)	
Polypharmacy (the use of ≥5 medications)	75 (88.2)
Antihypertensive medication	79 (92.9)
Beta-blockers	44 (51.8)
Diuretics	50 (58.8)
Objective measures, mean ± SD	
Blood pressure (mmHg)	
Systolic	150.3 ± 22.2
Diastolic	81.6 ± 11.8
eGFR (ml/min/1.73m^2)	15.8 ± 4.2
Urea (mg/dL)	21.3 ± 6.3
Phosphate (mmol/L)	1.32 ± 0.29
Albuminuria (mg/24 hours)	771 ± 882
Troponin (ng/L)	0.052 ± 0.070
NT-proBNP (ng/L)	879 ± 1208
Cardiovascular function, measured by MRI, median [IQR]	
Pulse wave velocity (m/s)	9.6 [7.8-13.0]
Ejection fraction (%)	62 [51-66]
Cardiac index (l/min/m^2)	2.5 [2.1-3.0]
Cerebrovascular changes, measured by MRI, n (%) or mean ± SD	
Presence of microbleeds	
Non-lobar	18 (21.2)
Lobar	32 (37.6)
Presence of lacunes*	39 (45.9)
Total white matter hyperintensities	16.1 ± 8.0

Table 1. (Continued) Baseline Characteristics Total Population (n=85)	
Cognitive function performance, mean ± SD or median [IQR]	
Global cognition	
Mini-Mental State Examination (points)	28 [27-30]
Visuoconstruction	
Clock drawing	12 [11-13]
Memory	
15-WVLT immediate recall	32.3 ± 10.1
15-WVLT delayed recall	6.3 ± 3.0
Visual Association Test	12 [11-12]
Executive function	
TMT-B (sec)	157.0 ± 72.5
SCWT III (sec)	166.0 ± 90.0
SCWT III corrected for SCWT II (sec)	84.0 ± 81.6
Psychomotor Speed	
LDST (correct in 60 sec)	22.9 ± 7.1
TMT-A (sec)	62.0 ± 39.0
SCWT II (sec)	82.5 ± 33.7

*Lacunes; both gliotic and hemorrhagic parenchymal defects subcortical, in brain stem and basal ganglia. Abbreviations: 15-WVLT, 15-Word Verbal Learning Test; eGFR, estimated glomerular filtration rate; LDST, Letter Digit Substitution Test; NT-proBNP, N-terminal pro b-type natriuretic peptide; SD, standard deviation; SCWT, Stroop Color Word Test; TMT, Trail Making Test.

COGNITIVE FUNCTION

Table 3 shows the association between cardiac parameters and cognitive function in three different domains, namely memory, executive function and psychomotor speed. The scores of all cognitive function tests were worse for patients with a high compared to low PWV in all three domains, statistical significance was reached for the Trail Making Test B (TMTB) with a difference of 39 seconds (p=0.009), for Trail Making Test A (TMTA) with a difference of 23 seconds (p=0.008) and for the Letter Digit Substitution Test (LDST) with a difference of 4 correct answers in 60 seconds (p=0.021). After adjustment for age, sex and education, only the TMTA, measuring psychomotor speed, remained statistically significant (p=0.030).

No clinically relevant nor statistically significant associations were found in cognitive function comparing patients with low compared to high EF. Patients with a low compared to high CI had a worse memory function (both immediate and delayed recall), which remained statistically significant for delayed recall after adjustment (p=0.003). No statistically significant differences were found in executive function or psychomotor speed. A sensitivity analysis based on median PWV, EF and CI and a sensitivity analysis excluding patients with a history of CVA yielded similar results.

Table 2. Association of Cardiovascular Function Parameters and Cerebrovascular Changes

	Better Cardiovascular Function	**Worse Cardiovascular Function**		
	Pulse Wave Velocity ≤10m/s n = 45	**Pulse Wave Velocity >10m/s n = 38**	**p-value**	
			Crude	**Adjusted**
Presence of microbleeds, %				
Non-lobar	7 (16.7)	11 (28.9)	0.189	0.575
Lobar	13 (31.0)	18 (47.4)	0.132	0.105
Presence of lacunes, %	18 (42.9)	19 (50.0)	0.522	0.616
Total WMH, mean ± SE	15.5 ± 1.2	16.6 ± 1.4	0.561	0.438
	Ejection Fraction ≥50% n = 65	**Ejection Fraction <50%** n = 19		
Presence of microbleeds, %				
Non-lobar	13 (20.6)	5 (27.8)	0.520	0.563
Lobar	22 (34.9)	10 (55.6)	0.114	0.121
Presence of lacunes, %	29 (46.0)	10 (55.6)	0.476	0.767
Total WMH, mean ± SE	15.8 ± 0.9	16.9 ± 2.4	0.609	0.778
	Cardiac Index >2.2l/min/m2 n = 56	**Cardiac Index ≤2.2l/min/m2** n = 27		
Presence of microbleeds, %				
Non-lobar	13 (23.6)	5 (20.0)	0.718	0.457
Lobar	23 (41.8)	8 (32.0)	0.403	0.382
Presence of lacunes, %	28 (50.9)	10 (40.0)	0.365	0.443
Total WMH, mean ± SE	17.0 ± 1.2	14.2 ± 1.3	0.151	0.166

P-values are assessed using linear or logistic regression models, unadjusted and adjusted for age and sex, comparing low versus high pulse wave velocity, ejection fraction and cardiac index. Lacunes include both gliotic and hemorrhagic parenchymal defects subcortical, in brain stem and basal ganglia. Abbreviations: SE, standard error; WMH, white matter hyperintensities.

Table 3. Association of Cardiovascular Function Parameters and Cognitive Function				
	Better Cardiovascular Function	**Worse Cardiovascular Function**	**p-value**	
			Crude	**Adjusted**
	PWV ≤10m/s n = 45	**PWV >10m/s n = 38**		
Memory				
15-Word Verbal Learning Test immediate recall ↓	33.3 ± 1.2	31.6 ± 2.0	0.455	0.899
15-Word Verbal Learning Test delayed recall ↓	6.6 ± 0.4	5.9 ± 0.6	0.307	0.719
Executive function				
Trail Making Test B (sec) ↑	140.6 ± 9.6	179.3 ± 12.2	0.009	0.184
SCWT III (sec) ↑	158.3 ± 15.6	173.5 ± 11.8	0.439	0.339
SCWT III corrected for SCWT II (sec) ↑	80.5 ± 14.0	87.6 ± 11.1	0.692	0.148
Psychomotor Speed				
Letter Digit Substitution Test (correct in 60 sec) ↓	24.6 ± 0.9	20.9 ± 1.3	0.021	0.179
Trail Making Test A (sec) ↑	51.6 ± 2.5	74.6 ± 8.7	0.008	0.030
Stroop Color Word Test II (sec) ↑	77.8 ± 3.9	87.0 ± 6.7	0.241	0.361
	EF ≥50% n = 65	**EF <50% n = 19**		
Memory				
15-Word Verbal Learning Test immediate recall ↓	32.1 ± 1.2	32.5 ± 2.8	0.881	0.621
15-Word Verbal Learning Test delayed recall ↓	6.4 ± 0.4	5.7 ± 0.8	0.426	0.664
Executive function				
Trail Making Test B (sec) ↑	160.4 ± 8.9	153.4 ± 19.2	0.809	0.728
SCWT III (sec) ↑	166.7 ± 11.7	164.8 ± 19.1	0.940	0.669
SCWT III corrected for SCWT II (sec) ↑	86.4 ± 10.3	75.9 ± 19.8	0.633	0.392
Psychomotor Speed				
Letter Digit Substitution Test (correct in 60 sec) ↓	23.4 ± 0.8	21.3 ± 1.9	0.265	0.383
Trail Making Test A (sec) ↑	59.2 ± 4.4	73.4 ± 12.3	0.186	0.274
Stroop Color Word Test II (sec) ↑	80.9 ± 3.2	88.9 ± 12.8	0.377	0.459

Table 3. (Continued) Association of Cardiovascular Function Parameters and Cognitive Function

	Better Cardiovascular Function	Worse Cardiovascular Function	p-value	
			Crude	Adjusted
	CI >2.2 l/min/m2 n = 56	CI ≤2.2 l/min/m2 n = 27		
Memory				
15-Word Verbal Learning Test immediate recall ↓	33.2 ± 1.4	30.1 ± 1.9	0.207	0.219
15-Word Verbal Learning Test delayed recall ↓	6.9 ± 0.4	4.9 ± 0.5	0.004	0.003
Executive function				
Trail Making Test B (sec) ↑	149.6 ± 8.7	176.6 ± 16.6	0.156	0.191
SCWT III (sec) ↑	164.5 ± 13.1	171.4 ± 15.6	0.748	0.978
SCWT III corrected for SCWT II (sec) ↑	85.1 ± 12.9	84.3 ± 10.0	0.966	0.712
Psychomotor Speed				
Letter Digit Substitution Test (correct in 60 sec) ↓	23.4 ± 0.9	21.5 ± 1.5	0.266	0.349
Trail Making Test A (sec) ↑	59.9 ± 4.4	68.1 ± 10.1	0.384	0.414
Stroop Color Word Test II (sec) ↑	80.2 ± 4.6	87.1 ± 6.5	0.387	0.428

Values are mean ± SE. ↓↑ indicates that a higher (↑) or lower (↓) score means a worse cognitive function.

P-values are assessed using linear regression models, unadjusted and multivariate adjusted for age, sex and education, comparing low versus high pulse wave velocity, ejection fraction and cardiac index. Abbreviations: CI, cardiac index; EF, ejection fraction; PWV, pulse wave velocity

DISCUSSION

The main findings of this explorative study are as follows. First, higher PWV associated with all measures of cognitive impairment, albeit this was only statistically significant for the association of PWV with the TMTA, measuring psychomotor speed. Second, no statistically significant differences in the association between cardiovascular structure and function and structural cerebrovascular changes were found.

Although the association of arterial stiffness and brain pathology has been described previously in patients with ESRD, studies are limited, and included in general only cerebrovascular changes [38] or cognition [39], and in case of the latter limited tests for global cognition, instead of differentiating between various functional domains [40, 41]. In the general population, the influence

of arterial stiffness on both cerebrovascular changes and cognitive function has been more extensively studied. Cardiovascular risk factors such as hypertension result in arterial stiffening that can be measured in the aorta as increased PWV, an important and independent determinant of arterial disease [11, 12]. Due to an impaired Windkessel effect aortic stiffness might increase the impact of cardiac pulsations on the cerebral microvasculature leading to cerebral small vessel diseases and cognitive impairment [13], as was recently confirmed by a systematic review [42]. Studies have shown independent associations of impaired systolic heart function on cerebrovascular changes or cognitive function, probably due to cerebral ischemia because of hypoperfusion in the brain due to decreased cardiac output [14-17], including in patients with ESRD [43]. However, it might have a more multifactorial dependency, like for instance whether patients clinically have heart failure. Previous studies have shown associations of heart failure [14], and also associations of both EF and CI in patients with heart failure [15], with cerebrovascular changes and cognitive impairment. Furthermore, treatment of heart failure, with for example cardiac resynchronization therapy or heart transplantation, can improve cognitive function, partly due to improvement of cerebral blood flow [44-46]. Taken together, our findings that arterial stiffness may be an underlying mechanism of development of cognitive impairment is in line with known literature in both the general population, but also in patients with ESRD.

We investigated two hypotheses as underlying pathophysiological mechanisms in the heart-kidney-brain axis, namely arterial stiffness and systolic heart function (Figure 1). Few associations reached statistical significance, possibly due to the relatively low number of participants in the study. However, for PWV, all 4 cerebrovascular changed parameters of structure and function, and all 8 cognitive associates point in the same direction. The magnitudes of association in most cognitive parameters were well above what could be considered clinically relevant, which is unlikely the result of chance. Therefore, we conclude that our results suggest that PWV is a potential predictor of cognitive function in older patients with ESRD. These results, however, should be considered as "suggestive" and need replication in larger cohorts. The contributing role of systolic cardiac function on cerebrovascular changes

and cognitive impairment seemed limited in our COPE population, possibly due to a low percentage of patients with manifest symptoms of clinical heart failure (7%). In addition, although EF and CI are both parameters of systolic cardiac function, values within the same patients are not always concordant. In our population, patients with a low EF had a relatively normal mean CI and vice versa. Cardiac output has been pointed out previously to be a superior reflection of systemic blood flow and cerebral blood flow than EF, especially in patients without heart failure [47]. It might explain that low CI was a better predictor of cognitive impairment (memory domain) than low EF in our population.

STRENGTHS AND LIMITATIONS

The COPE study is a unique observational study in older patients with ESRD with a mean eGFR of 16ml/min/1.73m2, prior to either dialysis or conservative care, with comprehensive measurements of cardiovascular function, cerebrovascular changes and cognitive function.

However, some limitations should be mentioned. Patient numbers were relatively limited, and although cardiovascular comorbidity was common, patients had a relatively normal cardiac function, with only 19 patients available with EF ≤50% and 27 patients with CI <2.2l/min/m2, limiting the power to find an association between cardiovascular function and cerebrovascular changes or cognitive function of a smaller magnitude. This limited us to merely observe possible trends suitable for future research.

CONCLUSIONS

In conclusion, this exploratory study suggests that a higher PWV is associated with lower cognitive function, but not with increased cerebrovascular changes in older patients with ESRD, suggesting that arterial stiffness may be an underlying mechanism of development of cognitive impairment.

Larger studies should replicate and extend on these findings, as identifying the mechanisms involved in cerebrovascular changes and cognitive impairment can be the first step towards prevention strategies. Prevention is of utmost importance, as the Framingham Heart Study have showed that earlier diagnosis and effective treatment of risk factors or proven vascular disease, can possibly lead to a decline in incidence of dementia [48]. Furthermore, future research should also focus on other potential biomarkers as miRNAs or metabolomics to unravel specific pathophysiological mechanisms in this interaction between the heart, kidney and brain and thereafter on potential interventions to prevent cerebrovascular changes and cognitive impairment.

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SUPPLEMENTAL MATERIAL

Supplemental Table 1. Determinants of Pulse Wave Velocity			
	Better Cardiovascular Function	**Worse Cardiovascular Function**	
	Pulse Wave Velocity ≤10 m/s n = 45	**Pulse Wave Velocity >10 m/s n = 38**	**p-value**
Pulse wave velocity (m/s), mean ± SD	7.8 ± 1.4	14.1 ± 3.8	
Male gender, n (%)	33 (73.3)	22 (57.9)	0.138
Age, years; mean ± SD	73.0 ± 6.4	78.4 ± 6.4	<0.0001
Race, Caucasian, n (%)	40 (88.9)	34 (89.5)	0.696
Higher educational level, n (%)	15 (33.3)	15 (39.5)	0.562
Primary kidney disease, n (%)			0.652
Non-vascular cause	16 (35.6)	14 (36.8)	
Vascular cause	28 (62.2)	24 (63.2)	
Comorbidity, n (%)			
Diabetes mellitus	17 (37.8)	15 (39.5)	0.874
Peripheral vascular disease	8 (17.8)	7 (18.4)	0.940
Cerebral vascular accident	13 (28.9)	10 (26.3)	0.794
Heart failure	3 (6.7)	3 (7.9)	0.830
Coronary heart disease	12 (26.7)	8 (21.1)	0.551
Atrial fibrillation	8 (17.8)	9 (23.7)	0.506
Alcohol consumption, n (%)	27 (61.4)	17 (44.7)	0.132
Current smoking, n (%)	10 (22.7)	4 (10.5)	0.143
History of smoking, n (%)	26 (76.5)	22 (66.7)	0.373
Objective measures, mean ± SD			
eGFR (ml/min/1.73m^2)	16.2 ± 4.5	15.4 ± 3.8	0.393
Urea (mg/dL)	20.3 ± 5.6	22.5 ± 7.1	0.134
Phosphate (mmol/L)	1.31 ± 0.30	1.33 ± 0.28	0.800
Albuminuria (mg/24 hours)	815 ± 900	712 ± 874	0.683
Troponin (ng/L)	0.056 ± 0.083	0.048 ± 0.053	0.715
NT-proBNP (ng/L)	1054 ± 1386	707 ± 985	0.691
Blood pressure (mmHg)			
Systolic	149.2 ±22.9	151.9 ± 21.9	0.601
Diastolic	82.8 ± 10.2	80.2 ± 13.7	0.342
Ejection fraction (%)	58.5 ± 10.9	59.4 ± 9.8	0.686
Cardiac index (l/min/m^2)	2.6 ± 0.6	2.6 ± 0.4	0.652

P-values are assessed using an independent t-test, Mann-Whitney U test or chi-square test. Abbreviations: eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal pro b-type natriuretic peptide; SD, standard deviation;

Supplemental Table 2. Determinants of Ejection Fraction			
	Better Cardiovascular Function	**Worse Cardiovascular Function**	
	Ejection Fraction >50% n = 65	**Ejection Fraction <50% n = 19**	**p-value**
Ejection fraction (%), mean ± SD	63.2 ± 6.2	42.3 ± 7.3	
Male gender, n (%)	40 (61.5)	15 (78.9)	0.160
Age, years; mean ± SD	75.4 ± 6.8	76.6 ± 7.5	0.471
Race, Caucasian, n (%)	55 (84.6)	19 (100.0)	0.506
Higher educational level, n (%)	21 (32.3)	10 (52.6)	0.106
Primary kidney disease, n (%)			0.860
Non-vascular cause	23 (35.4)	7 (36.8)	
Vascular cause	41 (63.1)	12 (63.2)	
Comorbidity, n (%)			
Diabetes mellitus	27 (41.5)	5 (26.3)	0.229
Peripheral vascular disease	10 (15.4)	6 (31.6)	0.114
Cerebral vascular accident	14 (21.5)	8 (42.1)	0.073
Heart failure	3 (4.6)	4 (21.1)	0.023
Coronary heart disease	15 (23.1)	6 (31.6)	0.452
Atrial fibrillation	12 (18.5)	6 (31.6)	0.220
Alcohol consumption, n (%)	32 (50.0)	13 (68.4)	0.157
Current smoking, n (%)	3 (15.8)	11 (17.2)	0.886
History of smoking, n (%)	33 (63.5)	16 (100.0)	0.004
Objective measures, mean ± SD			
eGFR (ml/min/1.73m^2)	16.0 ± 4.4	14.9 ± 3.4	0.303
Urea (mg/dL)	21.2 ± 6.3	21.7 ± 6.7	0.763
Phosphate (mmol/L)	1.31 ± 0.30	1.40 ± 0.23	0.243
Albuminuria (mg/24 hours)	754 ± 849	893 ± 1101	0.674
Troponin (ng/L)	0.050 ± 0.077	0.060 ± 0.032	0.008
NT-proBNP (ng/L)	572 ± 915	2020 ± 1482	<0.0001
Blood pressure (mmHg)			
Systolic	151.3 ± 22.3	148.0 ± 22.3	0.588
Diastolic	80.1 ± 11.7	85.8 ± 12.1	0.103
Pulse wave velocity (m/s)	10.5 ± 4.1	11.3 ± 4.8	0.447
Cardiac index (l/min/m^2)	2.6 ± 0.7	2.5 ± 0.7	0.498

P-values are assessed using an independent t-test, Mann-Whitney U test or chi-square test. Abbreviations: eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal pro b-type natriuretic peptide; SD, standard deviation;

Supplemental Table 3. Determinants of Cardiac Index			
	Better Cardiovascular Function	**Worse Cardiovascular Function**	
	Cardiac Index >2.2 l/min/m² **n = 56**	**Cardiac Index ≤2.2 l/min/m²** **n = 27**	**p-value**
Cardiac index (l/min/m²), mean ± SD	2.9 ± 0.5	1.9 ± 0.3	
Male gender, n (%)	39 (69.6)	16 (59.3)	0.349
Age, years; mean ± SD	75.4 ± 6.4	76.2 ± 7.7	0.675
Race, Caucasian, n (%)	48 (85.7)	26 (96.3)	0.373
Higher educational level, n (%)	21 (37.5)	10 (37.0)	0.967
Primary kidney disease, n (%)			0.690
Non-vascular cause	19 (33.9)	10 (38.5)	
Vascular cause	37 (66.1)	16 (61.5)	
Comorbidity, n (%)			
Diabetes mellitus	23 (41.1)	9 (33.3)	0.497
Peripheral vascular disease	13 (23.2)	3 (11.1)	0.190
Cerebral vascular accident	16 (28.6)	7 (25.9)	0.801
Heart failure	3 (5.4)	4 (14.8)	0.146
Coronary heart disease	14 (25.0)	7 (25.9)	0.928
Atrial fibrillation	9 (16.1)	9 (33.3)	0.074
Alcohol consumption, n (%)	29 (52.7)	15 (55.6)	0.809
Current smoking, n (%)	8 (14.5)	5 (18.5)	0.643
History of smoking, n (%)	32 (69.6)	17 (77.3)	0.508
Objective measures, mean ± SD			
eGFR (ml/min/1.73m²)	15.3 ± 4.0	17.0 ± 4.4	0.077
Urea (mg/dL)	21.4 ± 6.2	21.3 ± 6.8	0.972
Phosphate (mmol/L)	1.33 ± 0.31	1.32 ± 0.25	0.925
Albuminuria (mg/24 hours)	744 ± 754	836 ± 1160	0.737
Troponin (ng/L)	0.049 ± 0.048	0.038 ± 0.031	0.751
NT-proBNP (ng/L)	944 ± 1208	799 ± 1266	0.308
Blood pressure (mmHg)			
Systolic	149.5 ± 20.4	153.0 ± 26.6	0.516
Diastolic	80.0 ± 12.8	84.6 ± 9.0	0.110
Pulse wave velocity (m/s)	10.4 ± 3.9	11.2 ± 4.8	0.417
Ejection fraction (%)	59.6 ± 10.2	56.0 ± 12.5	0.168

P-values are assessed using an independent t-test, Mann-Whitney U test or chi-square test. Abbreviations: eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal pro b-type natriuretic peptide; SD, standard deviation;

Supplemental Table 4. Univariate Associations of Blood Pressure and Pulse Pressure with Cerebrovascular Changes						
	Systolic blood pressure		**Diastolic blood pressure**		**Pulse Pressure**	
	beta	**P-value**	**beta**	**P-value**	**beta**	**P-value**
Presence of microbleeds						
Non-lobar	0.021±0.01	0.100	0.000±0.02	0.999	0.023±0.01	0.085
Lobar	0.025±0.01	0.033	0.023±0.02	0.255	0.019±0.01	0.103
Presence of lacunar infarction	0.005±0.01	0.628	-0.007±0.02	0.701	0.008±0.01	0.468
Total WMH	0.024±0.04	0.559	-0.120±0.08	0.120	0.066±0.04	0.135
Values are beta ± SE. P-values are assessed using linear or logistic regression models. Lacunes include both gliotic and hemorrhagic parenchymal defects subcortical, in brain stem and basal ganglia. Abbreviations: WMH, white matter hyperintensities.						

Supplemental Table 5. Univariate Associations of Blood Pressure and Pulse Pressure with Cognitive Function						
	Systolic blood pressure		**Diastolic blood pressure**		**Pulse Pressure**	
	beta	**P-value**	**beta**	**P-value**	**beta**	**P-value**
Memory						
15-WVLT immediate recall	-0.030±0.05	0.564	0.124 ±0.10	0.204	-0.071±0.05	0.189
15-WVLT delayed recall	0.003±0.02	0.842	0.044±0.03	0.139	-0.010±0.02	0.548
Executive function						
Trail Making Test B (sec)	1.134±0.33	0.001	0.312±0.68	0.645	1.143±0.35	0.002
SCWT III (sec)	0.575±0.45	0.207	-1.231±0.87	0.163	0.973±0.46	0.038
SCWT III corrected for SCWT II (sec)	0.271±0.43	0.527	-1.609±0.81	0.051	0.754±0.44	0.088
Psychomotor Speed						
LDST (correct in 60 sec)	-0.066±0.03	0.058	-0.022±0.07	0.741	-0.066±0.04	0.072
Trail Making Test A (sec)	0.531±0.20	0.009	0.418±0.39	0.287	0.438±0.21	0.037
Stroop Color Word Test II (sec)	0.261±0.16	0.100	0.215±0.30	0.472	0.221±0.17	0.187
Values are beta ± SE. P-values are assessed using linear regression models. Abbreviations: 15-WVLT, 15-Word Verbal Learning Test; LDST, Letter Digit Substitution Test; SCWT, Stroop Color Word Test.						

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CHAPTER 5.

KIDNEY FUNCTION AND COGNITIVE DECLINE IN OLDER PATIENTS WITH VASCULAR DISEASE

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ABSTRACT

INTRODUCTION

Chronic kidney disease (CKD) has been identified as a significant direct marker for cognitive decline, but controversy exists regarding the magnitude of the association of kidney function with cognitive decline across the different CKD stages. Therefore, the aim of this study was to investigate the association of kidney function with cognitive decline in older patients at high risk of cardiovascular disease, using data from the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER).

METHODS

Data of 5796 patients of PROSPER were used. Strata were made according to clinical stages of CKD based on estimated glomerular filtration rate; <30ml/min/1.73m2 (stage 4), 30-45ml/min/1.73m2 (stage 3b), 45-60ml/min/1.73m2 (stage 3a) and ≥60ml/min/1.73m2 (stage 1-2). Cognitive function and functional status was assessed at six different time points and means were compared at baseline and over time, adjusted for multiple prespecified variables. Stratified analyses for history of vascular disease were executed.

RESULTS

Mean age was 75.3 years and 48.3% participants were male. Mean follow-up was 3.2 years. For all cognitive function tests CKD stage 4 compared to the other stages had the worst outcome at baseline and a trend for faster cognitive decline over time. When comparing stage 4 versus stage 1-2 over time the estimates (95% CI) were 2.23 (0.60–3.85; p=0.009) for the Stroop-Colour-Word test, -0.33 (-0.66–0.001; p=0.051) for the Letter-Digit-Coding test, 0.08 (-0.06–0.21; p=0.275) for the Picture-Word-Learning test with immediate recall and -0.07 (-0.02–0.05; p=0.509) for delayed recall. This association was most present in patients with a history of vascular disease. No differences were found in functional status.

CONCLUSION

In older people with vascular burden, only severe kidney disease (CKD stage 4), but not mild to modest kidney disease (CKD stage 3a and b), seem to be associated with cognitive impairment at baseline and cognitive decline over time. The association of severe kidney failure with cognitive impairment and decline over time was more outspoken in patients with a history of vascular disease, possibly due to a higher probability of polyvascular damage, in both kidney and brain, in patients with proven cardiovascular disease.

INTRODUCTION

Both chronic kidney disease (CKD) and cognitive impairment are increasingly prevalent with advancing age and partly share a common cause as the kidney and the brain share similar hemodynamic characteristics (1-3). Both organs are low resistance end organs exposed to high-volume blood flow and therefore predisposed for vascular damage (4). Next to aging, classical vascular risk factors like hypertension, diabetes and a history of cardiovascular diseases are associated with microvascular damage and small vessel disease in both the kidney and the brain (5-10).

Furthermore, CKD has been identified as a significant marker for cognitive impairment (11, 12). Although the underlying pathophysiological mechanisms of cognitive dysfunction in CKD remain largely unknown, several candidate mechanisms have been suggested apart from cardiovascular risk factors, which are the same for kidney and brain. In addition, nephrogenic risk factors as uremic toxins, oxidative stress, anaemia, albuminuria and inflammation can lead to cognitive impairment (10, 13). Also in end-stage kidney disease determinants related to dialysis, such as intradialytic hypotension or cerebral oedema can lead to cerebral hypoperfusion or neuronal damage (10, 12).

The prevalence and magnitude of the association of cognitive impairment across different CKD stages is still subject of debate. Whereas the relationship has been firmly established in patients with end-stage kidney disease, the association of mild to modest impaired kidney function with cognitive function remains questionable (14).

We hypothesized that with decreasing kidney function, cognitive function declines faster over time. Therefore, the aim of this study was to investigate the association of the different stages of CKD and cognitive decline and functional status in a high-risk population of older patients and furthermore, to investigate this association in patients with a history of vascular disease, or patients with only vascular risk factors, using data from the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER).

METHODS

The study population comes from PROSPER, a double-blind, randomized, placebo-controlled trial, designed to investigate the relationship between statin treatment and the risk of cardiovascular and cerebrovascular events. In summary, 5804 older participants (70-82 years) were enrolled in Ireland, Scotland and The Netherlands. Patients were included if they had a history of, or an increased risk for vascular disease and a baseline cholesterol between 4.0 – 9.0 mmol/l. A history of vascular disease included stroke, transient ischemic attack, myocardial infarction, arterial surgery, or amputation for vascular disease less than 6 months before study entry. Increased risk for vascular disease included current smoking, hypertension, known diabetes mellitus or fasting blood glucose levels over 7 mmol/L. Mean follow-up was 3.2 years. Detailed description of this population, including all in- and exclusion criteria, has been published previously (15). The study was approved by the institutional ethics review boards of centres of Cork University (Ireland), Glasgow University (Scotland) and Leiden University Medical Center (the Netherlands). Consent has been obtained from each patient or subject after full explanation of the purpose and nature of all procedures used.

KIDNEY FUNCTION

At baseline creatinine levels were measured. Individuals with baseline creatinine levels over 200 µmol/l were excluded. GFR was estimated using the Modification of Diet in Renal Disease equation: eGFR = 186 x Scr(-1.154) x age(-0.203) x 0.742 [if female], where Scr denotes serum creatinine level in mg/dl. It is assumed that all participants were of Northern European descent. (16) Statistical analysis of baseline characteristics was based on a comparison among subgroups of clinical stages of kidney failure based on eGFR, namely <30 (CKD stage 4), 30-45 (CKD stage 3b), 45-60 (CKD stage 3a) and ≥60 ml/min/1.73m2 (CKD stage 1-2) (17).

COGNITIVE FUNCTION AND FUNCTIONAL STATUS

Detailed description of the cognitive function and functional status measurements in PROSPER has been published previously (18, 19). Measurement of cognitive function and functional status were prespecified endpoints. One of the exclusion criteria was a poor cognitive function at baseline, measured by the Mini-Mental State Examination (MMSE). We used the generally used cut-off point of 24 (of 30) points.

Outcome variables were derived from three other widely used neuropsychological performance tests in different cognitive domains and two functional status tests, as decline in functional status is largely driven by cognitive impairment. First, executive functioning was assessed using the Stroop Colour Word Test (Stroop) and the Letter-Digit Coding Test (LDT). Selective attention was assessed using Stroop, which consist of three parts, namely colour names, coloured patches and colour names printed in incongruously coloured ink. The time in seconds required to read the names or to identify colours is recorded (20). We used an abbreviated version of the test with 40 elements (21). Processing speed of general information was assessed using the LDT, which is a modification of the procedurally identical Symbol-Digits Modalities Test, which has an outcome variable of total number of correct entries completed in 60 seconds (22, 23). Second, memory was assessed using the Picture-Word Learning Test. This verbal learning test is derived from the Groningen Fifteen Words Test. Outcomes are measured in three different trials and divided in recall (PLTi) and delayed recall (PLTd) after 20 minutes (24-26). Functional status was assessed using two questionnaires, namely the Barthel Index and the Lawton Instrumental Activities of Daily Living Scale (IADL) (27, 28). Barthel measures performance in basic activities of daily living and consists of 10 items. Barthel scores range from 0 to 20, with lower scores indicating more dependence. IADL evaluates more complex instrumental activities and includes 7 items. IADL scores range from 0 to 14, with again lower scores indicating more dependence. Cognitive function and functional status were tested at several time points, namely at baseline and after 9, 18 and 30 months, and at the end of the study.

STATISTICAL ANALYSIS

All categorical data are presented as numbers with percentages and were compared using the chi-square test. All continuous data are presented as mean ± standard deviation or median with interquartile range and were compared using an one-way ANOVA or Kruskal-Wallis test.

Means of cognitive function test scores and functional status scores at baseline were compared between the different CKD stages using a one-way ANOVA test. Furthermore, severe CKD stage 4 was compared to stage 1-2 (no CKD) using an independent t-test. For follow-up linear mixed models for repeated measurements were used, including the interim measures taken between the baseline and the final assessment. This last measurement varies between all participants between 36 and 48 months. Therefore all statistical analyses are performed with their individually varying time point, but we graphically display the results for the mean of these time points at 42 months. To preclude possible learning effects the pre-randomized measurement was discarded in all analyses. From the first PROSPER article about cognitive function, we know that all cognitive tests show a significant decline over time, confirming their adequate sensitivity to pick up deterioration of cognitive function in old age.(19) In the mixed model analyses CKD stages, time and CKD stage * time were included. Furthermore, to correct for confounders multiple prespecified fixed effects were included, namely sex, age, educational status, country, statin treatment, vascular confounders including history of vascular disease, hypertension, diabetes and current smoking, and other known confounders including objective measures as blood pressure, BMI, baseline lipids, haemoglobin, urea, NT-proBNP and troponin, all assessed as previously reported (15). A log-transformation will be used for the variables with skewed distribution. Analyses will be repeated stratified for subjects with or without a history of vascular disease. The data were analysed using IBM SPSS Statistics version 23. P-values lower than 0.05 were considered statistically significant.

RESULTS

Of the 5804 randomised patients, baseline creatinine levels were available for 5796 participants (99.9%). Participants had a mean age of 75.3 years and 48.3% were male. Mean eGFR was 60.0 ± 14.6 ml/min/1.73m2. In the prespecified stages of CKD, based on eGFR, 19 subjects (0.33%) had a baseline eGFR of <30 ml/min/1.73m2 (CKD stage 4), 786 (13.6%) an eGFR between 30-45 ml/min/1.73m2 (stage 3b), 2306 (39.8%) an eGFR between 45-60 ml/min/1.73m2 (stage 3a), and 2685 (46.3%) an eGFR ≥60ml/min/1.73m2 (stage 1-2).

Baseline characteristics are shown in Table 1, over strata of CKD stage and overall. Lower eGFR was significantly associated with older age, female sex, less years of education, more history of hypertension, vascular disease and medication use, and less history of diabetes and current smoking. Furthermore, lower eGFR was associated with an unfavorable lipid profile, higher levels of CRP, urea, NT-proBNP and troponin-T and lower levels of hemoglobin.

A higher score for Stroop or a lower score for the other five tests indicate a worse cognitive function or functional status. Non-adjusted baseline cognition and functional status scores are shown in Table 2, over strata of CKD stage and overall. The participants with the most impaired kidney function (CKD stage 4) had the worst cognitive function and functional status in all domains at baseline. When comparing the CKD stage 4 versus stage 1-2 mean scores (± SE) were 74.2 ± 6.7 vs 69.3 ± 0.6 for Stroop (p=0.514), 21.9 ± 1.2 vs 21.9 ± 0.2 for LDT (p=0.951), 8.6 ± 4.7 versus 9.2 ± 0.03 for PLTi (p=0.146), 9.8 ± 0.8 vs 10.1 ± 0.04 for PLTd (p=0.600), 19.7 ± 0.13 vs 19.8 ± 0.01 for Barthel (p=0.792) and 13.3 ± 0.31 vs 13.6 ± 0.02 for IADL (p=0.172).

Mean follow-up was 42 months with a range of 36-48 months. Figure 1 shows the effect of CKD stage on the different cognitive function and functional status tests over time. The mean cognition and functional status scores are adjusted for all prespecified confounders. In all cognitive function tests, a trend was seen for faster cognitive decline over time in CKD stage 4 compared to the other CKD groups. No differences were seen for functional status.

Table 1. Baseline Characteristics Split by Baseline CKD stages and Overall						
	CKD stages based on eGFR (ml/min/1.73m2)					
	Total n = 5796	**Stage 4 n = 19**	**Stage 3b n = 786**	**Stage 3a n = 2306**	**Stage 1 and 2 n = 2685**	**p-value***
Age (years)	75.3 ± 3.3	77.4 ± 3.1	76.8 ± 3.4	75.3 ± 3.3	74.9 ± 3.2	<0.001
Male gender	2799 (48.3)	0	223 (28.4)	1029 (44.6)	1547 (57.6)	<0.001
Education (years)	15.1 ± 2.0	15.1 ± 1.8	14.8 ± 1.6	15.0 ± 1.8	15.4 ± 2.3	<0.001
History of hypertension	3585 (61.9)	18 (94.7)	568 (72.3)	1471 (63.8)	1528 (56.9)	<0.001
History of diabetes	622 (10.7)	1 (5.3)	68 (8.7)	221 (9.6)	332 (12.4)	0.002
History of vascular disease	2561 (44.2)	7 (36.8)	400 (50.9)	1062 (46.1)	1092 (40.7)	<0.001
History of stroke or TIA	647 (11.2)	1 (5.3)	86 (10.9)	271 (11.8)	289 (10.8)	0.584
Current smoker	1558 (26.9)	4 (21.1)	131 (16.7)	558 (24.2)	865 (32.2)	<0.001
Number of medications	3.6 ± 2.3	5.2 ± 2.8	4.5 ± 2.3	3.7 ± 2.3	3.2 ± 2.2	<0.001
SBP (mmHg)	154.7 ± 21.8	156.1 ± 27.3	154.8 ± 22.2	154.6 ± 21.5	154.6 ± 22.0	0.728
DBP (mmHg)	83.8 ± 11.4	82.5 ± 11.2	83.1 ± 10.7	83.6 ± 11.4	84.1 ± 11.6	<0.001
BMI (kg/m2)	26.8 ± 4.2	26.6 ± 3.8	27.6 ± 4.4	26.8 ± 4.2	26.6 ± 4.1	<0.001
LDL-C (mmol/l)	3.79 ± 0.80	4.06 ± 0.93	3.92 ± 0.81	3.85 ± 0.81	3.71 ± 0.78	<0.001
HDL-C (mmol/l)	1.28 ± 0.35	1.29 ± 0.38	1.25 ± 0.34	1.28 ± 0.35	1.29 ± 0.35	<0.001
Total cholesterol (mmol/l)	5.68 ± 0.91	6.02 ± 1.03	5.88 ± 0.94	5.74 ± 0.87	5.57 ± 0.87	<0.001
Triglyceride (mmol/l)	1.54 ± 0.70	1.85 ± 0.74	1.76 ± 0.77	1.57 ± 0.69	1.46 ± 0.68	<0.001
Glucose (mmol/l)	5.5 ± 1.4	5.13 ± 0.94	5.56 ± 1.40	5.50 ± 1.31	5.4 ± 1.6	<0.001
CRP at 6 months (mg/l)	2.3 [1.1-4.5]	4.2 [2.1-10.1]	2.8 [1.4-5.7]	2.3 [1.1-4.6]	2.1 [1.0-4.1]	<0.001
Urea (mg/dL)	6.3 ± 1.8	10.5 ± 1.8	7.9 ± 2.3	6.4 ± 1.6	5.8 ± 1.5	<0.001
Hb (mmol/L)	8.7 ± 0.8	8.4 ± 0.7	8.4 ± 0.8	8.7 ± 0.8	8.8 ± 0.8	<0.001
NT-proBNP at 6 months (ng/l)	148.7 [79.5-289.3]	417.0 [283.9-789.0]	230.2[122.1-489.1]	151.5 [81.4-293.9]	127.6 [70.0-238.8]	<0.001
Troponin at 6 months (µg/l)	0.010 ± 0.036	0.016 ± 0.010	0.013 ± 0.017	0.010 ± 0.042	0.010 ± 0.036	<0.001

All values are presented as n (%), mean ± SD or median [IQR]. * p-values of categorical data were assessed using the chi-square test and p-values of the continuous data were assessed using an one-way ANOVA test or a Kruskal-Wallis test. Abbreviations: BMI, body mass index; CKD, chronic kidney disease; CRP, C-reactive protein; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NT-proBNP, N-terminal pro b-type natriuretic peptide; SBP, systolic blood pressure; SD, standard deviation; TIA, transient ischemic attack.

Table 2. Cognitive Function at Baseline Over Strata of CKD stages and Overall						
	CKD stages based on eGFR (ml/min/1.73m2)					
	Total n = 5796	**Stage 4 n = 19**	**Stage 3b n = 786**	**Stage 3a n = 2306**	**Stage 1 and 2 n = 2685**	**p-value***
Stroop-Colour-Word Test	66.5 ± 0.4	74.2 ± 6.7	66.4 ± 0.9	63.2 ± 0.5	69.3 ± 0.6	<0.001
Letter-Digit Coding Test	23.1 ± 0.1	21.9 ± 1.2	23.3 ± 0.3	24.4 ± 0.2	21.9 ± 0.2	<0.001
Picture-Word Learning Test – immediate	9.3 ± 0.03	8.6 ± 4.7	9.3 ± 0.07	9.4 ± 0.04	9.2 ± 0.03	0.001
Picture-Word Learning Test – delayed	10.1 ± 0.04	9.8 ± 0.8	10.0 ± 0.1	10.2 ± 0.05	10.1 ± 0.04	0.197
The Barthel index	19.8 ± 0.01	19.7 ± 0.13	19.7 ± 0.03	19.8 ± 0.01	19.8 ± 0.01	0.004
Instrumental Activities of Daily Living	13.6 ± 0.01	13.3 ± 0.31	13.5 ± 0.04	13.7 ± 0.02	13.6 ± 0.02	0.008

All values are presented as mean ± SE. * p-values of differences between groups were assessed using an one-way ANOVA test. Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; SE, standard error.

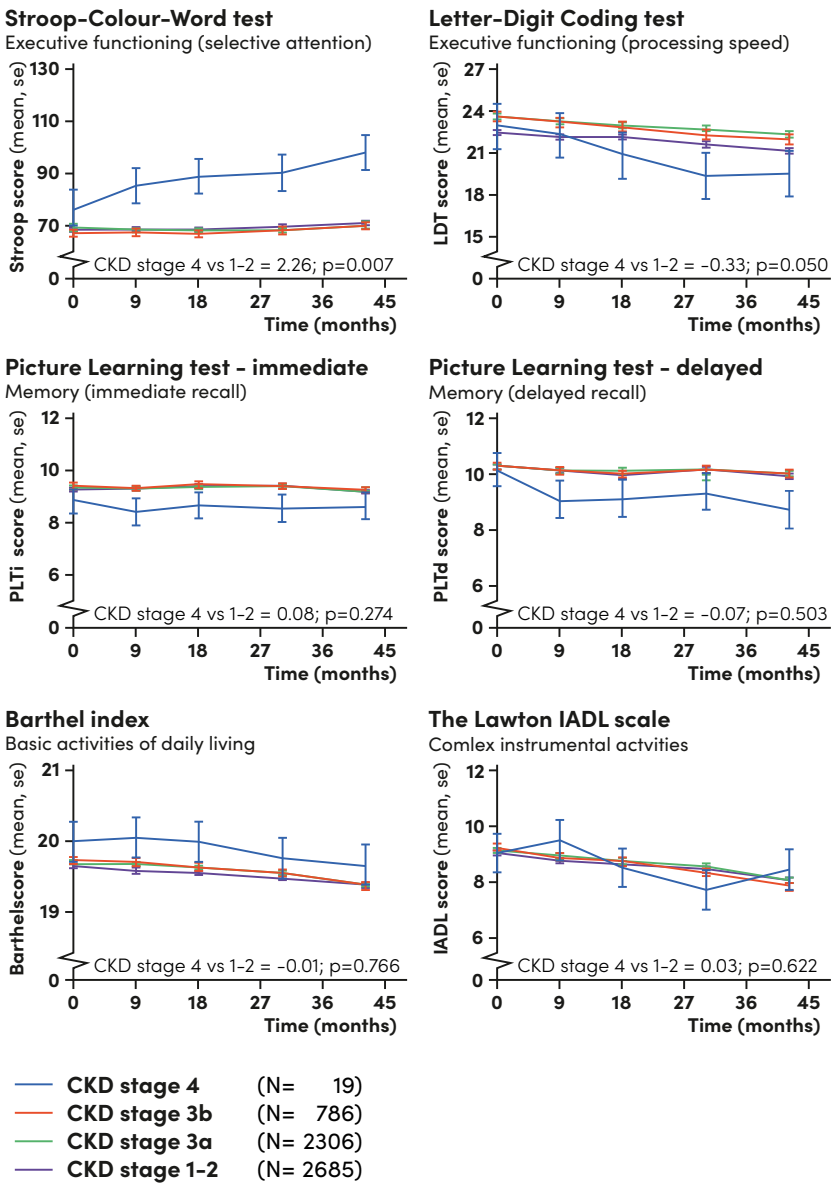
When comparing the most severe CKD stage 4 (<30 ml/min/1.73m2) versus stage 1-2 (>60ml/min/1.73m2) over time the estimates (95% confidence interval (CI)) are 2.26 (0.63 – 3.88; p=0.007) for Stroop, -0.33 (-0.66 – 0.00; p=0.050) for LDT, 0.08 (-0.06 – 0.21; p=0.274) for PLTi, -0.07 (-0.27 – 0.13; p=0.503) for PLTd, -0.01 (-0.11 – 0.08; p=0.766) for Barthel and 0.03 (-0.09 – 0.15; 0.622) for IADL, see also Figure 1. Participants with mild to modest CKD stage 3 compared to CKD stage 1-2 had no worse cognitive function, which is also seen in Figure 1, displaying practically parallel lines for CKD stages 3 to 1.

STRATIFICATION FOR HISTORY OF VASCULAR DISEASE

In Figure 2 the analysis was stratified according to the history of vascular disease. The trend of faster cognitive decline over time in CKD stage 4 compared to the other CKD groups was most prevalent in patients with a history of vascular disease compared to patients without a history of vascular disease, see Figure 2 and Table 3. No differences were found for functional status. Estimates (95% CI) of CKD stage 4 versus stage 1-2 in patients with a history of vascular disease are 6.52 (3.94 – 9.10; p<0.0001) for Stroop, -1.00 (-1.62 – -0.37; p=0.002) for LDT, 0.16 (-0.08 – 0.40; p=0.180) for PLTi, -0.02 (-0.37 – 0.34; p=0.930) for PLTd, 0.01 (-0.16 – 0.18; p=0.940) for Barthel

FIGURE 1. EFFECT OF CKD STAGE ON COGNITIVE FUNCTION AND FUNCTIONAL STATUS OVER TIME

* Means were assessed using linear mixed models adjusted for prespecified variables including sex, age, educational status, country, statin treatment and multiple other known vascular confounders. P-values represent the statistical significance of the difference in cognitive test score changes over time between CKD stage 4 (eGFR <30ml/min/1.73m2) versus CKD stage 1-2 (eGFR>60ml/min/1.73m2). Abbreviations: Barthel, the Barthel index; eGFR, estimated glomerular filtration rate; IADL, Instrumental Activities of Daily Living; LDT, Letter-Digit Coding Test; PLTd, Picture-Word Learning Test – delayed; PLTi, Picture-Word Learning Test – immediate; Stroop, Stroop-Colour-Word Test.



and 0.06 (-0.15 – 0.28; p=0.562) for IADL. Estimates (95% CI) of CKD stage 4 versus stage 1-2 in patients without a history of vascular disease are -0.11 (-2.21 – 1.99; p=0.919) for Stroop, -0.08 (-0.47 – 0.32; p=0.694) for LDT, 0.03 (-0.13 – 0.20; p=0.695) for PLTi, -0.09 (-0.33 – 0.15; p=0.485) for PLTd, -0.02 (-0.13 – 0.18; p=0.642) for Barthel and 0.01 (-0.12 – 0.15; p=0.868) for IADL, see also Table 3. Corresponding p-values for interaction of vascular disease and cognitive decline or functional decline over time were 0.016 for Stroop, 0.115 for LDT, 0.529 for PLTi, 0.123 for PLTd, 0.737 for Barthel and 0.064 for IADL.

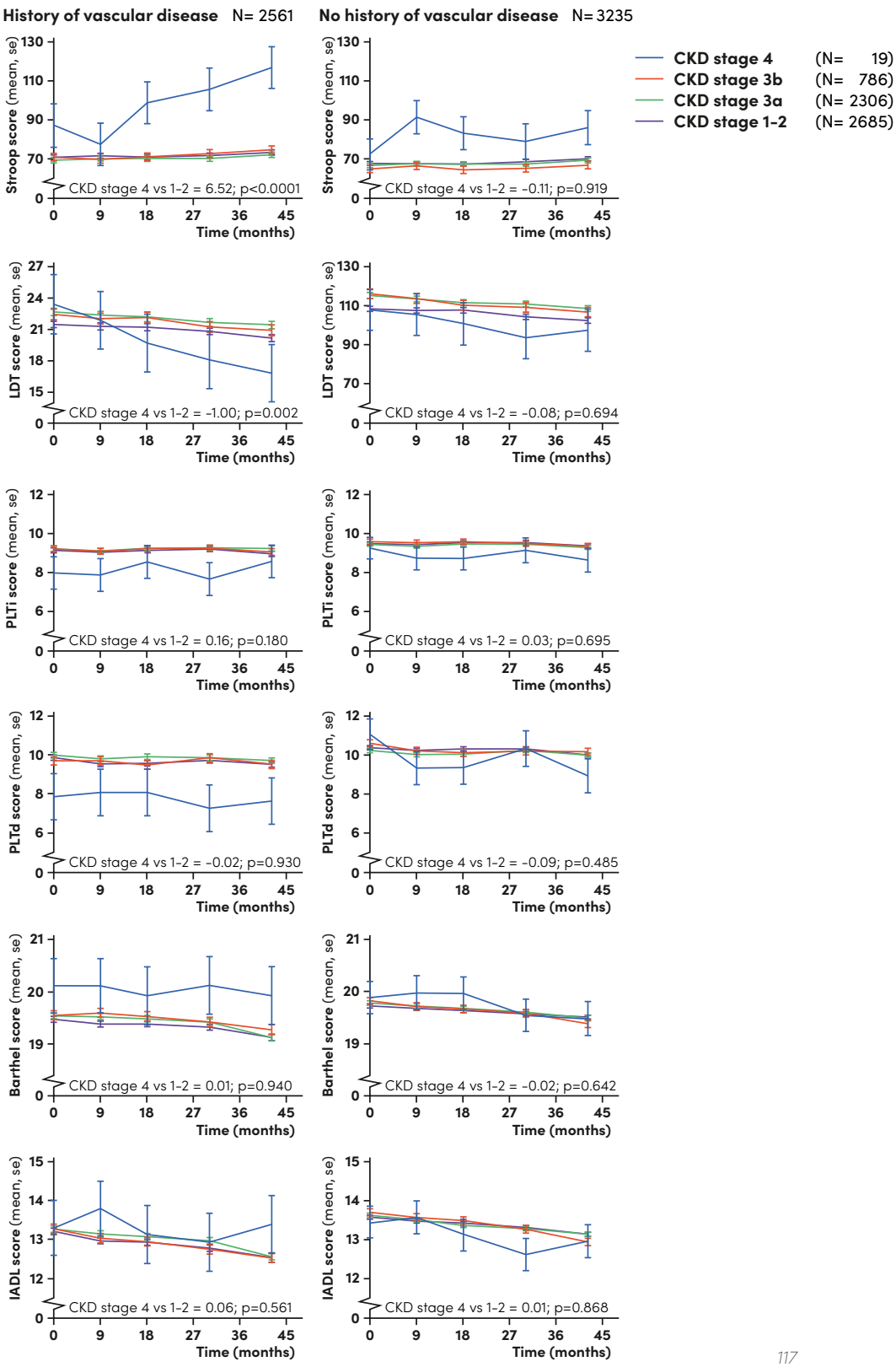
Table 3. Effect of CKD stage 4 versus stage 1-2 on cognitive function over time

	Total		History of vascular disease		No history of vascular disease		Interaction
	Beta (95%CI)	**p-value**	**Beta (95%CI)**	**p-value**	**Beta (95%CI)**	**p-value**	**p-value**
Stroop-Colour-Word Test	2.26 (0.63 – 3.88)	0.007	6.52 (3.94 – 9.10)	<0.0001	-0.11 (-2.21 – 1.99)	0.919	0.016
Letter-Digit Coding Test	-0.33 (-0.66 – 0.00)	0.050	-1.00 (-1.62 – -0.37)	0.002	-0.08 (-0.47 – 0.32)	0.694	0.115
Picture-Word Learning Test – immediate	0.08 (-0.06 – 0.21)	0.274	0.16 (-0.08 – 0.40)	0.180	0.03 (-0.13 – 0.20)	0.695	0.529
Picture-Word Learning Test – delayed	-0.07 (-0.27 – 0.13)	0.503	-0.02 (-0.37 – 0.34)	0.930	-0.09 (-0.33 – 0.15)	0.485	0.123
The Barthel index	-0.01 (-0.11 – 0.08)	0.766	0.01 (-0.16 – 0.18)	0.940	-0.02 (-0.13 – 0.08)	0.642	0.737
Instrumental Activities of Daily Living	0.03 (-0.09 – 0.15)	0.622	0.06 (-0.15 – 0.28)	0.561	0.01 (-0.12 – 0.15)	0.868	0.064

* p-values of differences in cognitive test score changes over time were assessed between CKD stage 4 (eGFR <30ml/min/1.73m^2) versus CKD stage 1-2 (eGFR>60ml/min/1.73m^2) using linear mixed models adjusted for prespecified variables including sex, age, educational status, country, statin treatment and multiple other known vascular confounders.

FIGURE 2. EFFECT OF CKD STAGE ON COGNITIVE FUNCTION AND FUNCTIONAL STATUS OVER TIME STRATIFIED FOR HISTORY OF VASCULAR DISEASE

* Means were assessed using linear mixed models adjusted for prespecified variables including sex, age, educational status, country, statin treatment and multiple other known vascular confounders. P-values represent the statistical significance of the difference in cognitive test score changes over time between CKD stage 4 (eGFR <30ml/min/1.73m2) versus CKD stage 1-2 (eGFR>60ml/min/1.73m2). Abbreviations: Barthel, the Barthel index; eGFR, estimated glomerular filtration rate; IADL, Instrumental Activities of Daily Living; LDT, Letter-Digit Coding Test; PLTd, Picture-Word Learning Test – delayed; PLTi, Picture-Word Learning Test – immediate; Stroop, Stroop-Colour-Word Test.


History of vascular disease N= 2561
No history of vascular disease N= 3235
CKD stage 4 (N= 19)
CKD stage 3b (N= 786)
CKD stage 3a (N= 2306)
CKD stage 1-2 (N= 2685)
Stroop score (mean, se)
CKD stage 4 vs 1-2 = 6.52; p<0.0001
CKD stage 4 vs 1-2 = -0.11; p=0.919
LDT score (mean, se)
CKD stage 4 vs 1-2 = -1.00; p=0.002
CKD stage 4 vs 1-2 = -0.08; p=0.694
PLTi score (mean, se)
CKD stage 4 vs 1-2 = 0.16; p=0.180
CKD stage 4 vs 1-2 = 0.03; p=0.695
PLTd score (mean, se)
CKD stage 4 vs 1-2 = -0.02; p=0.930
CKD stage 4 vs 1-2 = -0.09; p=0.485
Barthel score (mean, se)
CKD stage 4 vs 1-2 = 0.01; p=0.940
CKD stage 4 vs 1-2 = -0.02; p=0.642
IADL score (mean, se)
CKD stage 4 vs 1-2 = 0.06; p=0.561
CKD stage 4 vs 1-2 = 0.01; p=0.868
Time (months)

DISCUSSION

In this large cohort of older people with an increased risk for, or a history of, vascular disease, only severe kidney disease (CKD stage 4), but not mild to modest kidney disease (CKD stage 3a and b), was associated with cognitive impairment at baseline and cognitive decline over time. The association of severe kidney disease with cognitive impairment and decline over time was more outspoken in patients with a history of vascular disease. No association was found between kidney function and functional status.

Severe kidney failure as independent risk factor for cognitive dysfunction, in combination with lack of effect of the association of mild to modest kidney failure, has been found in previous research (29, 30). This might be due to the fact that nephrogenic risk factors only start playing a role in more advanced CKD (13). Patients that do have cognitive dysfunction in earlier stages of CKD might have worse cognitive function mainly related to vascular damage. Because it is known from previous studies that a history of vascular disease or risk factors as hypertension can lead to microvascular damage and small vessel disease in the brain, CKD can ultimately lead to cognitive dysfunction via this pathway (8, 9). Furthermore, impaired cardiac function, measured by NT-proBNP or Troponin, is associated with cognitive dysfunction independently from other cardiovascular risk factors (31-33). As expected, also in this study, a potential predictor for worse cognitive function at baseline in multivariate analysis was a higher vascular burden, as shown before in this cohort (19, 34).

The contribution of kidney failure to cognitive dysfunction on top of a high vascular burden versus no vascular burden remained hitherto mostly unknown. In general, it is difficult to make a distinction between cognitive decline in CKD patients with or without vascular risk factors, because many CKD patients have a vascular aetiology of their kidney failure, for instance due to hypertension or diabetes mellitus. In this cohort 44.2% of participants were included with a history of vascular disease, 61.9% had hypertension and 10.7% had diabetes. Therefore, we used stratification to distinguish patients with proven vascular disease from patients with only vascular risk factors. Cognitive function declined faster over time in patients with CKD stage 4

(<30ml/min/1.73m2) especially together with a history of vascular disease. In a comparable subanalysis, Seidel et al. also showed increasing prevalence of depression and cognitive dysfunction in the CVD group (defined as coronary heart disease or myocardial infarction) compared to no CVD in the higher CKD-stages compared to controls (35).

As previous research has been shown, decline in kidney function associates with a higher risk of stroke, partly due to a higher incidence in atrial fibrillation (36, 37). Approximately 10% of patients develop new-onset dementia after first stroke, and even more after recurrent stroke (38, 39). According to the U.S. Renal Data System prevalence of atrial fibrillation in patients with or without CKD is 24.0% versus 9.5% and prevalence of CVA or TIA is 19.4% versus 7.7%. Prevalence of both diseases increases with increasing stage of kidney failure (40). Therefore, a history of stroke or TIA, which is one of the inclusion criteria in this study, could be a confounder considering the influence on cognitive impairment. In the total group 11.2% had a history of stroke or TIA, which did not differ significantly between groups (p=0.584); 1 (5.3%), 86 (10.9%), 271 (11.8%) and 289 (10.8%) in the CKD stage 4, stage 3b, stage 3a and stage 1-2 respectively. Therefore, the worse cognitive function in CKD stage 4, appears unlikely to be explained by the prevalence of stroke or TIA.

Established previously in PROSPER, impaired kidney function was independently associated with increased risk of all-cause mortality, fatal vascular events and with composite fatal and nonfatal coronary and heart failure outcomes. This effect was most prevalent in eGFR <40ml/min/1.73m2. Notably, the PROSPER investigators did not find an association between an impaired eGFR and a higher risk of stroke (41). However, not only impaired kidney function increases the risk of mortality, but it is also known, that next to the importance to prevent cognitive decline for better quality of life of patients, a worse cognitive function associates with higher morbidity and mortality rates in older patients reaching end-stage kidney disease (42-44).

The difference in cognitive decline for CKD stage 4 and CKD stage 1-2 might

be partially explained by the sex difference between the groups. However, a sex-difference in cognitive decline is debated. A review showed that sex did not determine the rate of cognitive decline between ages of 60-80 years, an age that is comparable to our cohort that included patients between 70-82 years. (45). In our cohort univariate male sex correlated with worse cognitive function, however multivariate analysis showed still worse memory tests compared to females, but better cognitive function in executive function (data not shown). These results are comparable with a study by Proust-Lima et al, whereby older women showed better outcomes in memory tests, whereas men had a better visuospatial ability (46). Therefore, the lack of males in the CKD stage 4 cannot explain the worse outcome in that category.

LIMITATIONS

Our study has several limitations. First of all, our population was selected for a specific clinical trial and do not represent the general population.

Another limitation is that we were restricted to eGFR in our measures of kidney function, other predictors as albuminuria or cystatin C are not available. A recent systematic review and meta-analysis of prospective, population-based studies, showed that albuminuria was most consistent as marker of CKD in the association with cognitive decline with an odds ratio of 1.35 (95% CI 1.06-1.73). An eGFR <60ml/min/1.73m2 showed no significant association with cognitive dysfunction, consistent with our results, with an odds ratio of 1.28 (95% CI 0.99-1.65) (47). In our cohort the same analyses with creatinine instead of eGFR yielded similar results that were inversely proportional as expected (data not shown). Furthermore, no distinction can be made between acute or chronic kidney failure. Creatinine was only measured at baseline, so no follow-up measurements are available, therefore it is not known whether patients with a normal kidney function at baseline deteriorated or patients with an impaired kidney function improved over time. In addition, it would be interesting to see whether patients with a faster cognitive decline also have a faster decline in kidney function, considering that both the brain and kidney share similar hemodynamic characteristics, since both are low resistance

end organs exposed to high-volume blood flow and therefore predisposed for vascular damage (4, 48).

Whereas one of the exclusion criteria was a high creatinine at baseline and the groups of eGFR were divided based on clinical stages, the group containing CKD stage 4 was relatively small (after stratification only seven participants with and twelve participants without a history of vascular disease). Therefore, although the results of CKD stage 4 appear clinically relevant, especially in the cognitive function analysis over time (Figure 1 and 2), significance remained partly absent and therefore results needs to be interpreted with caution. Furthermore, although the results of the cognitive function tests show the same trend in both cognitive domains, the effect on executive functioning seems statistically larger than the effect on memory. Possible explanation could be that executive functioning seems most often sooner affected than memory, partly due to more sensitive executive function tests, and memory would be more affected over a longer follow-up period.

CONCLUSIONS

In this study, only severe kidney disease (CKD stage 4) seem to be associated with cognitive impairment at baseline and cognitive decline over time. A mild to modest impaired kidney function appeared not to be independently associated with cognitive decline during a 3.2 year follow-up period. For better understanding of the mechanisms involved in cognitive decline in CKD, especially end-stage kidney disease, additional studies are necessary, which may contribute to interventions for prevention. Combined (metabolic) parameters of kidney function, beside eGFR, should be taken into account, as well as albuminuria. An observational study is currently under way to gain insight in the potential different mechanisms of cognitive decline in older patients with end-stage kidney disease to identify modifiable risk factors (49). Furthermore, it would be of great interest to determine whether newer agents which seem to meaningfully slow renal function decline such as SGLT2 inhibitors, also slow decline in cognitive function, although we accept such agents may also lessens risks in cardiovascular disease (50).

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6

CHAPTER 6.

KIDNEY FUNCTION, SUBCLINICAL THYROID DISEASE AND CARDIOVASCULAR OUTCOMES IN OLDER PATIENTS WITH VASCULAR DISEASE

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ABSTRACT

INTRODUCTION

Thyroid hormones have been implicated to play a role in cardiovascular disease, along with studies linking thyroid hormone to kidney function. The aim of this study is to investigate whether kidney function modifies the association of subclinical thyroid dysfunction and the risk of cardiovascular outcomes.

METHODS

In total, 5804 patients were included in the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER). For the current analysis, 426 were excluded because of overt thyroid disease at baseline or 6 months, 266 because of inconsistent thyroid function at baseline and 6 months, 294 because of medication use that could influence thyroid function, and 16 because of missing kidney or thyroid values. Participants with normal fT4 were classified, based on TSH both at inclusion and 6 months, into 3 groups: subclinical hypothyroidism (TSH>4.5 mIU/L); euthyroidism (TSH=0.45–4.5 mIU/L); and subclinical hyperthyroidism (TSH<0.45 mIU/L). Strata of kidney function were made based on estimated glomerular filtration rate into 3 clinically relevant groups: <45; 45–60; and >60ml/min/1.73m2. The primary endpoint consists of death from coronary heart disease, non-fatal myocardial infarction and (non)fatal stroke.

RESULTS

Mean age was 75.3 years and 49.0% patients were male. Mean follow-up was 3.2 years. Of all participants 109 subjects (2.2%) had subclinical hypothyroidism, 4573 (94.0%) had euthyroidism, and 182 (3.7%) subclinical hyperthyroidism. For patients with subclinical hypothyroidism, euthyroidism and subclinical hyperthyroidism, primary outcome occurred in 9 (8.3%), 712 (15.6%) and 23 (12.6%) patients, respectively. No statistically significant

relationship was found between subclinical thyroid dysfunction and primary endpoint with adjusted hazard ratios of 0.51 (0.24-1.07) comparing subclinical hyperthyroidism and 0.90 (0.58-1.39) comparing subclinical hypothyroidism with euthyroidism. Neither was this relationship present in any of the strata of kidney function, nor did kidney function interact with subclinical thyroid dysfunction in the association with primary endpoint (p-interaction=0.602 for subclinical hyperthyroidism and 0.388 for subclinical hypothyroidism).

CONCLUSIONS

In this secondary analysis from PROSPER, we found no evidence that the potential association between thyroid hormones and cardiovascular disease is modified by kidney function in older patients with subclinical thyroid dysfunction.

INTRODUCTION

Subclinical hyperthyroidism has been linked to atrial fibrillation and coronary artery calcification, whereas subclinical hypothyroidism has been associated with hypercholesterolemia and atherosclerosis (1,2). Despite these observations, data from large prospective cohorts regarding thyroid function and cardiovascular events and mortality are conflicting (3,4). The discrepancies between the study results may be explained by a small number of participants with subclinical thyroid disease and variations in the definitions of subclinical thyroid disease among different studies. In an attempt to tackle these limitations, recent large individual participant data meta-analyses were performed and associations were found between increasing thyroid stimulating hormone (TSH) levels and increased risk of stroke, high circulating free thyroxin (fT4) and increased risk of atrial fibrillation, and subclinical hypo- and hyperthyroidism and increased risk of coronary heart disease and mortality (5-7).

Chronic kidney disease (CKD) is accompanied by a substantial cardiovascular disease risk (8,9). Associations of thyroid hormones and CKD have also been described. Multiple cross-sectional studies have linked lower thyroid function to a lower estimated glomerular filtration rate (eGFR) and increased prevalence of CKD (10-12). Longitudinal studies have presented conflicting results, linking kidney function decline to low (13) or high thyroid function (14). Whereas a recent individual participant data analysis found no role for thyroid hormones in renal function decline, it does not exclude reverse causality, indicating that previously found cross-sectional associations between kidney and thyroid may be explained by kidney dysfunction causing thyroid hormone changes (15). Whether or not kidney function causally alter thyroid hormones, several studies have shown an increased prevalence of subclinical thyroid disease and nonthyroidal illness in patients with CKD (16,17), and this may be associated with higher mortality (18).

We hypothesize that there might be a role for kidney function in the relation between thyroid function and clinical cardiovascular outcomes, which may partly explain the conflicting results from large observational studies.

Therefore, the aim of this study is to investigate whether kidney function modifies the association of subclinical thyroid dysfunction and the risk of cardiovascular outcomes in older patients.

METHODS

All subjects were participants of the 'PROspective study of Pravastatin in the Elderly at Risk' (PROSPER), a double-blind, randomized, placebo-controlled trial, designed to investigate the relationship between statin treatment and the risk of cardiovascular and cerebrovascular events. In summary, 5804 older participants (70-82 years) were enrolled in Ireland, Scotland and The Netherlands. Patients were included if they had a history of, or an increased risk for, vascular disease and a baseline cholesterol between 4.0 – 9.0 mmol/l. A history of vascular disease included stroke, transient ischemic attack, myocardial infarction, arterial surgery, or amputation for vascular disease less than six months before study entry. Increased risk for vascular disease included current smoking, hypertension, diabetes mellitus or fasting blood glucose levels over 7 mmol/L. Detailed description of this population, including all in- and exclusion criteria, has been published previously (19). The study was approved by the institutional ethics review boards of centres of Cork University (Ireland), Glasgow University (Scotland) and Leiden University Medical Center (the Netherlands). Consent has been obtained from each patient or subject after full explanation of the purpose and nature of all procedures used.

THYROID FUNCTION

TSH was measured at baseline in all participants and fT4 levels were only determined in case of abnormal TSH levels. Measurements were performed in three accredited laboratory centers (Cork in Ireland, Glasgow in Scotland and Leiden in the Netherlands). TSH and fT4 levels were measured using state-of-the-art immunoassays (third-generation assays with a functional sensitivity < 0.05 mIU/L). For both measurements, estimated inter- and intra-assay coefficients of variation were less than 5%. TSH and fT4 were

determined in all participants after six months of follow-up in available frozen plasma samples, which were stored at the University of Glasgow. The same electrochemiluminescence immunodetection method on a Roche Elecsys 2010 (Burgess Hill, United Kingdom) was used. The limits of detection were < 0.005 mIU/L for TSH and 0.3 pmol/L for free T4. A reference range between 0.45-4.50 mIU/L was used for TSH and 12-22 pmol/L for fT4.

Participants with overt hyperthyroidism or hypothyroidism were excluded from the initial PROSPER trial. In the current study, participants were excluded when biochemical data regarding TSH was missing. In addition, participants using antithyroid medication, thyroxine supplementation, amiodarone, or lithium were also excluded from the final analyses, see Figure 1. Participants were classified into three groups: subclinical hyperthyroidism, euthyroidism, and subclinical hypothyroidism. Subclinical hyperthyroidism was defined at TSH levels < 0.45 mIU/L with normal fT4 levels. Euthyroidism was defined as normal TSH levels (0.45–4.5 mIU/L). Subclinical hypothyroidism was defined as TSH levels > 4.5 mIU/L with normal fT4 levels.

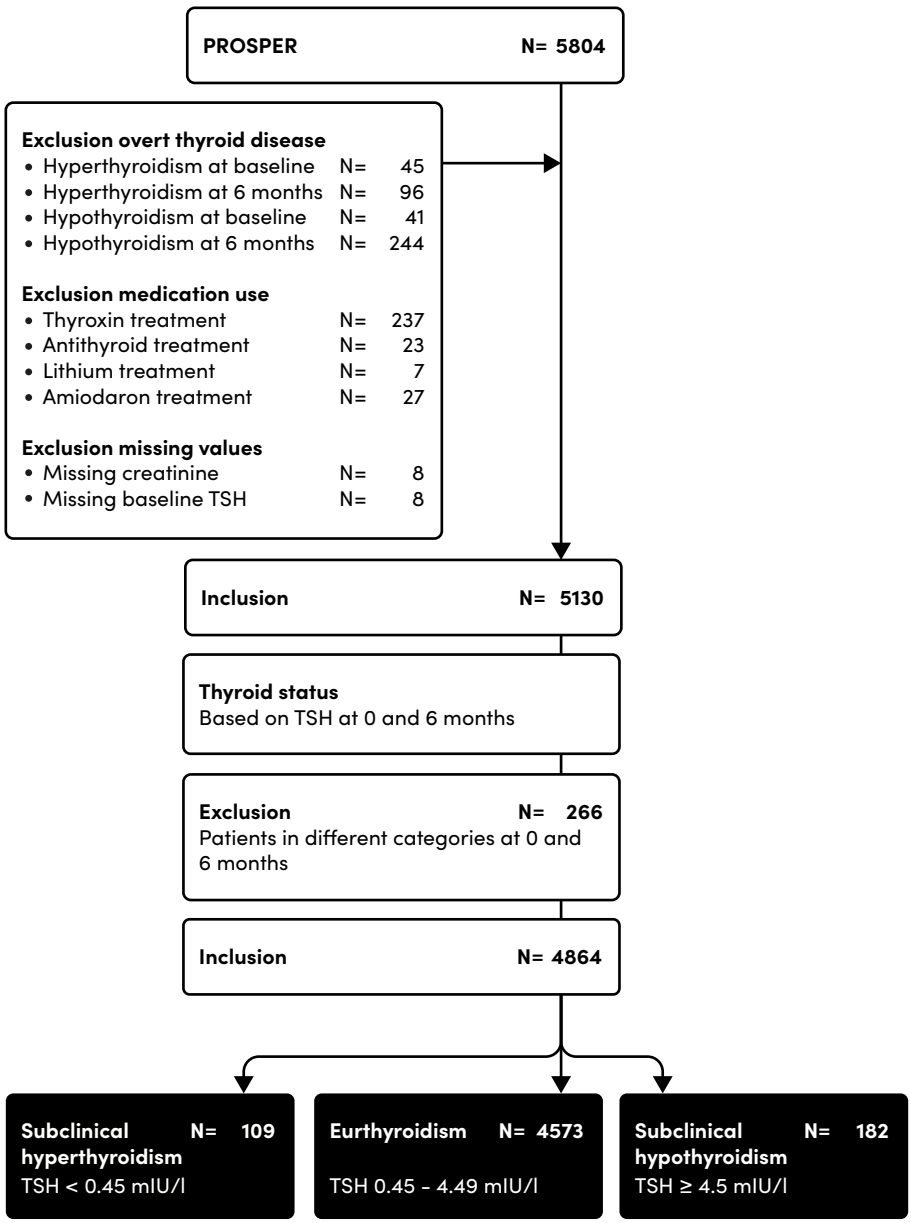
Subclinical thyroid dysfunction may spontaneously resolve over time (20) and euthyroid participants may have developed subclinical thyroid dysfunction during the study follow up period. As only participants with persistent subclinical thyroid dysfunction are of interest in this study, participants with the same thyroid function classification, based on TSH, at both baseline and 6 months of follow up were included in the final study population.

KIDNEY FUNCTION

Serum creatinine levels were measured at central laboratories, one in each of the three participating countries. Participants with baseline creatinine levels over 200 µmol/l were excluded from PROSPER and for the current study, participants were also excluded when biochemical data was missing regarding kidney function. GFR was estimated using the Modification of Diet in Renal Disease equation: eGFR = 186 x Scr(-1.154) x age(-0.203) (x 0.742 [if female]), where Scr denotes serum creatinine level in mg/dL. It is assumed

FIGURE 1. FLOWCHART OF STUDY POPULATION

Inclusion and exclusion criteria of the current substudy of PROSPER (the PROspective Study of Pravastatin in the Elderly at Risk.



that all participants were of Northern European descent (21). Participants were stratified by eGFR into three clinically relevant groups: eGFR <45 ml/min/1.73m2, eGFR 45 – 60 ml/min/1.73m2, and eGFR >60 ml/min/1.73m2 (22).

ENDPOINT

The primary endpoint of PROSPER is a combined end point which consists of death from coronary heart disease, non-fatal myocardial infarction and fatal or non-fatal stroke. All clinical endpoints were adjudicated by an expert study endpoints committee blinded to randomised study medication and using predefined criteria.

STATISTICAL ANALYSIS

Baseline characteristics were reported for the three different classifications of thyroid function. Data are presented as mean ± standard deviation or median [interquartile range] depending on the distribution of data. One-way ANOVA, Kruskal Wallis, or Pearson chi square tests were used to assess differences in baseline characteristics. Cox proportional hazard analysis was performed to assess the risk of the primary endpoint attributed to subclinical thyroid dysfunction within different subgroups of eGFR, including an interaction analysis between thyroid function and kidney function and the risk of primary endpoint. Analyses were adjusted for multiple prespecified variables including country, age, gender, use of pravastatin, history of vascular disease, history of diabetes mellitus, history of hypertension, current smoking, alcohol in units/week, BMI, total cholesterol/HDL-ratio and albumin. The data were analysed using IBM SPSS Statistics version 23. P-values were considered statistically significant if lower than 0.05 for baseline characteristics and hazard ratios (HR, 95% confidence interval [CI]), or lower than 0.10 for interaction analyses.

ROLE OF THE FUNDING SOURCES

The Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) trial was supported by an unrestricted investigator-initiated grant from Bristol-Myers Squibb, USA. The funding source had no involvement in study design; in the collection, analysis, and interpretation of data; in writing of the report; and in the decision to submit the paper for publication.

RESULTS

Of the 5804 randomised participants, 4864 were suitable for inclusion in the current analysis, see Figure 1 and supplemental figure 1. A proportion of the participants with subclinical thyroid dysfunction had a spontaneous resolution when biochemical measurements were repeated after six months or vice versa (5.2%). Participants had a mean age of 75.3 years and 49.0% were male. Mean follow-up was 3.2 years. Of all participants 109 subjects (2.2%) had subclinical hypothyroidism, 4573 (94.0%) had euthyroidism, and 182 (3.7%) subclinical hyperthyroidism.

Baseline characteristics are shown in Table 1 per thyroid function group and overall. Age and gender were comparable between groups. Furthermore, eGFR category did not differ (p=0.417). A history of vascular disease was present in 33 (30.3%), 2059 (45.0%), and 69 (37.9%) patients with subclinical hyperthyroidism, euthyroidism and subclinical hypothyroidism, respectively (p=0.002). Use of pravastatin, beta-blockers and antiarrhythmics were comparable. Patients with subclinical hyperthyroidism had highest blood pressure, both systolic with a mean difference of 6 mmHg (p=0.015) and diastolic with a mean difference of 3 mmHg (p=0.001).

Table 1. Baseline Characteristics Split by Baseline Thyroid status and Overall					
	Subcl hyperth n = 109	**Euthyroidism n = 4573**	**Subcl hypoth n = 182**	**Total n = 4864**	**p-value**
Age (years; mean ± SD)	74.7 ± 3.2	75.3 ± 3.4	75.2 ± 3.5	75.3 ± 3.4	0.160
Male gender, n (%)	64 (58.7)	2226 (48.7)	95 (52.2)	2385 (49.0)	0.080
Education (years; mean ± SD)	15.1 ± 1.9	15.1 ± 2.1	15.1 ± 2.1	15.1 ± 2.1	0.966
History of hypertension, n (%)	70 (64.2)	2804 (61.3)	110 (60.4)	2984 (61.3)	0.801
History of diabetes, n (%)	9 (8.3)	490 (10.7)	20 (11.0)	519 (10.7)	0.706
History of vascular disease, n (%)	33 (30.3)	2059 (45.0)	69 (37.9)	2161 (44.4)	0.002
eGFR categories, n (%)					0.417
<45 ml/min/1.73m^2	14 (12.8)	615 (13.4)	27 (14.8)	656 (13.5)	
45-60 ml/min/1.73m^2	35 (32.1)	1830 (40.0)	68 (37.4)	1933 (39.7)	
>60 ml/min/1.73m^2	60 (55.0)	2128 (46.5)	87 (47.8)	2275 (46.8)	
Smoking status, n (%)					0.665
Never	32 (29.4)	1545 (33.8)	54 (29.7)	1631 (33.5)	
Former	46 (42.2)	1776 (38.8)	73 (40.1)	1895 (39.0)	
Current	31 (28.4)	1252 (27.4)	55 (30.2)	1338 (27.5)	
Alcohol in units/week, n (%)	6.2 ± 9.2	5.3 ± 9.3	6.4 ± 12.1	5.3 ± 9.4	0.173
Medication use, n (%)					
Pravastatin	56 (51.4)	2292 (50.1)	90 (49.5)	2438 (50.1)	0.951
Aspirin	28 (25.7)	1672 (36.6)	61 (33.5)	1761 (36.2)	0.049
Beta-blockers	25 (22.9)	1191 (26.0)	58 (31.9)	1274 (26.2)	0.159
Antiarrhythmics	3 (2.8)	117 (2.6)	4 (2.2)	124 (2.5)	0.946
Objective measures (mean ± SD)					
SBP (mmHg)	161 ± 21	155 ± 22	155 ± 21	155 ± 22	0.015
DBP (mmHg)	88 ± 10	84 ± 12	84 ± 10	84 ± 12	0.001
BMI (kg/m^2)	26.5 ± 4.3	26.8 ± 4.2	26.6 ± 4.0	26.8 ± 4.2	0.552
Total cholesterol/HDL-ratio (mmol/l)	4.7 ± 1.3	4.7 ± 1.3	4.7 ± 1.3	4.7 ± 1.3	0.967
Glucose (mmol/l)	5.4 ± 1.2	5.5 ± 1.5	5.5 ± 1.2	5.5 ± 1.5	0.922
CRP (mg/l)	4.0 ± 5.1	4.2 ± 6.5	3.7 ± 5.4	4.2 ± 6.5	0.560
Urea (mg/dL)	6.3 ± 1.8	6.3 ± 1.8	6.2 ± 1.6	6.3 ± 1.8	0.627
Hb (mmol/L)	8.9 ± 0.7	8.7 ± 0.8	8.8 ± 0.8	8.7 ± 0.8	0.018

Abbreviations: BMI, body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; Hb, haemoglobin; HDL, high-density lipoprotein; SBP, systolic blood pressure; SD, standard deviation; Subcl hyper, subclinical hyperthyroidism; Subl hypo, subclinical hypothyrodism

SUBCLINICAL THYROID DYSFUNCTION

Table 2 shows the univariate and multivariate association between thyroid function groups and the primary endpoint. Primary endpoint occurred in 9 (8.3%), 712 (15.6%), and 23 (12.6%) patients with subclinical hyperthyroidism, euthyroidism and subclinical hypothyroidism, respectively. Multivariate adjusted HRs (95% CI) were 0.51 (0.24-1.07) comparing subclinical hyperthyroidism with euthyroidism and 0.90 (0.58-1.39) comparing subclinical hypothyroidism with euthyroidism.

Table 2. Univariate and Multivariate Influence of Thyroid Status on the Primary Endpoint

	Thyroid status based on TSH at baseline and 6 months	
	Subcl hyperth vs euth n = 109 vs 4573	**Subcl hypo vs euth n = 182 vs 4573**
No. of events (%)	9 (8.3) vs 712 (15.6)	23 (12.6) vs 721 (15.6)
Univariate HR (95% CI)	0.52 (0.27-1.00)	0.80 (0.52-1.22)
Age and sex adjusted HR (95% CI)	0.52 (0.27-0.99) ‡	0.30 (0.53-1.22)
Multivariate adjusted HR (95% CI)	0.51 (0.24-1.07)	0.90 (0.58-1.39)

* Primary endpoint includes coronary heart disease death or non-fatal myocardial infarction or fatal or non-fatal stroke. ‡ p-value of hazard ratio <0.05

Abbreviations: CI, confidence interval; HR, hazard ratio; Multivariate adjusted analysis include prespecified variables: country, age, gender, use of pravastatin, history of vascular disease, history of diabetes mellitus, history of hypertension, current smoking, alcohol in units/week, BMI, total cholesterol/HDL-ratio, albumin.

The univariate and multivariate association of thyroid status with primary endpoint over strata of baseline eGFR category is shown in Table 3. In the prespecified eGFR categories 656 subjects (13.5%) had an eGFR <45ml/min/1.73m2, 1933 (39.7%) 45-60ml/min/1.73m2, and 2275 (46.8%) >60ml/min/1.73m2. The levels of TSH and fT4 did not differ between this categories. Mean ± standard error TSH was 2.1±0.06; 2.1±0.03; and 2.0±0.03 in patients with eGFR <45 ml/min/1.73m2; eGFR 45-60 ml/min/1.73m2; and eGFR >60 ml/min/1.73m2, respectively (p=0.383). Mean ± standard error fT4 was 16.4±0.3; 16.5±0.1; and 16.7±0.1 in patients with eGFR <45 ml/min/1.73m2; eGFR 45-60 ml/min/1.73m2; and eGFR >60 ml/min/1.73m2, respectively (p=0.413). When comparing subclinical hyperthyroidism with euthyroidism, multivariate adjusted HRs (95% CI) were 1.07 (0.26-4.40), 0.25 (0.03-1.76), and 0.54 (0.20-1.45) in eGFR <45ml/min/1.73m2, 45-60ml/min/1.73m2,

and >60ml/min/1.73m2, respectively, without a significant interaction (p-interaction=0.602). When comparing subclinical hypothyroidism with euthyroidism multivariate adjusted HRs (95% CI) were 1.41 (0.57-3.51), 0.24 (0.06-0.95), and 1.43 (0.83-2.46) in eGFR <45ml/min/1.73m2, 45-60ml/min/1.73m2, and >60ml/min/1.73m2, respectively, also without a significant interaction (p-interaction=0.388).

Table 3. Univariate and Multivariate Influence of Thyroid Status on the Primary Endpoint Split by Baseline eGFR Category

	eGFR <45, n = 656		**eGFR 45-60, n = 1933**		**eGFR >60, n = 2275**	
	Subcl hyperth vs euth n = 14 vs 615	**Subcl hypo vs euth n = 27 vs 615**	**Subcl hyperth vs euth n = 35 vs 1830**	**Subcl hypo vs euth n = 68 vs 1830**	**Subcl hyperth vs euth n = 60 vs 2128**	**Subcl hypo vs euth n = 87 vs 2128**
No. of events (%)	6 (33.3) vs 123 (18.8)	8 (20.5) vs 123 (18.8)	4 (12.9) vs 280 (15.0)	8 (7.8) vs 280 (15.0)	4 (9.8) vs 338 (16.0)	16 (13.0) vs 338 (16.0)
Univariate HR (95% CI)	0.72 (0.18-2.93)	1.13 (0.50-2.56)	0.37 (0.09-1.47)	0.28 (0.09-0.88) ‡	0.55 (0.23-1.33)	1.10 (0.64-1.87)
Age and sex adjusted HR (95% CI)	0.79 (0.20-3.12)	1.18 (0.52-2.69)	0.36 (0.09-1.43)	0.27 (0.09-0.86) ‡	0.54 (0.22-1.31)	1.10 (0.65-1.89)
Multivariate adjusted HR (95% CI)	1.07 (0.26-4.40)	1.41 (0.57-3.51)	0.25 (0.03-1.76)	0.24 (0.06-0.95) ‡	0.54 (0.20-1.45)	1.43 (0.83-2.46)

* Primary endpoint includes coronary heart disease death or non-fatal myocardial infarction or fatal or non-fatal stroke. ‡ p-value of hazard ratio <0.05. *Abbreviations:* CI, confidence interval; HR, hazard ratio. Multivariate adjusted analysis include prespecified variables: country, age, gender, use of pravastatin, history of vascular disease, history of diabetes mellitus, history of hypertension, current smoking, alcohol in units/week, BMI, total cholesterol/HDL-ratio, albumin.

DISCUSSION

In this secondary analysis of the PROSPER study we aimed to explore whether kidney function explains the association of thyroid function and the risk of cardiovascular events. In these older patients at high risk of cardiovascular disease, no relationship was found between subclinical thyroid dysfunction and cardiovascular events, nor was this relationship present after stratification by kidney function. Furthermore, kidney function did not interact with subclinical thyroid dysfunction in relation with cardiovascular events.

Although the association of thyroid dysfunction on cardiovascular events and mortality has been studied in patients with CKD (23,24), this is the first time this association has been studied over different strata of CKD to explore a possible interaction between thyroid and kidney function, which we did not find.

There are two hypotheses regarding the interaction of kidney and thyroid. Firstly, thyroid hormone changes may predispose to kidney dysfunction, due to possible alterations in kidney hemodynamics and structure, both direct and indirect via the cardiovascular system (25). Secondly, kidney dysfunction may predispose to thyroid hormone changes, via influence on the metabolism of thyroid hormones or due to chronic illness (as CKD) leading to nonthyroidal illness as potential underlying pathophysiological mechanisms (23,26). A recent individual participant data analysis from 16 independent cohorts of the Thyroid Studies Collaboration found no role for thyroid hormones in kidney dysfunction (15). However, it does not exclude that previously found cross-sectional associations between kidney and thyroid (10,11) may be explained by kidney dysfunction causing thyroid hormone changes. Another explanation could be that there might be a common pathway to thyroid and kidney dysfunction, due to shared cardiovascular risk factors, especially in older people.

The prevalence of subclinical thyroid dysfunction within this study (2.2% patients with subclinical hypothyroidism and 3.7% with subclinical hyperthyroidism) was lower than the prevalence of subclinical thyroid

dysfunction among the general population within the same geographical location (27). In addition, contrary to what has been reported in literature, the prevalence of subclinical thyroid dysfunction did not change with increasing degrees of kidney dysfunction (28), nor did the levels of TSH and fT4 differ between groups stratified by kidney function (data not shown) (12).

STRENGTHS AND LIMITATIONS

The presence of two thyroid measurements is a major strength which allowed us to more accurately assess subclinical thyroid disease by excluding those without persistent thyroid disease after six months of follow up. In most studies regarding thyroid function and cardiovascular disease risk, all measurements are performed at baseline and compared to follow up data. We showed that a proportion of participants with subclinical thyroid dysfunction has a spontaneous resolution when biochemical measurements are repeated after six months or vice versa (5.2%), explaining why for instance the TRUST study 'Thyroid hormone Replacement for Untreated older adults with Subclinical hypothyroidism - a randomised placebo controlled Trial' had a relatively long run-in phase, as patients with subclinical thyroid disease spontaneously resolved to euthyroidism before the trial started (20). Using two separate measurements, as in our study, thus filters bias which is otherwise present in studies utilizing only baseline measurements. Furthermore, we excluded patients who used medication that could be of influence on thyroid function such as both thyroxin and antithyroid treatment, but also lithium and amiodaron, although amiodaron could have long-lasting effects and previous use is unknown. Despite the large number of participants within the PROSPER study, the number of patients with subclinical thyroid dysfunction within this study was relatively small, limiting the power to find an association between subclinical thyroid disease and cardiovascular events, and an interaction between thyroid and kidney function, of a smaller magnitude. Whereas one of the exclusion criteria was a high creatinine (>200 µmol/l) at baseline, the influence of severe or end-stage CKD (eGFR <30 ml/min/1.73m2) on the association of thyroid function and cardiovascular events is unknown. Furthermore, the larger proportion of euthyroid patients

with a history of vascular disease compared to those with subclinical thyroid dysfunction may have masked the risk of cardiovascular events in subclinical thyroid dysfunction, but adjustment in linear regression models yielded similar results.

CONCLUSION

In this secondary analysis from PROSPER, kidney function did not interact in the association of thyroid function and cardiovascular disease. Therefore, we found no evidence that the potential association between thyroid hormones and cardiovascular disease is modified by kidney function in older patients with subclinical thyroid dysfunction.

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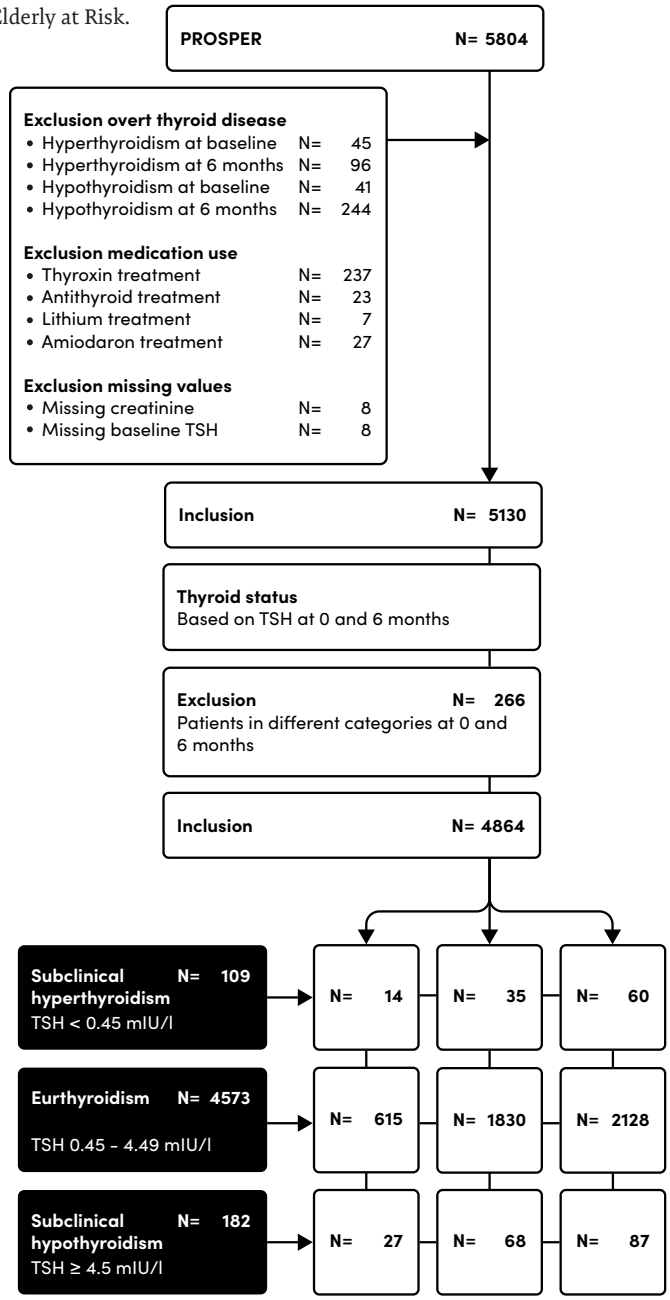
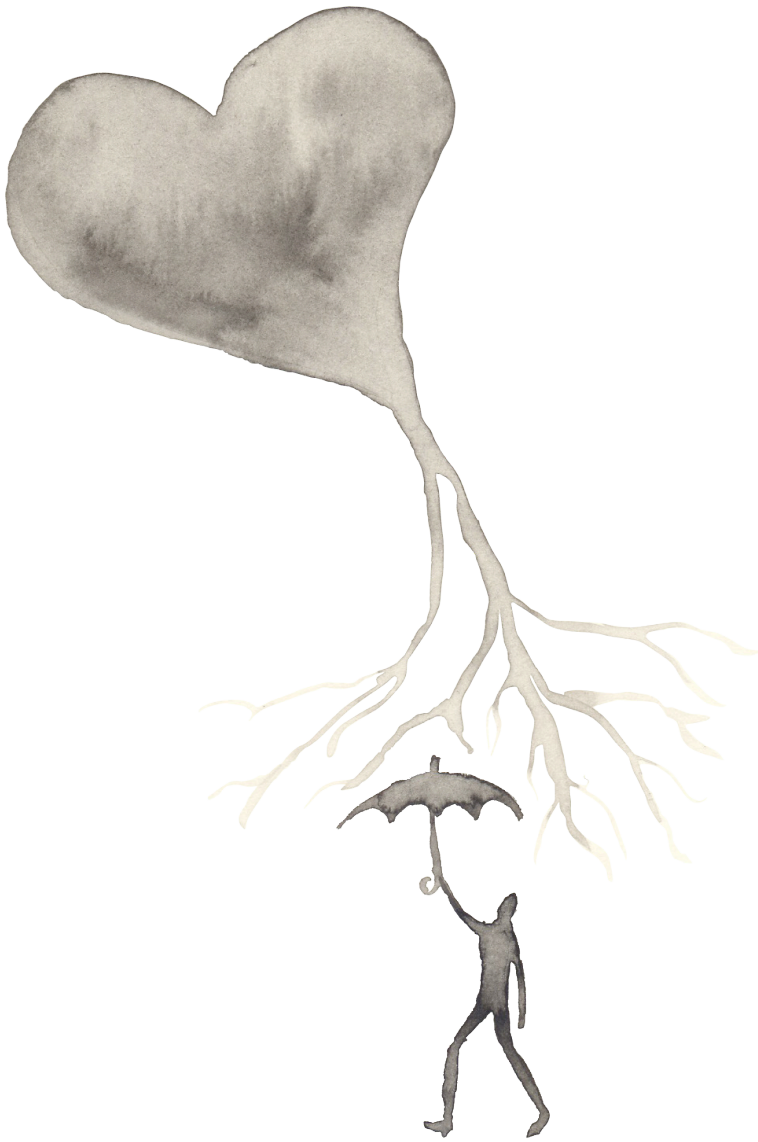
SUPPLEMENTAL MATERIAL

SUPPLEMENTAL FIGURE 1. EXTENDED FLOWCHART OF STUDY POPULATION

Inclusion and exclusion criteria of the current substudy of PROSPER (the PROspective Study of Pravastatin in the Elderly at Risk.





PART 2.

TREATMENT OF HIGH-RISK CARDIOVASCULAR PATIENTS

7

CHAPTER 7.

PCSK9 INHIBITION IN POLYVASCULAR DISEASE

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Based on: Alirocumab in Patients With Polyvascular Disease and Recent Acute Coronary Syndrome: ODYSSEY OUTCOMES Trial. J Am Coll Cardiol. 2019;74(9):1167-76.

ABSTRACT

INTRODUCTION

Patients with acute coronary syndrome (ACS) and concomitant noncoronary atherosclerosis have a high risk of major adverse cardiovascular events (MACE) and death. The impact of lipid-lowering by proprotein convertase subtilisin–kexin type 9 (PCSK9) inhibition in such patients is undetermined. This pre-specified analysis from ODYSSEY OUTCOMES determined whether polyvascular disease influenced risks of MACE and death and their modification by alirocumab in patients with recent ACS and dyslipidemia despite intensive statin therapy.

METHODS

Patients were randomized to alirocumab or placebo 1–12 months after ACS. The primary MACE endpoint was the composite of coronary heart disease death, nonfatal myocardial infarction, fatal/nonfatal ischemic stroke, or unstable angina requiring hospitalization. All-cause death was a secondary endpoint.

RESULTS

Median follow-up was 2.8 years. Of 18,924 patients, 17,370 had monovascular (coronary) disease, 1,405 had polyvascular disease in two beds (coronary and peripheral artery or cerebrovascular), and 149 had polyvascular in three beds (coronary, peripheral artery, cerebrovascular). With placebo, the incidence of MACE by respective vascular categories was 10.0%, 22.2%, and 39.7%. With alirocumab, corresponding absolute risk reduction (ARR [95% confidence interval]) was 1.4% (0.6, 2.3), 1.9% (−2.4%, 6.2%), and 13.0% (−2.0, 28.0). With placebo, the incidence of death by respective vascular categories was 3.5%, 10.0%, and 21.8%; ARR with alirocumab was 0.4% (−0.1, 1.0), 1.3% (−1.8%, 4.3%), and 16.2% (5.5, 26.8).

CONCLUSIONS

In patients with recent ACS and dyslipidemia despite intensive statin therapy, polyvascular disease is associated with high risks of MACE and death. The large absolute reductions in those risks with alirocumab are a potential benefit for this population.

INTRODUCTION

Patients with peripheral artery disease (PAD) or cerebrovascular disease (CeVD) have an elevated risk of major adverse cardiovascular events (MACE) and death compared with patients without these conditions, irrespective of a concurrent history of coronary artery disease (1-3). The risk of future MACE and death also remains high among patients with an acute coronary syndrome (ACS), despite application of evidence-based secondary prevention measures including statins and dual antiplatelet therapy (4). When PAD or CeVD is concurrent with ACS, risk may be particularly elevated, warranting more intensive approaches to secondary prevention (5, 6).

Lowering of atherogenic lipoproteins, reflected in part by reduction of low-density lipoprotein cholesterol (LDL-C), favorably modifies the risks of MACE and death (7). Accordingly, statin treatment is broadly recommended for patients with coronary atherosclerosis, PAD, or CeVD in the guidelines of the American College of Cardiology/American Heart Association, American College of Cardiology/American Stroke Association and European Society of Cardiology/European Atherosclerosis Society (8-11).

The advent of inhibitors of proprotein convertase subtilisin–kexin type 9 (PCSK9) provided an opportunity to lower LDL-C to levels not previously achievable with statins and/or ezetimibe. The FOURIER trial compared the PCSK9 inhibitor evolocumab with placebo in patients with established, stable atherosclerotic cardiovascular disease, including coronary artery disease, PAD, or CeVD. Evolocumab reduced MACE, but not death. Benefits were particularly pronounced among patients with PAD at entry into the trial (12).

The ODYSSEY OUTCOMES trial showed that MACE was reduced with the PCSK9 inhibitor alirocumab compared with placebo in 18,924 patients with recent ACS and elevated atherogenic lipoproteins despite intensive statin therapy. In addition, fewer deaths occurred among patients treated with alirocumab. The aim of this pre-specified analysis of the ODYSSEY OUTCOMES trial was to determine whether the benefits of alirocumab on MACE and death were influenced by presence of polyvascular disease, defined as concomitant

PAD, CeVD, or both, and thus to identify preferred candidates for alirocumab treatment.

METHODS

Details of the study design (13) and primary efficacy and safety results have been published (14). In brief, ODYSSEY OUTCOMES was a multicenter, double-blind, placebo-controlled trial in 18,924 patients at least 40 years of age who provided written informed consent and had been hospitalized with an ACS (defined as myocardial infarction or unstable angina) 1 to 12 months prior to randomization. Qualifying patients had a level of LDL-C at least 70 mg/dl (1.81 mmol/l), non–high-density lipoprotein cholesterol at least 100 mg/dl (2.59 mmol/l), or apolipoprotein B at least 80 mg/dl, measured after a minimum of 2 weeks of stable treatment with atorvastatin 40 to 80 mg daily, rosuvastatin 20 to 40 mg daily, or the maximum tolerated dose of either statin (including no statin in case of documented intolerance). Patients were randomly assigned in a 1:1 ratio stratified by country to receive treatment with alirocumab 75 mg subcutaneously every 2 weeks or matching placebo.

CATEGORIES OF POLYVASCULAR DISEASE

In this analysis, three subgroups of patients with recent ACS were defined based upon the distribution of other evident vascular disease: 1) monovascular disease (coronary artery disease without known PAD or CeVD); 2) polyvascular disease in two vascular beds (coronary artery disease and either PAD or CeVD); and 3) polyvascular disease in three vascular beds (coronary artery disease with both PAD and CeVD). Two additional sensitivity analyses were performed. The first considered two vascular disease categories: 1) monovascular disease (coronary artery disease without known PAD or CeVD); and 2) polyvascular disease (coronary artery disease with any combination of PAD or CeVD). The second considered four subgroups of patients with ACS: 1) those with monovascular disease, as defined above; 2) all patients with PAD, with or without concurrent CeVD; 3) all patients with CeVD, with or without concurrent PAD; and 4) those with disease in all three vascular beds, as

defined above. PAD included arterial disease of the extremities or abdominal aortic aneurysm. CeVD was defined as a history of carotid endarterectomy, carotid stenting, prior stroke or transient ischemic attack.

ENDPOINTS

The primary MACE endpoint was a composite of coronary heart disease death, nonfatal myocardial infarction, fatal or nonfatal ischemic stroke, or unstable angina requiring hospitalization. All-cause death was a secondary endpoint.

STATISTICAL CONSIDERATIONS

Analyses of clinical outcomes and LDL-C levels were performed according to the intention-to-treat principle, including all patients, events, and measurements from randomization to the common study end date (November 11, 2017). Hazard ratios (HR) and 95% confidence intervals (CI) were estimated using a Cox proportional-hazards model, stratified by geographic region; p-values were determined using stratified log-rank tests. Endpoint rates were based on observed incidences. Alirocumab treatment effect heterogeneity by categories of polyvascular disease was assessed by Cox models with interaction terms for relative risk reduction and Gail-Simon tests for ARR. Analyses were performed in SAS version 9.4 (IBM, Armonk, New York).

RESULTS

Of 18,924 randomized patients, 9,462 were assigned to the alirocumab group and 9,462 to the placebo group, with a median (quartile 1, quartile 3) follow-up of 2.8 years (2.3, 3.4). At baseline, 17,370 patients had monovascular disease (91.8%), 1,405 patients had polyvascular disease in two vascular beds (7.4%; 3.2% PAD and 4.2% CeVD), and 149 had polyvascular disease in three vascular beds (0.8%).

BASELINE CHARACTERISTICS

Table 1 summarizes the baseline characteristics of patients with monovascular (coronary) disease, polyvascular disease in two beds (split by PAD only and CeVD only), and polyvascular disease in three beds. Compared to patients with monovascular disease, those with coronary and PAD, coronary and CeVD, and polyvascular disease in three beds were older (median ages 58, 62, 62, and 66 years, $p<0.0001$); those with coronary and PAD or coronary and CeVD were more likely to be female (26.7% and 33.2%, respectively) than those with monovascular disease (24.7%, $p<0.0001$). Of all patients with CeVD, 526 (66.2%) had a history of stroke. Patients with polyvascular disease in three beds had more comorbidities, including a history of hypertension, myocardial infarction and coronary artery bypass grafting, compared to patients with monovascular disease (all $p<0.0001$). Furthermore, patients with polyvascular disease in three beds versus patients with monovascular disease had a higher prevalence of diabetes (43.6% vs. 27.7%; $p<0.0001$) and were more likely to be current or former smokers (81.9% vs. 64.9%; $p<0.0001$). More patients with polyvascular disease in three beds versus patients with monovascular disease had an estimated glomerular filtration rate (eGFR) of <60ml/min/1.73m2 (39.6% vs. 12.3%) with median eGFRs of 78.5, 73.5, 72.3, and 67.0 ml/min/1.73m2 in patients with monovascular disease, coronary and PAD, coronary and CeVD, and polyvascular disease in three beds, respectively ($p<0.0001$).

Table 1. Baseline Characteristics by History of PAD or CeVD Category

Coronary without PAD or CeVD (n=17,370)	Monovascular Disease	Disease in Two Vascular Beds		Disease in Three Vascular Beds	p-value
	Coronary and PAD (n=610)	Coronary and CeVD (n=795)	Coronary, PAD, and CeVD (n=149)		
Age, years	58 (51,65)	62 (56, 68)	62 (56, 69)	66 (60, 71)	<0.0001
Age category					<0.0001
<65 years	12,956 (74.6)	368 (60.3)	456 (57.4)	60 (40.3)	
65 to <75 years	3,575 (20.6)	178 (29.2)	252 (31.7)	72 (48.3)	
≥75 years	839 (4.8)	64 (10.5)	87 (10.9)	17 (11.4)	
Female	4,298 (24.7)	163 (26.7)	264 (33.2)	37 (24.8)	<0.0001
Region					<0.0001
Western Europe	3,852 (22.2)	152 (24.9)	142 (17.9)	29 (19.5)	
Eastern Europe	4,993 (28.7)	189 (31.0)	215 (27.0)	40 (26.8)	
North America	2,513 (14.5)	134 (22.0)	170 (.4)	54 (36.2)	
South America	2,413 (13.9)	64 (10.5)	101 (12.7)	10 (6.7)	
Asia	2,170 (12.5)	22 (3.6)	92 (11.6)	9 (6.0)	
Rest of world	1,429 (8.2)	49 (8.0)	75 (9.4)	7 (4.7)	
Index event					<0.0001
NSTEMI	8,300 (47.9)	342 (56.3)	439 (55.4)	94 (63.1)	
STEMI	6,080 (35.1)	195 (31.1)	227 (28.6)	34 (22.8)	
Unstable angina	2,963 (17.1)	71 (11.7)	127 (16.0)	21 (14.1)	
Time from index event to randomization, months	2.6 (1.7, 4.3)	3.0 (1.8, 5.4)	2.7 (1.7, 4.8)	3.0 (2.1, 3.9)	0.0003
Lipid-lowering therapy at randomization					<0.0001
High-dose atorvastatin or rosuvastatin	15,486 (89.2)	525 (86.1)	679 (85.4)	121 (81.2)	
Other LLT	1,734 (10.0)	75 (12.3)	102 (12.8)	24 (16.1)	
No LLT	150 (0.9)	10 (1.6)	14 (1.8)	4 (2.7)	
LDL-C, mg/dl	86 (73, 103)	91 (76, 108)	90 (75, 109)	95 (80, 115)	<0.0001
LDL-C ≥100 mg/dl	5,060 (29.1)	218 (35.7)	290 (36.5)	61 (40.9)	<0.0001
HDL-C, mg/dl	42 (36, 50)	42 (36, 50)	43 (36, 51)	43 (37, 51)	NS
Non–HDL-C, mg/dl	114 (99, 136)	121 (105, 143)	120 (103, 144)	124 (108, 143)	<0.0001
Triglycerides, mg/dl	128 (94, 181)	134 (99, 187)	136 (98, 190)	135 (94, 182)	0.002
Apolipoprotein B, mg/dl	79 (69, 93)	83 (72, 96)	83 (71, 96)	82 (75, 95)	<0.0001
Lipoprotein(a), mg/dl	20.8 (6.6, 59.4)	25.5 (7.5, 68.1)	23.0 (7.1, 61.7)	29.4 (9.4, 74.5)	0.004
C-reactive protein, mg/dl	0.16 (0.08, 3.73)	0.26 (0.11, 0.55)	0.22 (0.10, 0.48)	0.21 (0.11, 0.49)	<0.0001

Body mass index, kg/m2	27.9 (25.2, 31.1)	27.7 (24.9, 31.0)	28.1 (25.4, 31.5)	27.7 (24.5, 30.7)	NS
HbA1c, %	5.8 (5.5, 6.3)	6.0 (5.6, 6.7)	6.1 (5.7, 7.0)	6.0 (5.7, 6.7)	<0.0001
eGFR, ml/min per 1.73 m2	78.5 (68.1, 90.4)	74.1 (61.6, 86.7)	72.9 (59.5, 85.8)	67.0 (52.2, 84.4)	<0.0001
eGFR <60 ml/min per 1.73 m2	2139 (12.3)	135 (22.1)	206 (25.9)	59 (39.6)	<0.0001
Diabetes status					<0.0001
Diabetes	4,805 (27.7)	225 (36.9)	349 (43.9)	65 (43.6)	
Pre-diabetes	7,630 (43.9)	260 (42.6)	299 (37.6)	57 (38.3)	
Normoglycemia	4,935 (28.4)	125 (20.5)	147 (18.5)	27 (18.1)	
Smoking status					<0.0001
Current	4,181 (24.1)	189 (31.0)	147 (18.5)	43 (28.9)	
Former	7,095 (40.8)	302 (49.5)	335 (42.1)	79 (53.0)	
Never	6,093 (35.1)	119 (19.5)	313 (39.4)	27 (18.1)	
Medical history prior to index event					
Hypertension	10,930 (62.9)	489 (80.2)	694 (87.3)	136 (91.3)	<0.0001
Myocardial infarction	3,147 (18.1)	204 (33.4)	226 (28.4)	62 (41.6)	<0.0001
Stroke	0	0	526 (66.2)	85 (57.0)	<0.0001
Malignant disease	458 (2.6)	28 (4.6)	34 (4.3)	12 (8.1)	<0.0001
COPD	613 (3.5)	64 (10.5)	46 (5.8)	23 (15.4)	<0.0001
CABG	826 (4.8)	82 (13.4)	91 (11.4)	48 (32.2)	<0.0001
PAD	0	610 (100)	0	149 (100)	<0.0001
CeVD	0	0	795 (100)	149 (100)	<0.0001
Revascularization for index event	12,596 (72.5)	436 (71.5)	540 (67.9)	105 (70.5)	0.04
Medications					
Aspirin	16,647 (95.8)	564 (92.5)	737 (92.7)	138 (92.6)	<0.0001
P2Y12 antagonist	15,223 (87.6)	525 (86.1)	664 (83.5)	129 (86.6)	0.005
ACE inhibitor/ARB	13,444 (77.4)	494 (81.0)	655 (82.4)	123 (82.6)	0.0008
Beta-blocker	14,687 (84.6)	507 (83.1)	672 (84.5)	124 (83.2)	NS
Ezetimibe	473 (2.7)	38 (6.2)	30 (3.8)	13 (8.7)	<0.0001
Treatment Variables Among Alirocumab Treated Patients	n=8,683	n=302	n=406	n=71	
% switched to placebo	691 (8.0)	12 (4.0)	25 (6.2)	2 (2.8)	0.01

Values are median (quartile 1, quartile 3) or n (%). P values reflect the statistical comparison between the four vascular disease subgroups (coronary without PAD or CeVD; coronary and PAD; coronary and CeVD; coronary, PAD, and CeVD). ARB, angiotensin receptor blocker; CABG, coronary artery bypass graft; CeVD, cerebrovascular disease; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LLT, lipid-lowering therapy; NS, not significant (p > 0.05); NSTEMI, non–ST-elevation myocardial infarction; PAD, peripheral artery disease; STEMI, ST-elevation myocardial infarction.

LDL-C LOWERING

At baseline, median LDL-C (quartile 1, quartile 3) was higher in patients with polyvascular disease, with values of 86 (73, 103) in patients with monovascular disease, 91 (76, 108) in coronary and PAD disease, 90 (75, 109) in coronary and CeVD, and 95 mg/dl (80, 115) with polyvascular disease in three beds ($p<0.0001$). In the placebo group, LDL-C at 4 months was 87 (72, 106) in patients with monovascular disease, 90 (73, 108) in only PAD, 90 (73, 115) in only CeVD, and 93 mg/dl (78, 118) in polyvascular disease in three beds. In patients treated with alirocumab, LDL-C at 4 months was 30 (20, 47), 34 (23, 50), 34 (21, 52), and 31 (20, 42) in the same four vascular disease categories.

PRIMARY MACE ENDPOINT AND ALL-CAUSE DEATH

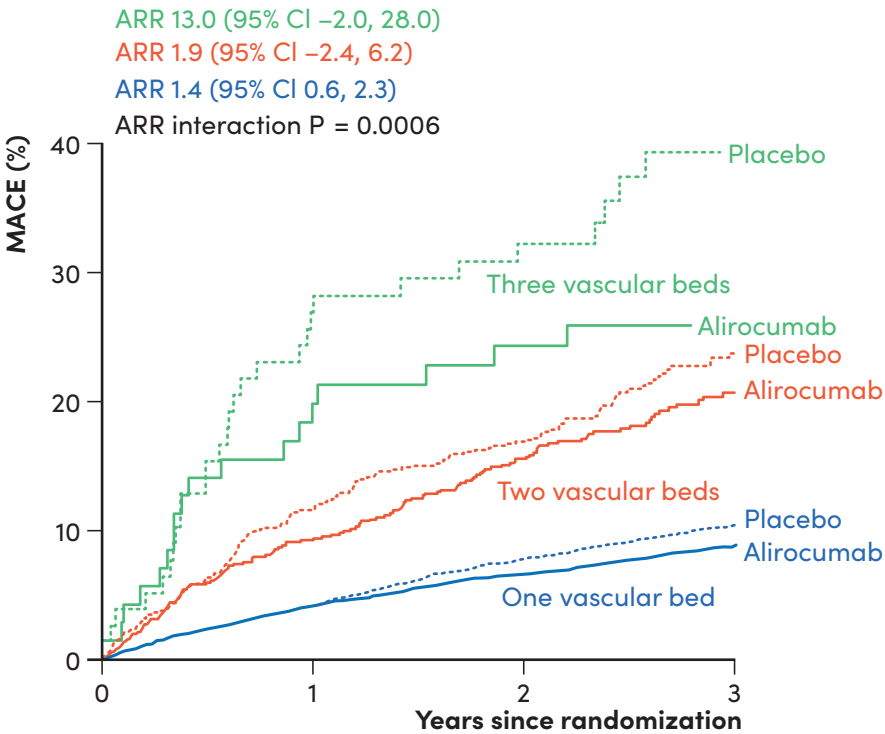
Overall in the ODYSSEY OUTCOMES trial, the incidence of MACE in the placebo and alirocumab groups was 11.1% and 9.5%, respectively, with a corresponding ARR of 1.6% (95% CI, 0.7%, 2.4%; $p=0.0003$) (14). Figure 1 shows that this overall efficacy reflects a gradient of absolute risk and ARR according to the number of diseased vascular beds. For patients in the placebo group with one, two, or three diseased vascular beds, the incidence of MACE was 10.0%, 22.2%, and 39.7%, respectively. The corresponding ARR with alirocumab was 1.4% (0.6%, 2.3%), 1.9% (−2.4%, 6.2%), and 13.0% (−2.0%, 28.0%), with an interaction $p=0.0006$.

For all-cause death in ODYSSEY OUTCOMES, the overall incidence of death in the placebo and alirocumab groups was 4.1% and 3.5%, respectively, with a corresponding ARR of 0.6% (95% CI, 0.2%, 1.2%) (14). Similar to MACE, there was a gradient of absolute risk and ARR with alirocumab. In the placebo group, the incidence of death with one, two, or three diseased vascular beds was 3.5%, 10.0%, and 21.8%, respectively. With alirocumab, the corresponding ARR was 0.4% (−0.1%, 1.0%), 1.3% (−1.8%, 4.3%), and 16.2% (5.5%, 26.8%), with an interaction $p=0.002$.

FIGURE 1. PRIMARY MACE ENDPOINT

Kaplan-Meier curves for primary MACE endpoint in patients with arterial disease in, respectively, 1 (CAD and no PAD or CeVD), 2 (CAD and PAD or CeVD), or 3 (CAD and PAD and CeVD) vascular beds.

ALI, alirocumab; ARR, absolute risk reduction; CAD, coronary artery disease; CeVD, cerebrovascular disease; CI, confidence interval; HR, hazard ratio; MACE, major adverse cardiovascular events; PAD, peripheral artery disease; PBO, placebo.



Number at risk

Placebo	8687	8155	7612	3242
Alirocumab	8683	8167	7741	3304
Placebo	697	594	541	206
Alirocumab	708	624	554	242
Placebo	78	57	50	24
Alirocumab	71	56	51	29

Details of the primary MACE endpoint and all-cause death are shown in Table 2, including the total number of events with corresponding HR and ARR of alirocumab versus placebo for primary endpoint and all-cause death for patients with monovascular disease, polyvascular disease in two or three beds, polyvascular disease in two beds (split by PAD or CeVD), and polyvascular disease in three beds. Supplemental tables 1 and 2 show these details for both sensitivity analyses (monovascular versus polyvascular and the four overlapping vascular groups based on PAD or CeVD).

SAFETY OUTCOMES

Overall, there were no differences in the incidence of adverse events or laboratory abnormalities between alirocumab and placebo groups, with the exception of local injection-site reactions, which occurred more often in the alirocumab group (14). Table 3 shows all safety endpoints for alirocumab versus placebo for patients with monovascular disease, polyvascular disease in two beds (categorized as PAD or CeVD), and polyvascular disease in three beds. No major differences were observed between the groups.

Table 2. Primary MACE Endpoint and All-Cause Death by History of PAD or CeVD Category						
	Alirocumab n/N (%)	**Placebo n/N (%)**	**HR* (95% CI)**	**HR Interaction p-value**	**ARR (95% CI)**	**ARR interaction p-value**
Primary composite						
Monovascular disease (CAD without PAD or CeVD)	740/8,683 (8.5)	866/8,687 (10.0)	0.85 (0.77, 0.93)		1.4 (0.6, 2.3)	
Disease in two vascular beds						
CAD and PAD	69/302 (22.8)	73/308 (23.7)	0.93 (0.67, 1.30)	0.40	0.9 (−5.9, 7.6)	0.0006
CAD and CeVD	75/406 (18.5)	82/389 (21.1)	0.87 (0.63, 1.19)		2.6 (−2.9, 8.2)	
Disease in three vascular beds (CAD, PAD, and CeVD)	19/71 (26.8)	31/78 (39.7)	0.64 (0.35, 1.12)		13.0 (−2.0, 28.0)	
All patients	903/9,462 (9.5)	1,052/9,462 (11.1)	0.85 (0.78, 0.93)		1.6 (0.7, 2.4)	
All-cause death						
Monovascular disease (CAD without PAD or CeVD)	268/8,683 (3.1)	305/8,687 (3.5)	0.88 (0.75, 1.04)		0.4 (−0.1, 1.0)	
Disease in two vascular beds						
CAD and PAD	28/302 (9.3)	27/308 (8.8)	1.03 (0.60, 1.75)	0.06	−0.5 (−5.1, 4.0)	0.002
CAD and CeVD	34/406 (8.4)	43/389 (11.1)	0.68 (0.44, 1.08)		2.7 (−6.8, 1.4)	
Disease in three vascular beds (CAD, PAD, and CeVD)	4/71 (5.6)	17/78 (21.8)	0.23 (0.08, 0.68)		16.2 (5.5, 26.8)	
All patients	334/9,462 (3.5)	392/9,462 (4.1)	0.85 (0.77, 0.98)		0.6 (0.1, 1.2)	

*HRs reflect stratification by geographic region in models with interaction between treatment and the three disease bed subgroups (monovascular disease, disease in two beds, and disease in three beds). ARR, absolute risk reduction; CAD, coronary artery disease; CeVD, cerebrovascular disease; CI, confidence interval; HR, hazard ratio; MACE, major adverse cardiovascular event; PAD, peripheral artery disease.

Table 3. Safety Endpoints

	Monovascular Disease		Disease in Two Vascular Beds				Disease in Three Vascular Beds	
	CAD without PAD or CeVD		CAD and PAD		CAD and CeVD		CAD, PAD, and CeVD	
	Alirocumab (n=8,672)	Placebo (n=8,668)	Alirocumab (n=302)	Placebo (n=308)	Alirocumab (n=406)	Placebo (n=389)	Alirocumab (n=71)	Placebo (n=78)
Any adverse event	6,532 (75.3)	6,619 (76.4)	250 (82.8)	262 (85.1)	321 (79.1)	328 (84.3)	62 (87.3)	73 (93.6)
Serious adverse event	1,905 (22.0)	2,012 (23.2)	124 (41.1)	142 (46.1)	136 (33.5)	151 (38.8)	37 (52.1)	45 (57.7)
Adverse event that led to death	143 (1.6)	175 (2.0)	15 (5.0)	15 (4.9)	22 (5.4)	24 (6.2)	1 (1.4)	8 (10.3)
Adverse event that led to treatment discontinuation	298 (3.4)	285 (3.3)	21 (7.0)	15 (4.9)	19 (4.7)	18 (4.6)	5 (7.0)	6 (7.7)
Local injection-site reaction	339 (3.9)	185 (2.1)	8 (2.6)	4 (1.3)	9 (2.2)	11 (2.8)	4 (5.6)	3 (3.8)
General allergic reaction	670 (7.7)	643 (7.4)	31 (10.3)	38 (12.3)	41 (10.1)	45 (11.6)	6 (8.5)	10 (12.8)
Diabetes worsening or diabetic complication in patients with diabetes at baseline	444/2,369 (18.7)	521/2,427 (21.5)	23/99 (2.3)	28/126 (22.2)	29/188 (15.4)	32/161 (19.9)	10/32 (31.3)	2/33 (6.1)
New-onset diabetes among patients without diabetes at baseline*	595/6,303 (9.4)	617/6,241 (9.9)	26/203 (12.8)	22/182 (12.1)	24/218 (11.0)	32/228 (14.0)	3/39 (7.7)	5/45 (11.1)
Neurocognitive disorder	120 (1.4)	143 (1.6)	6 (2.0)	10 (3.2)	9 (2.2)	10 (2.6)	8 (11.3)	4 (5.1)
Hepatic disorder	450 (5.2)	493 (5.7)	19 (6.3)	18 (5.8)	24 (5.9)	21 (5.4)	7 (9.9)	2 (2.6)
Cataracts	99 (1.1)	117 (1.3)	8 (2.6)	9 (2.9)	10 (2.5)	5 (1.3)	3 (4.2)	3 (3.8)
Hemorrhagic stroke, adjudicated (fatal and nonfatal)	10 (0.1)	13 (0.1)	1 (0.3)	1 (0.3)	2 (0.5)	3 (0.8)	0	0

Values are n/N (%) or n (%). *New-onset diabetes was defined according to the presence of one or more of the following, with confirmation of the diagnosis by blinded external review by experts in the field of diabetes: an adverse-event report, a new prescription for diabetes medication, a glycated hemoglobin level of ≥6.5% on two occasions (and a baseline level of <6.5%), or a fasting serum glucose level of ≥126 mg/dl (7.0 mmol/l) on two occasions (and a baseline level of <126 mg/dl). CAD, coronary artery disease; CeVD, cerebrovascular disease; PAD, peripheral artery disease

DISCUSSION

In patients with recent ACS and dyslipidemia despite intensive statin therapy and high rates of guideline-directed medical therapy, polyvascular disease is associated with high risks of MACE and death. The large absolute reductions in both MACE and death with alirocumab therapy are a potential benefit for this population of patients.

This analysis of ODYSSEY OUTCOMES defines easily identifiable subsets of patients with ACS with high absolute risk and marked absolute benefit of PCSK9 inhibition with alirocumab. Identification of patient subsets likely to derive large absolute benefit is important (15, 16).

Increasing risks of MACE and death in patients with an increasing number of affected vascular beds has been described previously in large cohorts such as the REACH, CRUSADE, and the American Heart Association Get With The Guidelines registries (2, 5, 17), but remains a therapeutic challenge. It is likely that the elevated cardiovascular risk associated with polyvascular disease is due in part to clustering of risk factors known to affect prognosis, including older age and more frequent history of hypertension, diabetes, prior myocardial infarction, coronary artery bypass surgery, and chronic kidney disease, as was observed in the present analysis. Dyslipidemia, including higher levels of LDL-C and lipoprotein(a), was also more pronounced in patients with polyvascular disease than in patients with monovascular (coronary) disease (Table 1). Studies have shown that high-intensity compared with low-to-moderate-intensity statin therapy reduces MACE and death in patients with polyvascular disease, including trials with ACS, but also PAD and CeVD (7, 18, 19). Our findings reinforce and extend this concept with reduction of LDL-C below levels achievable with statins using alirocumab. Although alirocumab produced a similar degree of LDL-C lowering in each vascular category, a particularly pronounced absolute reduction of MACE and death was observed in patients with ACS and concurrent disease in other vascular beds. Similar conclusions regarding MACE were drawn from an analysis of the FOURIER trial, employing the PCSK9 inhibitor evolocumab added to statin in patients with stable, established atherosclerotic cardiovascular disease (12).

In that analysis, evolocumab reduced the risk of cardiovascular events in patients with PAD. Of note, due to trial selection criteria, patients with PAD comprised 13.2% of the FOURIER cohort, compared with 3.2% in ODYSSEY OUTCOMES (12). However, some patients in FOURIER with PAD or CeVD had monovascular disease in those territories. On the other hand, because qualification for ODYSSEY OUTCOMES required ACS, all patients with PAD or CeVD had disease in at least two vascular beds.

LIMITATIONS

A substantial fraction of the patients categorized as having monovascular (coronary) disease may have had undetected PAD or CeVD, since they were not systematically evaluated for those conditions at baseline. However, the classification employed in the present analysis is representative of daily clinical practice and decision-making, as ACS patients are not routinely screened for polyvascular disease (20).

CONCLUSIONS

The present findings indicate that patients with polyvascular disease are an easily identifiable subgroup of patients with recent ACS with high absolute risk of MACE and death. The large absolute benefit of PCSK9 inhibition with alirocumab, when added to high-intensity statin therapy, is a potential benefit for this population of patients. However, further studies are needed to guide the selection of patients with ACS for treatment with a PCSK9 inhibitor in the context of other established and evolving therapies in atherosclerosis, so that efficacy and efficiency are optimized (21-23).

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Supplemental table 1. Primary MACE Endpoint and All-Cause Death by History of Vascular Disease Category						
	Alirocumab n/N (%)	**Placebo n/N (%)**	**HR* (95% CI)**	**HR Interaction p Value**	**ARR (95% CI)**	**ARR Interaction p Value**
Primary composite						
Monovascular disease (CAD without PAD or CeVD)	740/8,683 (8.5)	866/8,687 (10.0)	0.85 (0.77, 0.93)	0.92	1.4 (0.6, 2.3)	0.0005
Polyvascular disease (CAD with PAD and/or CeVD)	163/779 (20.9)	186/775 (24.0)	0.86 (0.69, 1.06)		3.1 (−1.1, 7.2)	
All patients	903/9,462 (9.5)	1,052/9,462 (11.1)	0.85 (0.78, 0.93)		1.6 (0.7, 2.4)	
All-cause death						
Monovascular disease (CAD without PAD or CeVD)	268/8,683 (3.1)	305/8,68 (3.5)	0.88 (0.75, 1.04)	0.24	0.4 (−0.1, 1.0)	0.0220
Polyvascular disease (CAD with PAD and/or CeVD)	66/779 (8.5)	87/775 (11.2)	0.71 (0.51, 0.97)		2.8 (−0.2, 5.7)	
All patients	334/9,462 (3.5)	392/9,462 (4.1)	0.85 (0.77, 0.98)		0.6 (0.1, 1.2)	

*HRs reflect stratification by geographic region in models with interaction between treatment and monovascular vs. polyvascular disease.
ARR, absolute risk reduction; CAD, coronary artery disease; CeVD, cerebrovascular disease; CI, confidence interval; HR, hazard ratio; MACE, major adverse cardiovascular event; PAD, peripheral artery disease.

Supplemental table 2. Primary MACE Endpoint and All-Cause Death by Subgroup of Patients with ACS

	Alirocumab n/N (%)	Placebo n/N (%)	HR (95% CI)	Log-Rank p-Value	ARR (95% CI)
Primary MACE					
Neither PAD nor CeVD	740/8,683 (8.5)	866/8,687 (10.0)	0.85 (0.77, 0.93)	0.0008	1.4 (0.6, 2.3)
PAD (with or without concurrent CeVD)	88/373 (23.4)	104/386 (26.9)	0.83 (0.62, 1.10)	0.19	3.4 (-2.8, 9.5)
CeVD (with or without concurrent PAD)	94/477 (19.7)	113/467 (24.2)	0.80 (0.61, 1.05)	0.10	4.5 (-0.8, 9.8)
Both PAD and CeVD	19/71 (26.8)	31/78 (39.7)	0.63 (0.35, 1.12)	0.11	13.0 (-2.0, 28.0)
All patients	903/9,462 (9.5)	1,052/9,462 (11.1)	0.85 (0.78, 0.93)	0.0003	1.6 (0.7, 2.4)
All-cause death					
Neither PAD nor CeVD	268/8,683 (3.1)	305/8,687 (3.5)	0.88 (0.75, 1.04)	0.12	0.4 (-0.1, 1.0)
PAD (with or without concurrent CeVD)	32/373 (8.6)	44/386 (11.4)	0.71 (0.45, 1.12)	0.14	2.8 (-1.4, 7.1)
CeVD (with or without concurrent PAD)	38/477 (8.0)	60/467 (12.8)	0.56 (0.37, 0.84)	0.0047	4.9 (1.0, 8.8)
Both PAD and CeVD	4/71 (5.6)	17/78 (21.8)	0.23 (0.08, 0.68)	0.0038	16.2 (5.5, 26.8)
All patients	334/9,462 (3.5)	392/9,462 (4.1)	0.85 (0.77, 0.98)	0.0261	0.6 (0.1, 1.2)

HRs and p-values reflect stratification by geographic region.

ACS, acute coronary syndrome; ARR, absolute risk reduction; CeVD, cerebrovascular disease; CI, confidence interval; HR, hazard ratio; MACE, major adverse cardiovascular event; PAD, peripheral artery disease.

CHAPTER 7. EDITORIAL

HOW THE COW ATE THE CABG.

AIM LOW, LIVE LONGER?

Jacques Genest, Alexandre M. Bélanger, Mandeep S. Sidhu.

Based on: How the Cow Ate the CABG: Aim Low, Live Longer? J Am Coll Cardiol. 2019;74(9):1187-9.

EDITORIAL

"To tell someone how the cow ate the cabbage" means to tell the person the unvarnished truth, even if the person would rather not hear it. (1)

More than 35 years ago, Dr. Lucien Campeau from the Montreal Heart Institute demonstrated that progression of atherosclerosis in bypassed or native coronary arteries was strongly influenced by patients who continue to smoke, did not take aspirin, and had elevated plasma levels of atherogenic lipoproteins. This study included 82 patients and radically changed the management of patients' status post coronary artery bypass grafting (CABG) (2). Shortly after this publication, statins became available and constitute, along with smoking cessation and aspirin use, the cornerstone of secondary prevention for atherosclerotic cardiovascular diseases (ASCVD).

The next 20 years confirmed the utility and effectiveness of statin therapy to decrease low-density lipoprotein-cholesterol (LDL-C) in secondary prevention of ASCVD (3).The use of moderate versus high doses of statins on clinical outcomes, including cardiovascular death, nonfatal myocardial infarction, stroke, and the need for revascularization (or atherosclerosis progression) created some controversy, mostly based in small, underpowered clinical trials (4). In the Cholesterol Treatment Trialists meta-analysis of statin trials, there was a significant trend ($p = 0.0002$) toward greater proportional reductions in coronary revascularization (CABG or angioplasty) associated with a greater mean absolute LDL-C reduction in the different trials (3). Lowering

LDL-C further with ezetimibe led to an additional reduction in major adverse cardiovascular events (MACE) in IMPROVE-IT (Improved Reduction of Outcomes: Vytorin Efficacy International Trial), albeit with no mortality benefit (5, 6). Further lowering of LDL-C with high-dose statins and ezetimibe had reached a plateau.

Then, the seminal discovery of an obscure protein, proprotein convertase subtilisin/kexin type 9 (PCSK9) that chaperones the LDL receptor to an intracellular degradation site led to the identification of the third genetic defect in familial hypercholesterolemia. Interestingly, loss-of-function genetic variants in the PCSK9 gene are associated with a marked decrease in LDL-C and protection against ASCVD. On the basis of these results, PCSK9 inhibition became a therapeutic target (7). Inhibiting this pathway leads to an increase in cell-surface LDL receptors and a marked decrease, by more than 50%, in circulating LDL-C. Large-scale clinical trials of PCSK9 inhibitors have led to the conclusion that lowering LDL-C and all atherogenic lipoproteins improves cardiovascular outcomes (8, 9).

Presented in this issue of the Journal are two secondary analyses of the ODYSSEY OUTCOMES (Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab) trial in patients with a recent acute coronary syndrome (ACS). The authors examined two extremely high-risk populations; Goodman et al. (10) evaluated the effects of PCSK9 inhibition in a population with prior CABG, Jukema et al. (11) evaluated whether polyvascular disease influenced risks of MACE and death with PCKS9 inhibition. The Goodman et al. study is a pre-specified analysis, investigating the effects of alirocumab on outcomes, according to CABG status. Patients were categorized as no CABG (n = 16,896); index CABG after the qualifying ACS (n = 1,025) or CABG before the qualifying ACS (n = 1,003). The prior CABG patients had a greater burden of cardiovascular (CV) risk factors and were at higher CV risk than the no-CABG or index CABG participants, as reflected by increased age, a higher burden of atherogenic lipoproteins, an increased prevalence of hypertension and diabetes, and an increased burden of atherosclerosis in noncoronary vascular beds. Of interest, fewer prior CABG patients were given

a high-dose statin (83.2% vs. 89.2% for the no-CABG group and 87.8% for the index CABG group) even though they showed the highest LDL-C burden with 41.7% of them having a LDL-C level above 100mg/dl (compared with 28.9% and32.5%, respectively). Despite the IMPROVE-IT study and the current guidelines (6, 12), few patients were on ezetimibe (8.9% in the prior CABG group). In each CABG category, the hazard ratios for MACE were: no CABG 0.86 (95% confidence interval [CI]: 0.78 to 0.95), index CABG 0.85 (95% CI: 0.54 to 1.35), and prior CABG 0.77 (95% CI: 0.61 to 0.98). Similarly, the hazard ratios for all-cause death were: no CABG subgroup 0.88 (95% CI: 0.75 to 1.03), index CABG subgroup 0.85 (95% CI: 0.46 to 1.59), and prior CABG subgroup 0.67 (95% CI: 0.44 to 1.01), respectively. Also in this issue, Jukema et al. (11) present a pre-specified secondary analysis of the ODYSSEY OUTCOMES trial in participants with ACS and noncoronary atherosclerosis defined as "polyvascular disease," investigating the effects of alirocumab on outcomes, according to their vascular disease status. Patients were categorized as monovascular (coronary) disease (n = 17,370); polyvascular disease in 2 vascular territories (coronary and peripheral artery or cerebrovascular) with n = 1,405; and polyvascular disease in 3 vascular territories (coronary, peripheral, and cerebrovascular) with n = 149. Compared with placebo, the incidence of MACE by respective involvement of vascular territories was 10.0%, 22.2%, and 39.7%, respectively. The absolute risk reduction with intervention with alirocumab therapy was 1.4% (95% CI: 0.6% to 2.3%), 1.9% (95% CI: –2.4% to 6.2%) and 13.0% (95% CI: –2.0% to 28.0%).Compared with placebo, the incidence of death by respective involvement of vascular territories was 3.5%, 10.0%, and 21.8%, respectively, with corresponding absolute risk reduction with intervention with alirocumab of 0.4% (95% CI: –0.1% to 1.0%), 1.3% (95% CI: –1.8% to 4.3%) and 6.2% (95% CI: 5.5% to 26.8%), respectively. Similar to the prior CABG participants, these participants with a recent ACS and polyvascular disease and an elevated LDL-C >70 mg/dl despite intensive statin therapy, were found to have higher risks of MACE and death. The large absolute risk reductions demonstrate the potential benefit of lowering LDL-C below current guideline recommendations (12).

The Goodman and Jukema analyses followed rigorous pre-specified analytical plans. The authors acknowledge that patients with a prior CABG and polyvascular disease were at very high CV risk judging by their risk factors in Table 1 of both analyses and further confirm that higher risk patients obtain the greatest absolute benefit of lipid-lowering therapy. In the original ODYSSEY OUTCOMES trial publication, the hierarchical analysis of main secondary efficacy endpoints in ODYSSEY OUTCOMES were coronary heart disease (CHD) event; major CHD event; CV event; death, myocardial infarction, and ischemic stroke; CHD death; CV death; and all-cause death. The latter was significant, but the question of "true" statistical significance was raised in light of this hierarchical analysis. The authors acknowledged this point.

These are important pre-specified secondary analyses, confirming that patients post CABG and polyvascular patients are at very high CV risk and benefit from aggressive lipid-lowering therapy. It is a concern that, despite a higher burden of cardiovascular risk factors and extent of atherosclerosis, patients with a prior CABG and those with a high burden of atherosclerosis in 2 or more arterial territories are seemingly less well treated that recent ACS patients. More than 40% of such participants have an LDL-C level ≥100 mg/dl at the time of randomization; 16% currently smoke, 40% have diabetes, and 87% have high blood pressure.

A reasonable first step in the approach to patient care is for physicians to be less complacent and apply, with enthusiasm, secondary prevention guidelines in post CABG and polyvascular disease patients (12, 13), to strive to eliminate smoking and enforce lifestyle modifications with known benefits in ASCVD. The studies by Goodman et al. (10) and Jukema et al. (11) show once again that patients at the highest risk derive the most benefit from aggressive LDL-C lowering. The significant reduction in mortality associated with a low LDL-C on alirocumab can no longer be ignored. Returning to Campeau et al.'s (2) 1984 paper, the lessons learned are critical for patient care: decreasing atherogenic lipoproteins in patients with extensive atherosclerosis to a very low-level decreases cardiovascular risk and mortality. Finally, it would be interesting to conduct an economic analysis as such a reduction in both MACE events and

all-cause deaths with alirocumab should have an effect on health care costs and on society in general.

To conclude, these are both very important studies that reinforce the role of PCSK9 inhibitors in the treatment of patients post-ACS who had a prior CABG or in patients with polyvascular disease. Although each study has limitations as acknowledged, it provides further evidence that should lead us to strongly consider the use of PCSK9 inhibitors in patients who had a previous CABG or a history of polyvascular disease (14).

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CHAPTER 7. LETTER TO THE EDITOR

ALIROCUMAB IN POLYVASCULAR ATHEROSCLEROTIC DISEASE

Mohammad Alkhalil

Based on: Alirocumab in Polyvascular Atherosclerotic Disease. J Am Coll Cardiol. 2020;75(2):240-1.

LETTER TO THE EDITOR

Jukema et al demonstrated in a pre-specified analysis from the ODYSSEY-OUTCOMES trial that alirocumab was associated with absolute risk reduction (ARR) in cardiovascular events related to the distribution of evident vascular disease [1.4% for monovascular disease (MVD), 1.9% for polyvascular in two beds (PVD-2), and 13% in polyvascular in three beds (PVD-3)]. (1) Nonetheless, the relative risk reduction using alirocumab did not follow the same trend (15% for MVD, 8.6% for PVD-2, and 36% in PVD-3). This was in contrast to statin trials whereby lowering LDL-c produced a consistent reduction in vascular events among patients with different clinical characteristics. (2) Importantly, the magnitude of LDL-c reduction across the three subgroups was relatively comparable (1.4 mmol/L for MVD, 1.5 mmol/L for PVD-2, and 1.7 mmol/L in PVD-3) and may suggest that monitoring response to intensive lipid-lowering therapy (additional to statin) can no longer be guided using LDL-c only. Such observation should not be surprising since other biomarkers have demonstrated strong associations with cardiovascular events irrespective of LDL-c. (3) Furthermore, the ARR in patients with PVD-2 was relatively modest when compared to the other two subgroups, despite having an 'in-between' baseline absolute risk (10%, 22.2%, 39.7% respectively). Such mismatch between ARR and baseline absolute risk maybe related to the used method of classifying patients based on their disease category (i.e. stroke or myocardial infarction) without mechanistic insight into how alirocumab reduced their risk. (4, 5)

The mechanism of how reducing LDL-c is translated into reduction of cardiovascular events is believed to be related to the reduction in atherosclerosis burden and/or changes in plaque composition. Therefore, plaque imaging techniques have the potential to offer mechanistic stratification tools that could identify patients who may benefit maximally from additional lipid lowering therapy. (4, 5)

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CHAPTER 7. REPLY LETTER TO THE EDITOR

ALIROCUMAB IN POLYVASCULAR ATHEROSCLEROTIC DISEASE

Laurien E. Zijlstra, Gregory G. Schwartz, Philippe Gabriel Steg, J. Wouter Jukema

Based on: Reply: Alirocumab in Polyvascular Atherosclerotic Disease. J Am Coll Cardiol. 2020; 75(2):241.

REPLY LETTER TO THE EDITOR

We appreciate the comments by Dr. Alkhalil on our paper (1). Measurement of plaque burden might provide information that would complement levels of circulating biomarkers, including low-density lipoprotein cholesterol (LDL-C), to identify subsets of patients who derive particular benefit from alirocumab treatment. Although LDL-C reduction and regression or slowed progression of coronary atheroma volume have been closely linked in prior investigations (e.g. REVERSAL, SATURN, GLAGOV) (2), it is not established whether vascular imaging should guide therapy with PCSK9 inhibitors.

Although most patients in ODYSSEY OUTCOMES underwent coronary angiography and 72% underwent percutaneous coronary intervention or coronary bypass surgery for the qualifying acute coronary syndrome, systematic collection of angiographic data accumulated prior to randomization was not possible in this large, multicenter, multinational trial. Therefore, we cannot determine whether the burden of coronary artery disease predicted the therapeutic benefit of alirocumab. It is possible that other imaging techniques that assess volume, virtual histology, or inflammatory characteristics of atherosclerotic plaque (3) might contribute in that regard, but such hypotheses remain to be tested. Moreover, application of vascular imaging techniques may be limited by invasiveness, sensitivity, cost, availability or ease of implementation in daily clinical practice.

Risk stratification to guide optimal application of therapies in atherosclerosis is most often based on levels of lipid and inflammatory biomarkers and readily identified clinical features. The latter include diabetes, a history of coronary bypass grafting, or in the case of our analysis, polyvascular disease. Subanalyses of ODYSSEY OUTCOMES show that the presence of such high-risk characteristics is associated with absolute benefit of PCSK9 inhibition with alirocumab, when added to high-intensity statin therapy (1, 4, 5).

An important future research objective would be to determine whether vascular imaging adds further prognostic information that helps to predict the clinical benefit of PCSK9 inhibitor treatment.

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CHAPTER 8.

PCSK9 INHIBITION AND STROKE

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Based on: Effect of Alirocumab on Stroke in ODYSSEY OUTCOMES. Circulation. 2019;140(25):2054-62.

ABSTRACT

INTRODUCTION

Lowering of atherogenic lipoproteins, including low-density lipoprotein cholesterol (LDL-C), reduces the risk of ischemic stroke. However, concerns have been raised about very low LDL-C levels and a potential increased risk of hemorrhagic stroke. ODYSSEY OUTCOMES compared the PCSK9 inhibitor alirocumab with placebo in 18,924 patients with recent acute coronary syndrome (ACS) and elevated atherogenic lipoproteins, despite intensive statin therapy, targeting LDL-C levels of 25-50 mg/dL and avoiding sustained LDL-C <15 mg/dL. This prespecified analysis was designed to assess the effect of alirocumab on ischemic and hemorrhagic stroke. We hypothesized that for patients treated with alirocumab there would be a reduction in risk of ischemic stroke without increasing hemorrhagic stroke, irrespective of baseline LDL-C and of history of cerebrovascular disease (CeVD).

METHODS

Patients were randomized to alirocumab or placebo 1-12 months after ACS. The risk of nonfatal or fatal ischemic or hemorrhagic stroke was evaluated, stratified by baseline LDL-C concentration and history of CeVD. A potential association of very low achieved LDL-C with alirocumab treatment at month 4 and subsequent hemorrhagic stroke was assessed.

RESULTS

Median follow-up was 2.8 years. In total, 263 ischemic and 33 hemorrhagic strokes occurred. Alirocumab reduced the risk of any stroke [HR 0.72 (0.57–0.91)] and ischemic stroke [HR 0.73 (0.57–0.93)] without increasing hemorrhagic stroke [HR 0.83 (0.42–1.65)]. In total, 7164 (37.9%), 6128 (32.4%), and 5629 (29.7%) patients had a baseline LDL-C of <80, 80–100, >100 mg/dL, respectively. The treatment effect on stroke appeared numerically greater for patients with higher baseline LDL-C, but there was no formal

evidence of heterogeneity (Pinteraction=0.31). The effect of alirocumab on stroke was similar among 944 patients (5.0%) with a history of prior CeVD and among those without a history of CeVD (Pinteraction=0.37). There was no apparent adverse relationship between lower achieved LDL-C and incidence of hemorrhagic stroke in the alirocumab group.

CONCLUSIONS

In patients with recent ACS and dyslipidemia despite intensive statin therapy, alirocumab decreased the risk of stroke, irrespective of baseline LDL-C and history of CeVD, over a median follow-up of 2.8 years. Furthermore, risk of hemorrhagic stroke did not depend on achieved LDL-C levels within the alirocumab group.

INTRODUCTION

Lowering of atherogenic lipoproteins with statin treatment reduces the risk of first or recurrent stroke,(1-3) and the benefit has been shown by the Cholesterol Treatment Trialist meta-analysis to be directly proportional to the degree of absolute lowering of low-density lipoprotein cholesterol (LDL-C).(4) Accordingly, international guidelines recommend statin treatment for patients at high cardiovascular risk or with established cardiovascular disease with or without a history of cerebrovascular disease (CeVD), to prevent major cardiovascular events, including ischemic stroke.(5, 6)

Although some data have raised a potential association of very low LDL-C levels and risk of hemorrhagic stroke,(7, 8) the decrease in ischemic stroke outweighed the potential increase in hemorrhagic stroke.(4, 9) The advent of inhibitors of proprotein convertase subtilisin–kexin type 9 (PCSK9) provided an opportunity to lower LDL-C to levels not previously achievable in most patients with statins and/or ezetimibe. Two large cardiovascular outcomes trials have compared the effect of a fully human PCSK9 inhibitor with placebo on the risk of stroke in patients with atherosclerotic cardiovascular disease and elevated atherogenic lipoproteins despite background statin treatment.(10, 11) In both trials, treatment with the PCSK9 inhibitor lowered LDL-C by more than 50% below the statin-treated baseline. The FOURIER (Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Patients With Elevated Risk) trial(10) compared evolocumab with placebo in patients with established, stable, atherosclerotic cardiovascular disease. Evolocumab treatment reduced the risk of ischemic stroke, without a significant effect on hemorrhagic stroke. The ODYSSEY OUTCOMES (Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab) trial(11) compared alirocumab with placebo in 18 924 patients with recent acute coronary syndrome (ACS), and showed a reduction in major adverse cardiovascular events with alirocumab compared to placebo.

This prespecified analysis was designed to assess the effect of alirocumab on ischemic and hemorrhagic stroke. We hypothesized that for patients treated with alirocumab there would be a reduction in risk of ischemic stroke without

increasing hemorrhagic stroke, irrespective of baseline LDL-C and of history of CeVD.

METHODS

Details of the study design(12) and primary efficacy and safety results(11) have been published. In brief, ODYSSEY OUTCOMES was a multicenter, double-blind, placebo-controlled trial in 18 924 patients at least 40 years of age who provided written informed consent and had been hospitalized with an ACS (defined as myocardial infarction or unstable angina) 1 to 12 months before randomization. Qualifying patients had a level of LDL-C ≥70 mg/dL (1.81 mmol/L), or non–high-density lipoprotein cholesterol (non-HDL-C) ≥100 mg/dL (2.59 mmol/L), or apolipoprotein B ≥80 mg/dL (0.8 mmol/L), measured after a minimum of 2 weeks of stable treatment with atorvastatin 40–80 mg daily, rosuvastatin 20–40 mg daily, or the maximum tolerated dose of either statin (including no statin in case of documented intolerance). All patients provided written informed consent. All sites obtained institutional review board approval as per local and national guidelines.

Patients were randomly assigned in a 1:1 ratio stratified by country to receive treatment with alirocumab 75 mg subcutaneously every 2 weeks or matching placebo. In case of a persistent LDL-C ≥50 mg/dL, the dose of alirocumab was up-titrated to 150 mg/dL. When two consecutive measurements of LDL-C <25 mg/dL were identified, the alirocumab dose was reduced to 75 mg (if measurements were made on the 150-mg dose) and safety was monitored by an independent physician blinded to treatment allocation. In case of two consecutive measurements of LDL-C <15 mg/dL on alirocumab 75 mg, alirocumab was discontinued with blinded substitution of placebo for the remainder of the trial. The protocol did not specify any change to the background statin dose.

Patients were compared based on three prespecified categories of baseline LDL-C (ie, <80 mg/dL, 80–100 mg/dL, ≥100 mg/dL). We compared the effect of alirocumab on stroke among patients with or without a history of CeVD,

defined as a history of carotid endarterectomy, carotid stenting, prior stroke or transient ischemic attack. A multivariable prediction model of stroke risk was performed. Finally, a subgroup analysis was performed based on the treat-to-target design of the trial, where patients assigned to alirocumab treatment were classified in the following categories based on their achieved LDL-C value at month 4: below target range (<25 mg/dL), within target range (25 to <50 mg/dL), above target range (50 to <70 mg/dL) and very above target range (≥70 mg/dL). In this analysis, the incidence of hemorrhagic stroke after month 4 was summarized among patients in each of these achieved LDL-C categories.

ENDPOINTS

End points were classified as fatal or nonfatal ischemic or hemorrhagic stroke, adjudicated by physicians who were unaware of the study treatment group assignments. As part of the original analysis conventions, an ischemic or unclassified stroke that was followed by a death within 30 days with a cause of ischemic or undetermined stroke was considered a fatal ischemic stroke, with an event date of the initial event. For the purposes of the current report, this convention was applied for hemorrhagic strokes (ie, a hemorrhagic stroke that was followed by a death within 30 days was considered a fatal hemorrhagic stroke, with an event date of the initial event). Additionally, nine nonfatal strokes with an unclassified cause were grouped with nonfatal ischemic strokes, as pre-specified in the design of the study.

STATISTICAL ANALYSIS

Analyses of clinical outcomes and LDL-C levels were performed according to the intention-to-treat principle, including all patients, events, and measurements from randomization to the study end date (November 11, 2017). Hazard ratios (HR) and 95% confidence intervals (CIs) were estimated by Cox proportional hazards models, stratified by geographic region; P-values were determined using stratified log-rank tests. End point rates were based on observed incidences. The treatment proportional hazards assumption

for each type of stroke (any, ischemic, hemorrhagic) was assessed by a Kolmogorov-type supremum test. A multivariable model was performed to predict all-cause stroke with stepwise selection, using P=0.05 for entry or exit. Prespecified candidate variables were age category, sex, race, region, index event, lipid-lowering therapy at randomization, LDL-C, high-density lipoprotein cholesterol, lipoprotein(a), body mass index, systolic blood pressure, glomerular filtration rate (GFR), diabetes, hypertension, myocardial infarction, CeVD, malignant disease, percutaneous coronary intervention, chronic obstructive pulmonary disease, coronary artery bypass grafting, peripheral artery disease, chronic heart failure, venous thromboembolism, atrial fibrillation, current smoker, revascularization for index event, oral adenosine diphosphate receptor antagonist, oral anticoagulant, and alirocumab treatment. Relationships between categories of achieved month-4 LDL-C and subsequent hemorrhagic stroke in the alirocumab group were summarized by descriptive statistics. Analyses were performed in SAS 9.4 and S+ 8.2.

RESULTS

Of 18924 randomized patients, 9462 were assigned to the alirocumab group and 9462 to the placebo group, with a median (quartile 1, quartile 3) follow-up of 2.8 (2.3, 3.4) years. There were no major differences in baseline characteristics between the alirocumab group and the placebo group.(11) At baseline there were 944 (5.0%) patients with a history of CeVD and 17 980 (95.0%) without a history of CeVD.

Table 1 summarizes the baseline characteristics of patients with or without a history of CeVD. Compared to patients without a history of CeVD, those with CeVD were older (median age 63 vs 58 years) and included more women (31.9% vs 24.8%). Of all patients with CeVD, 611 (64.7%) had a history of stroke. Furthermore, compared to patients without a history of CeVD, those with CeVD had a higher systolic blood pressure and more often had comorbidities, including a history of diabetes, hypertension, myocardial infarction, atrial fibrillation, peripheral artery disease, venous thromboembolism, chronic obstructive pulmonary disease, heart failure, malignant disease, percutaneous coronary intervention, coronary artery bypass grafting, and a GFR <60 mL/min/1.73 m2. Median (quartile 1, quartile 3) baseline LDL-C was 91 (76, 110) mg/dL in patients with CeVD versus 86 (73, 104) mg/dL in those without CeVD.

Table 1. Baseline Characteristics by History of Cerebrovascular Disease			
Variable	**History of CeVD (n=944)**	**No History of CeVD (n=17,980)**	**p-value**
Age, years	63 (57, 70)	58 (52, 65)	<0.001
Women	301 (31.9)	4461 (24.8)	<0.001
Race			
White	754 (79.9)	14,270 (79.4)	0.004
Asian	107 (11.3)	2391 (13.3)	
Black	39 (4.1)	434 (2.4)	
Other	44 (4.7)	885 (4.9)	
Geographic region			
Western Europe	171 (18.1)	4004 (22.3)	<0.001
Eastern Europe	255 (27.0)	5182 (28.8)	
North America	224 (23.7)	2647 (14.7)	
South America	111 (11.8)	2477 (13.8)	
Asia	101 (10.7)	2192 (12.2)	
Rest of world	82 (8.7)	1478 (8.2)	
Risk factors/medical history			
Body mass index, kg/m^2	28.1 (25.2, 31.3)	27.9 (25.2, 31.1)	0.24
Systolic blood pressure, mmHg	130 (120, 141)	126 (117, 137)	<0.001
Diabetes	414 (43.9)	5030 (28.0)	<0.001
Current smoking	190 (20.1)	4370 (24.3)	0.003
Hypertension	830 (87.9)	11,419 (63.5)	<0.001
Myocardial infarction	288 (30.5)	3351 (18.6)	<0.001
Stroke	611 (64.7)	0	<0.001
Atrial fibrillation	54 (5.7)	357 (2.0)	<0.001
Peripheral artery disease	149 (15.8)	610 (3.4)	<0.001
Venous thromboembolism	17 (1.8)	182 (1.0)	0.021
Chronic obstructive pulmonary disease	69 (7.3)	677 (3.8)	<0.001
Heart failure	232 (24.6)	2583 (14.4)	<0.001
Malignant disease	46 (4.9)	486 (2.7)	<0.001
Percutaneous coronary intervention	277 (29.3)	2964 (16.5)	<0.001
Coronary artery bypass graft	139 (14.7)	908 (5.1)	<0.001
GFR <60 mL/min/1.73 m^2	265 (28.1)	2274 (12.7)	<0.001

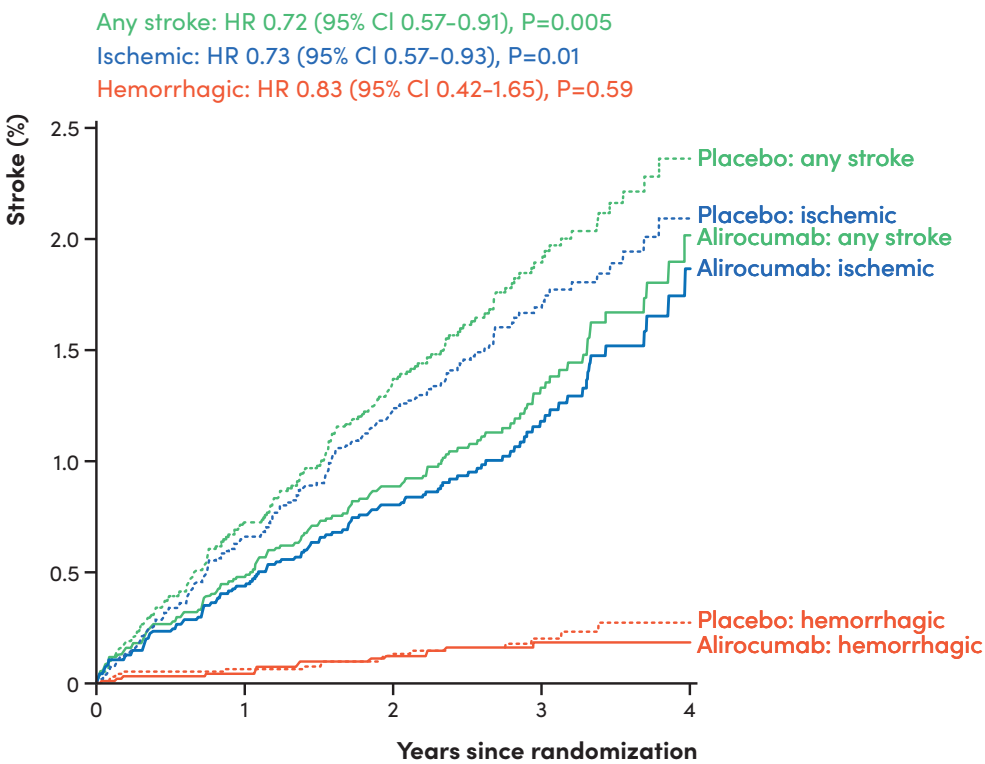
Table 1. (Continued) Baseline Characteristics by History of Cerebrovascular Disease			
Index event			
Time from ACS to randomization, months	2.8 (1.8, 4.7)	2.6 (1.7, 4.3)	0.04
Acute coronary syndrome type			
Non–ST-segment elevation myocardial infarction	533 (56.6)	8642 (48.1)	<0.001
ST-segment elevation myocardial infarction	261 (27.7)	6275 (35.0)	
Unstable angina	148 (15.7)	3034 (16.9)	
Revascularization	645 (68.3)	13,032 (72.5)	0.005
Medications			
Aspirin	875 (92.7)	17,211 (95.7)	<0.001
High-intensity statin	800 (84.7)	16,011 (89.0)	<0.001
Oral ADP receptor antagonist	922 (97.7)	17,782 (98.9)	0.001
Specific oral anticoagulant	101 (10.7)	680 (3.8)	<0.001
ACE inhibitor or ARB	778 (82.4)	13,938 (77.5)	<0.001
Beta-blocker	796 (84.3)	15,199 (84.5)	0.86
Lipoproteins, mg/dL			
Lipoprotein(a)	22.4 (7.6, 62.1)	21.2 (6.9, 57.5)	0.09
HDL-C	43 (36, 51)	42 (36, 50)	0.20
LDL-C	91 (76, 110)	86 (73, 104)	<0.001
Non–HDL-C	121 (104, 144)	115 (99, 137)	<0.001
Triglycerides	136 (97, 190)	129 (94, 182)	0.002
Total cholesterol	166 (148, 190)	159 (142, 182)	<0.001
Apolipoprotein B	83 (72, 96)	79 (69, 93)	<0.001
C-reactive protein, mg/dL	0.22 (0.10, 0.48)	0.16 (0.08, 0.38)	<0.001
Glycated hemoglobin A1c, %	6.1 (5.7, 7.0)	5.8 (5.5, 6.3)	<0.001
Switched to blinded placebo after randomization	27 (2.9)	703 (3.9)	0.10

Data presented as n (%), median (quartile 1, quartile 3). A Wilcoxon rank sum test was used to compare means for continuous variables and chi-square tests for categorical variables. To convert the values for cholesterol to mmol/L, multiply by 0.02586. To convert the values for triglycerides to mmol/L, multiply by 0.01129. ACE indicates angiotensin converting enzyme inhibitor; ACS, acute coronary syndrome; ADP, adenosine diphosphate receptor; ARB, angiotensin receptor blocker; CeVD, cerebrovascular disease; GFR, glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

The Kaplan–Meier curves for any stroke, ischemic stroke and hemorrhagic stroke are shown in Figure 1. In total, 263 ischemic strokes and 33 hemorrhagic strokes occurred. Of the 33 hemorrhagic strokes, 25 occurred in the safety population during the treatment-emergent adverse event reporting period,(11) and an additional 8 were captured in the intention-to-treat analysis. Alirocumab reduced the risk of any stroke (HR, 0.72; 95% CI, 0.57 to 0.91) and ischemic stroke (HR, 0.73; 95% CI, 0.57 to 0.93) without increasing hemorrhagic stroke (HR, 0.83; 95% CI, 0.42 to 1.65). There was no evidence of non-proportionality in the treatment effects (supremum test P = 0.56, 0.35, and 0.47 for any, ischemic, and hemorrhagic, respectively).

FIGURE 1. KAPLAN–MEIER CURVES FOR ANY STROKE, ISCHEMIC STROKE AND HEMORRHAGIC STROKE.

CI, confidence interval; HR, hazard ratio.



Number at risk

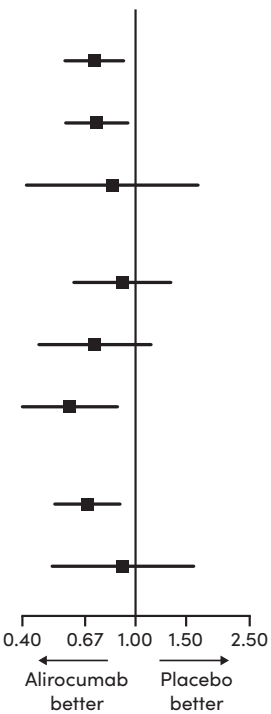
Placebo	9462	9162	8789	3838	724
Alirocumab	9462	9179	8856	3901	729

Figure 2 shows the HRs for stroke by baseline LDL-C category and history of CeVD. In total, 7164 (37.9%) patients had a baseline LDL-C of <80 mg/dL, 6128 (32.4%) had a value of 80–100 mg/dL and 5629 (29.7%) had value >100 mg/dL. The treatment effect appeared numerically greater for patients with higher baseline LDL-C, but there was no formal evidence of treatment effect heterogeneity (Pinteraction =0.31). An exploratory analysis was performed in which baseline LDL-C was categorized dichotomously (<100 mg/dL and ≥100 mg/dL) which also found no formal evidence of treatment effect heterogeneity (Pinteraction =0.18). Similarly, the effect of alirocumab on stroke appeared consistent regardless of the presence (n= 944 patients, 5.0 %) or absence of a history of CeVD, (Pinteraction=0.37).

FIGURE 2. STROKE BY HISTORY OF CEVD AND BASELINE LDL-C CATEGORY.

CeVD indicates cerebrovascular disease; CI, confidence interval; LDL-C, low-density lipoprotein cholesterol; HR, hazard ratio

Subgroup and endpoint	Alirocumab n/N (%)	Placebo n/N (%)	HR (95% CI)
All patients			
Any stroke	123/9462 (1.3)	170/9462 (1.8)	0.72 (0.57–0.91)
Ischemic	111/9462 (1.2)	152/9462 (1.6)	0.73 (0.57–0.93)
Hemorrhagic	15/9462 (0.2)	18/9462 (0.2)	0.83 (0.42–1.65)
Baseline LDL-C, mg/dL			
<80: any stroke	47/3581 (1.3)	52/3583 (1.3)	0.90 (0.61–1.34)
80–<100: any stroke	33/3066 (1.1)	46/3062 (1.3)	0.72 (0.46–1.12)
≥100: any stroke	43/2814 (1.5)	72/2815 (2.4)	0.59 (0.40–0.86)
History of CeVD			
No: any stroke	98/8985 (1.1)	144/8995 (1.6)	0.68 (0.53–0.88)
Yes: any stroke	25/477 (5.2)	26/467 (5.6)	0.90 (0.52–1.56)



LDL-C $P_{interaction}$ = 0.31

CeVD $P_{interaction}$ = 0.37

The multivariable baseline predictors of any stroke are shown in Table 2. History of CeVD was the strongest predictor, with a HR of 2.469 (95% CI 1.792 to 3.401; P<0.0001). In addition, GFR <60 mL/min/1.73 m2, diabetes, heart failure, myocardial infarction, oral anticoagulants, current smoking and peripheral artery disease, and increasing age, systolic blood pressure and LDL-C were associated with an increased risk of all-cause stroke (all P<0.05). Alirocumab had the strongest negative association with stroke (HR, 0.712; 95% CI, 0.564 to 0.898; P=0.0041). Per 1-mg/dL increment, high-density lipoprotein cholesterol also had a negative association with stroke (HR, 0.989; 95% CI, 0.978 to 1.000; P=0.0476).

Table 2. Multivariable Model Predicting Stroke

Variable	HR (95% CI)	P-value
History of cerebrovascular disease	2.469 (1.792, 3.401)	<0.0001
Glomerular filtration rate <60 mL/min/1.73 m2	1.751 (1.328, 2.309)	<0.0001
Age, per 1-year increment	1.027 (1.013, 1.042)	0.0001
History of diabetes	1.589 (1.251, 2.020)	0.0001
Systolic blood pressure, per 1-mmHg increment	1.012 (1.005, 1.019)	0.0008
LDL-C, per 1-mg/dL increment	1.005 (1.002, 1.008)	0.0009
Alirocumab treatment	0.712 (0.564, 0.898)	0.0041
History of heart failure	1.502 (1.124, 2.004)	0.0060
Geographic region		0.0065
Western Europe	Reference	
Eastern Europe	1.493 (1.010, 2.206)	
North America	1.569 (1.032, 2.386)	
South America	1.603 (1.013, 2.537)	
Asia	2.288 (1.434, 3.648)	
Rest of world	2.173 (1.371, 3.445)	
Myocardial infarction before index event	1.366 (1.056, 1.770)	0.0177
Specific oral anticoagulant	1.595 (1.054, 2.415)	0.0272
Current smoker	1.355 (1.024, 1.795)	0.0339
History of peripheral artery disease	1.497 (1.007, 2.223)	0.0461
HDL-C, per 1-mg/dL increment	0.989 (0.978, 1.000)	0.0476

Candidate predictors (stepwise selection), P=0.05 for entry or exit: age category, sex, race, geographic region, index event, lipid-lowering therapy at randomization, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), lipoprotein(a), body mass index, systolic blood pressure, glomerular filtration rate, diabetes, hypertension, myocardial infarction, cerebrovascular disease, malignant disease, percutaneous coronary intervention, chronic obstructive pulmonary disease, coronary artery bypass graft, peripheral artery disease, congestive heart failure, venous thromboembolism, atrial fibrillation, current smoker, revascularization for index event, oral adenosine diphosphate receptor antagonist, oral anticoagulant, alirocumab treatment.

Achieved LDL-C at month 4 by treatment group is shown in Figure 3. Among the 9462 alirocumab-assigned patients, 3397 (35.9%) achieved LDL-C concentrations at month 4 of <25 mg/dL, 3749 (39.6%) achieved 25 to <50 mg/dL, 1087 (11.5%) achieved 50 to <70 mg/dL and 1169 (12.4%) achieved ≥70 mg/dL. Table 3 shows the incidence of hemorrhagic stroke by ordered category of month 4 achieved LDL-C in the alirocumab group. There was no apparent adverse relationship between lower achieved LDL-C and incidence of hemorrhagic stroke, with a numerically lower proportion of patients in the lowest categories of achieved LDL-C (ie, <50 mg/dL) experiencing this outcome.

FIGURE 3. MONTH 4 LDL-C BY TREATMENT GROUP.
LDL-C indicates low-density lipoprotein cholesterol.

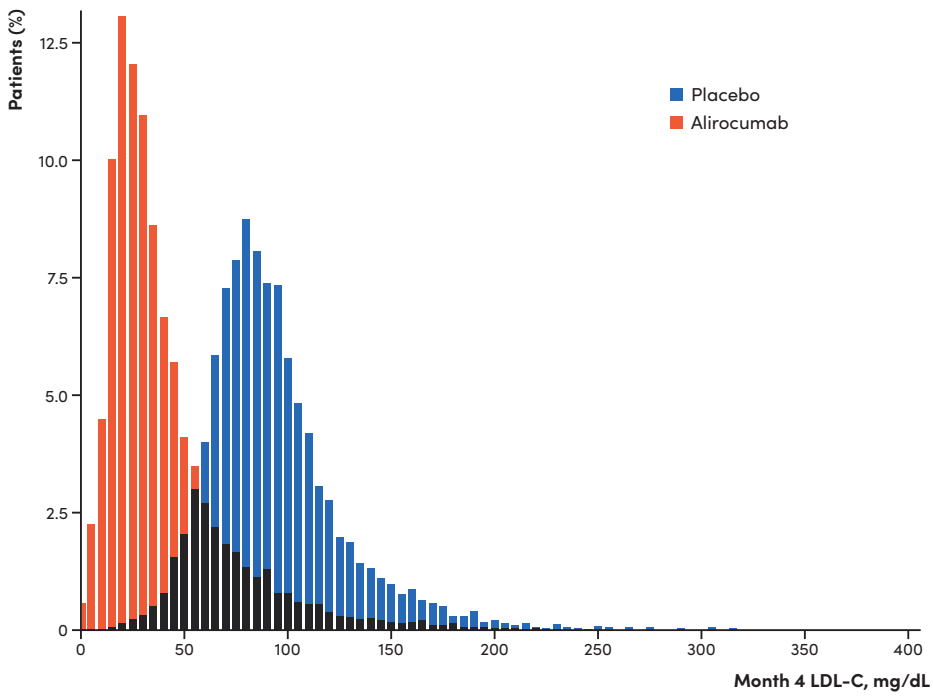


Table 3. Hemorrhagic Stroke by Achieved LDL-C Category at 4 Months in Patients Assigned to Alirocumab Treatment	
Month 4 LDL-C, mg/dL	**n/N (%)**
<25	2/3399 (0.1)
25 to <50	3/3754 (0.1)
50 to <70	3/1090 (0.3)
≥70	4/1177 (0.3)
To convert the values for cholesterol to mmol/L, multiply by 0.02586. LDL-C, low-density lipoprotein cholesterol.	

DISCUSSION

In patients with recent ACS and dyslipidemia despite intensive statin therapy, alirocumab decreased the risk of ischemic stroke without increasing hemorrhagic stroke. Furthermore, risk of hemorrhagic stroke did not depend on achieved LDL-C levels in the alirocumab group.

The treatment effect appeared numerically greater with lower HRs for patients with higher baseline LDL-C, suggesting that patients with a higher risk at baseline have a larger benefit of alirocumab. However, this linear trend was not statistically significant. Furthermore, as qualification for inclusion in ODYSSEY OUTCOMES required an ACS, patients with a history of CeVD all had polyvascular disease, which is associated with high risk of major adverse cardiovascular events and large absolute benefit of alirocumab in reducing such events.(13) Accordingly, stroke risk was markedly higher in patients with a history of CeVD, with a HR of 2.469 (95% CI, 1.792 to 3.401) in multivariable analysis. However, the treatment effect of alirocumab on stroke was similar in both patients with or without a history of CeVD. Therefore, alirocumab is a suitable therapy in patients with recent ACS, irrespective of baseline LDL-C and of history of CeVD.

The potential association of very low LDL-C with hemorrhagic stroke risk has been investigated primarily in epidemiologic studies,(14) but more recently two large prospective cohort studies tried to provide clarity on this matter in healthy participants and found increased risks of hemorrhagic stroke

with LDL-C <70 mg/dL.(7, 15) The Women's Health Study in the United states found an adjusted relative risk of 2.17 (95% CI, 1.05 to 4.48).(15) The Kailuan study in China reported adjusted HRs of 1.65 (95% CI, 1.32 to 2.05) for LDL-C 50–69 mg/dL and 2.69 (95% CI, 2.03 to 3.57) for LDL-C <50 mg/dL.(7) Despite concerns regarding hemorrhagic stroke, our findings that intensive reduction of LDL-C did not cause harm in terms of hemorrhagic stroke reinforces and extends other previous data. The SPARCL (Stroke Prevention by Aggressive Reduction in Cholesterol Levels) trial showed a reduction in overall strokes with high-intensity atorvastatin therapy, despite a small increase in hemorrhagic stroke in 4731 patients with recent stroke or transient ischemic attack.(16) SPARCL also demonstrated that achieving ≥50% LDL-C lowering was associated with a greater reduction in the risk of ischemic stroke without increasing hemorrhagic stroke, and that a higher risk of hemorrhagic stroke was largely in patients with a history of small vessel disease or nonatherothrombotic stroke.(1, 17)

Similar conclusions regarding stroke were drawn from the FOURIER trial, investigating the PCSK9 inhibitor evolocumab added to statin therapy in 27 564 patients with stable, established, atherosclerotic cardiovascular disease, including 19.4% with a history of nonhemorrhagic stroke. Evolocumab treatment significantly reduced the risk of ischemic stroke (HR, 0.75; 95% CI, 0.62 to 0.92), without a significant effect on hemorrhagic stroke (HR, 1.16; 95% CI 0.68 to 1.98).(10) Although PCSK9 inhibition may lower LDL-C to levels far below those achieved with statins alone, FOURIER found that lower achieved LDL-C did not increase the risk of hemorrhagic stroke, even when LDL-C levels were <0.5 mmol/L (20 mg/dL).(18)

As most lipid-trials have few hemorrhagic stroke events, a meta-analysis was performed recently of randomized trials, including all lipid-lowering trials with statins, ezetimibe and PCSK9 inhibition.(9) The investigators found a net benefit of lipid-lowering, with a rate ratio of 0.80 (95% CI, 0.76 to 0.84) for ischemic stroke and 1.17 (95% CI, 1.03 to 1.32) for hemorrhagic stroke, for each 1-mmol/L lower achieved LDL-C, at about 1 year of follow-up.(9) Of note, in these studies different types and doses of lipid-lowering therapies

were used in patients with or without proven vascular disease in various vascular beds, including coronary artery, peripheral artery and cerebrovascular disease. Our results are in line with this meta-analysis, as we found a large benefit of alirocumab in multivariable analysis (HR, 0.712; 95% CI, 0.564 to 0.898; P=0.0041) and no relationship between very low achieved LDL-C and incidence of hemorrhagic stroke. However, as only 33 patients had a hemorrhagic stroke, the confidence intervals were large, with an HR in all patients of 0.83 (95% CI, 0.42 to 1.65). The ongoing Treat Stroke to Target (TST) trial of patients with stroke of atherothrombotic origin treated with statins, is testing whether targeting a lower LDL-C level with statins and ezetimibe reduces cardiovascular event rates further, and will also provide additional prospective testing of the safety of that strategy.(19)

LIMITATIONS

Median follow-up was relatively brief, at 2.8 years, and one cannot exclude that the effects of alirocumab on ischemic or particularly hemorrhagic stroke might differ with much longer-term follow-up. Therefore, a relationship of alirocumab treatment to long-term risk of stroke is as yet unknown. A relatively small number of patients had a history of CeVD, and therefore the power to detect effects of alirocumab in this subgroup was limited. Only a dedicated randomized controlled trial among individuals with CeVD could reliably establish the efficacy and safety in this subgroup. As blood pressure was generally well controlled in the trial population, the present results may not necessarily apply in populations with uncontrolled blood pressure. In the analyses relating achieved LDL-C levels to subsequent risk of hemorrhagic stroke, patients who achieved lower LDL-C might have other prognostic characteristics placing them at lower risk for this outcome, on average, relative to patients with higher achieved LDL-C. This, in combination with few hemorrhagic strokes after month 4, might in part explain the lack of an observed adverse relationship.

CONCLUSIONS

This analysis of ODYSSEY OUTCOMES shows that in patients with recent ACS and dyslipidemia despite intensive statin therapy, the PCSK9 inhibitor alirocumab decreased the risk of stroke, irrespective of baseline LDL-C and of history of CeVD, over a median follow-up of 2.8 years. Furthermore, the present findings indicate that the risk of hemorrhagic stroke did not depend on achieved LDL-C levels in the alirocumab group.

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CHAPTER 8. EDITORIAL

ACHIEVEMENT OF VERY LOW LOW-DENSITY LIPOPROTEIN CHOLESTEROL LEVELS.

IS IT TIME TO UNLEARN CONCERN FOR HEMORRHAGIC STROKE?

Erin D. Michos, Seth S. Martin.

Based on: Achievement of Very Low Low-Density Lipoprotein Cholesterol Levels: Is It Time to unlearn Concern for Hemorrhagic Stroke? Circulation. 2019;140: 2063–2066.

EDITORIAL

Skepticism is as much the result of knowledge, as knowledge is of skepticism. To be content with what we at present know, is, for the most part, to shut our ears against conviction; …we must set aside old notions and embrace fresh ones; and as we learn, we must be daily unlearning something which it has cost us no small labor and anxiety to acquire. -Introduction to The Odyssey by Homer (1)

From the Greek epic poem by Homer, the word "odyssey" means an extended journey. Indeed, tremendous progress has been made in the journey, over the past few decades, in the management of blood cholesterol for the primary and secondary prevention of atherosclerotic cardiovascular disease (ASCVD). Overwhelming evidence from randomized controlled trials, meta-analyses of randomized controlled trials, and genetic studies have shown a dose–response relationship of low-density lipoprotein cholesterol (LDL-C) lowering and reduction in major ASCVD events.(2)

In the era of PCSK9 inhibition, there is now the ability to lower LDL-C to levels not previously achievable in most patients with statin (±ezetimibe) therapy. (3, 4)

Stroke is a leading cause of morbidity and mortality. The majority of strokes are ischemic, caused by thrombotic events arising from atherosclerosis in the aortic arch, carotid arteries, or cerebral arteries, from small vessel occlusions, or from cardioembolic sources (i.e., atrial fibrillation). Data from Mendelian randomization studies suggest that for every ~1 mmol/L (~40 mg/dL) genetically elevated LDL-C level, there is a 12% increased relative risk in ischemic stroke driven by a 28% increased relative risk in ischemic strokes from large artery atherosclerosis.(5) Hemorrhagic stroke, in contrast, is not caused by atherosclerosis but arises from vasculopathies (hypertension, cerebral amyloid, cerebral aneurysms, or other vascular malformations), systemic bleeding disorders, or anticoagulation use. There has been a longstanding controversy whether very low total cholesterol and LDL-C levels place individuals at greater risk for cerebral hemorrhage. This stems from several epidemiology studies that have found an association between low serum cholesterol level and increased risk of hemorrhagic stroke.(6-8)

The thought behind those studies is that some necessary threshold of serum cholesterol is needed for integrity of vessel walls in the brain. This concern has led to discomfort for some clinicians about achieving very low LDL-C levels in their patients. However, associations do not mean causations. Individuals with very low LDL-C levels may be less healthy than those with higher levels, in the setting of liver disease, significant alcohol use, poor nutrition, and frailty, which are conditions that also predispose to cerebral hemorrhage. Although observational studies attempt to control for some of these variables, there can still be residual confounding by other factors reflective of poor health status. To date, most data from clinical trials and genetic studies, which together form a stronger evidence base than observational studies, do not support a causal link between low LDL-C level and hemorrhagic stroke. Several meta-analysis of randomized controlled trials have found no association of statins and hemorrhagic stroke risk. (9, 10)

Instead, a reduction in all-cause stroke and mortality was found. Thus, if any association for hemorrhagic stroke was present, it is likely to be very small and

outweighed by the significant benefit of statins on reducing ASCVD events. In addition, genetic studies have not supported a causal link between lifetime low LDL-C level and hemorrhagic stroke risk. One study found that genetic variants leading to higher (rather than lower) LDL-C level was associated with intracranial hemorrhage.(11) Another analysis found that individuals with a missense variant in the PCSK9 gene (rs11591147), conferring PCSK9 loss of function and lifetime lower LDL-C level, had lower risks for coronary heart disease and ischemic stroke but no association with hemorrhagic stroke (P=0.81).(12)

Observational studies typically measure lipid levels at only one time point, when the individual may be in poorer health because of the aforementioned confounding factors, whereas genetic studies provide more reliable estimates of lifetime exposure to low blood lipid levels.

The FOURIER trial (Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk),3 which enrolled patients with established stable ASCVD, found that the US Food and Drug Administration–approved PCSK9 inhibitor drug evolocumab, in comparison with placebo, dramatically reduced LDL-C levels to a median of 30 mg/dL. Hemorrhagic stroke events were few (n=54 [0.19%]) and not statistically different between treatment groups. On the other hand, there was a 25% reduction in ischemic stroke conferred by evolocumab. However, follow-up in the trial was short, with a median duration of 2.2 years. Affirming the findings in the FOURIER trial, in this issue of Circulation, Jukema et al (13) present a prespecified analysis from the ODYSSEY Outcomes trial (ODYSSEY Outcomes: Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab) assessing the effect of alirocumab, the second Food and Drug Administration–approved PCSK9 inhibitor, on stroke. The analysis included 18 924 individuals with acute coronary syndrome within the past 1 to 12 months who had LDL-C level ≥70 mg/dL at enrollment, despite treatment with high-intensity statin (or maximally tolerated dose). At 4 months, the on-treatment LDL-C level was 48 versus 93 mg/dL in the alirocumab and placebo groups, respectively.(4)

The principal findings of the new analysis were that alirocumab reduced risk of all-cause stroke by a 0.5% absolute and 28% relative reduction and ischemic stroke by a 0.4% absolute and 27% relative reduction, over 2.8 years, which was statistically significant.(13)

The Kaplan-Meier curves separating the alirocumab versus placebo groups appear to widen over time, suggesting that an even greater benefit for stroke reduction may have been seen with longer follow-up. In the ODYSSEY Outcomes trial, hemorrhagic stroke was infrequent (33 cases) and occurred similarly in 0.2% of individuals in both placebo and alirocumab groups. By on-treatment LDL-C level in the alirocumab treated group at 4 months, hemorrhagic stroke events occurred in 2 (0.1%), 3 (0.1%), 3 (0.3%), and 4 (0.3%) cases for LDL-C of <25, 25 to <50, 50 to <70, and ≥70 mg/dL, respectively. Thus, there was reassuringly no signal for a dose response of hemorrhagic stroke with very low LDL-C levels. In a multivariable-adjusted model examining predictors of stroke, LDL-C level was positively associated with stroke, and treatment with alirocumab inversely so.

This important analysis from the ODYSSEY Outcomes trial provides additional reassurance in addressing this key controversy in lipid management—whether an LDL-C level that is too low can cause hemorrhagic stroke. However, there are some key limitations that should be noted. First, similar to the FOURIER trial, the ODYSSEY Outcomes trial was short in duration (median, 2.8 years). Although initial findings are reassuring, longer follow-up is needed to adequately monitor this safety concern. Second, hemorrhagic strokes were infrequent. The study is likely underpowered to truly address whether very low LDL-C level increases hemorrhagic stroke risk. Third, the subgroup with previous cerebrovascular disease, who would be most vulnerable to brain bleeding, was small (n=944 [5%]), and thus the analysis was especially underpowered to assess hemorrhagic stroke risk in this group. Last, the ODYSSEY Outcomes trial population was predominantly white, with only 13% of Asian ethnicity. More data are warranted in Asian populations, which are at greater risk for hemorrhagic stroke. In Asian societies, blood cholesterol levels tend to be lower than in Western societies, and although ischemic stroke

is more common, hemorrhagic strokes make up a larger proportion of total strokes than in Western societies.(14)

In the 1989 MRFIT observational study (Multiple Risk Factor Intervention Trial for the Prevention of Coronary Heart Disease), which found that men with serum cholesterol <160 mg/dL had 3-fold increased risk of death from hemorrhagic stroke, (6) findings were observed only in men with hypertension with a diastolic blood pressure (BP) ≥90 mm Hg. The SPARCL trial (Stroke Prevention by Aggressive Reduction in Cholesterol Levels) also reported higher numbers of hemorrhagic strokes among individuals treated with atorvastatin than placebo; however, hypertension increased the risk of hemorrhagic stroke.(15) Thus, BP control, as one of the core ABCDEs for ASCVD prevention (16) is particularly paramount for hemorrhagic stroke prevention as well. Indeed, BP was generally well controlled in ODYSSEY Outcomes trial participants. Clinicians treating patients with PCSK9 inhibitors should similarly be encouraged to pay close attention to BP control and not focused on just the lipids.

The ODYSSEY Outcomes trial findings are reassuring from a dose–response standpoint. If the hypothesis is that low LDL-C level increases hemorrhage stroke risk, then one would expect the signal to become stronger in PCSK9 inhibitor trials, where the LDL-C has been driven lower than in prior statin trials. Yet, now we have data from the FOURIER trial and the ODYSSEY Outcomes trial that do not exhibit any dose–response connection. This evidence is not only reassuring with respect to use of PCSK9 inhibitors but also from a mechanistic standpoint as it points away from the causal connection between low LDL-C and hemorrhagic stroke. Whereas the safety of very low LDL-C level among those without underlying cerebrovascular disease appears to be established, there remains some lingering concerns about risks among those with previous stroke. Stroke is a devastating consequence of ASCVD, and individuals with previous cerebrovascular disease warrant aggressive secondary prevention management, which includes effective LDL-C-lowering strategies. More data are needed for this subgroup, but being overly cautious about too low of an LDL-C level may lead to undertreatment in this high-risk

group, making them vulnerable to recurrent ischemic stroke and coronary heart disease events.

Ongoing PCSK9 inhibitor registries should further confirm the safety of PCSK9 inhibitor therapy among a larger spectrum of patients, including those with previous cerebrovascular disease, although ultimately, a dedicated, well-powered randomized controlled trial of PCSK9 inhibitors among patients with previous stroke would be needed to answer this question more definitively. For now, in our odyssey, it is important to not be afraid to set aside old notions and embrace fresh ones1 in our efforts to ward off the devastating consequences of recurrent ASCVD, including stroke, with aggressive LDL-C lowering. This should be performed in conjunction with other lifestyle and pharmacologic preventive strategies, including BP control.

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CHAPTER 9.

PCSK9 INHIBITION IN KIDNEY DISEASE

Laurien E. Zijlstra, Stella Trompet, Simon P. Mooijaart, Marjolijn van Buren, J. Wouter Jukema

Based on: Renal Impairment, Cardiovascular Disease, and the Short-Term Efficacy and Safety of PCSK9 Targeted by Inclisiran. Mayo Clin Proc. 2020;95(1):12-4.

EDITORIAL

This editorial refers to 'The effects of renal impairment on the pharmacokinetics, efficacy and safety of inclisiran: an analysis of the ORION-7 and ORION-1 studies', by R. Scott Wright et al.

Atherosclerotic cardiovascular diseases (CVD) result in high mortality and morbidity worldwide, especially when concomitant with chronic kidney disease (CKD). A common risk factor for both CVD and CKD is dyslipidemia. In CVD, lowering of atherogenic lipoproteins, reflected in part by reduction of LDL-C, favorably modifies cardiovascular outcomes, lowering both major adverse cardiovascular events (MACE) and even death. Accordingly, statin therapy is recommended for patients with CVD in the guidelines of the American College of Cardiology and American Heart Association 1, and the European Society of Cardiology and European Atherosclerosis Society2. In CKD, the effects of lipid-lowering therapies are less prominent. A possible explanation is that in CKD patients other mechanisms like vascular calcifications and inflammation play a more important role than lipoproteins in atherosclerosis. Dyslipidemia can not only cause CKD, but CKD, in turn, can also cause alterations in the lipid profile. These alterations are called the dyslipidemic profile of CKD, which usually exhibits variable (but mostly lower) levels of low-density lipoprotein cholesterol (LDL-C), increased triglycerides and decreased high-density lipoprotein cholesterol (HDL-C). Furthermore, CKD patients often have a lower tolerance for statins. The KDIGO (Kidney Disease Improving Global Outcomes) guidelines 3,4 recommend statin therapy for patients over 50 years with CKD and an estimated glomerular filtration rate (eGFR) ≥60 ml/min/1.73m2 and statin therapy with or without ezetimibe for patients with an eGFR between 30 ml/min/1.73m2 and 60 ml/min/1.73m2. In adult patients below 50 years with CKD (without chronic dialysis or kidney transplantation), statin therapy is only suggested in patients with high cardiovascular risk. In dialysis-dependent CKD there is no evidence for a benefit of statin therapy, and lipid-lowering therapy should not be initiated. However, it is suggested that lipid-lowering therapy be continued if patients already receive statin and/or ezetimibe at the time of dialysis initiation.

Among other established and evolving therapies in atherosclerosis, lipid-lowering therapy targeting proprotein convertase subtilisin-kexin type 9 (PCSK9) is on the rise, achieving LDL-C below levels achievable with statin therapy in most patients – such therapy is thus a therapeutic option for high-risk CVD patients, or for patients in whom current treatment is insufficient due to inadequate effect or intolerance for statins. Inhibition of PCSK9 can be obtained via various routes.

First, two fully human monoclonal antibodies that selectively inhibit PCSK9 are already on the market: evolocumab and alirocumab. Evidence for the efficacy and safety of these PCSK9 inhibitors in patients with CKD is still limited. A recent pooled analysis of 8 phase III ODYSSEY trials showed that alirocumab significantly improved the lipid profile of CKD patients, without affecting renal function.5 The FOURIER study group performed a subanalysis comparing the 8,077 individuals (16.1%) with preserved renal function (eGFR ≥90 ml/min/1.73 m2), 15,034 (54.6%) with mild impairment/stage 2 CKD (eGFR 60 to <90 ml/min/1.73 m2), and 4,443 (29.3%) with stage ≥3 CKD (eGFR <60 ml/min/1.73 m2). LDL-C lowering and relative clinical efficacy and safety of evolocumab versus placebo were consistently observed across CKD groups. However, given the higher event rates of cardiovascular death, myocardial infarction, or stroke at lower eGFR, the absolute reduction with evolocumab was greater with more advanced CKD. This is most often seen in other high risk groups, as polyvascular disease or diabetes, making high risk patients potentially more suitable for PCSK9 inhibiting.6 However, both trials investigating clinical outcomes using evolocumab (FOURIER)7 and alirocumab (ODYSSEY OUTCOMES)8 excluded patients with severe CKD. FOURIER included only patients with an eGFR ≥20 ml/min/1.73m2 and ODYSSEY OUTCOMES >30 mL/min/1.73m2, thereby leaving unanswered the effect of PCSK9 in more advanced stages of CKD.

Second, a new promising strategy is the administration of small interfering RNA (siRNA) targeting PCSK9, as inclisiran, currently in development phase II. Presented in this issue of Mayo Clinic Proceedings is a combined analysis from the phase 1 ORION-7 renal study and the phase 2 ORION-1 study,

investigating the pharmacodynamic properties of inclisiran in subjects with normal renal function, or mild, moderate or severe renal dysfunction. Wright et al. found that the pharmacodynamic effects and safety profile of inclisiran were similar comparing normal to impaired renal function. Although plasma concentrations of inclisiran are increased in parallel with the degree of renal impairment, circulating inclisiran was undetectable 48 hours after injection in all groups, whereas the LDL-C lipid-lowering effect persists for more than six months. Therefore, the authors concluded that dose-adjustments of inclisiran are not required in patients with impaired renal function. These findings are of clinical and practical importance, and the authors should be complimented for this valuable and relevant report. However, this combination of phase I and phase II ORION trials is, of course, just the beginning in assessing the efficacy and safety of inclisiran. The current study points to us the short-term renal clearance effects of inclisiran in a relatively small patient group with only seven patients with severe CKD, without addressing the effect on long-term renal function or the effect on clinical outcomes. Currently, larger cohorts of subjects with various degrees of CKD have been enrolled in the ongoing inclisiran phase III LDL-C lowering studies, and another cohort will be enrolled in the first trial investigating the effects of inclisiran on cardiovascular outcomes.

As may be seen in clinical trials, the patients with more severe CKD were probably healthier than patients with comparably severe CKD in the general population. At least patients with worse renal function were younger at baseline compared to those with less severe kidney disease. Safety in older age groups is yet to be determined, in particular because older patients with CKD are prone to multiple comorbidities and are therefore at a higher risk of CVD and other (competing) morbidity and mortality. Furthermore, LDL-C was lower at baseline for patients with lower renal function. As HDL-C and triglycerides were not reported, it is unknown whether this lower LDL-C matches the above mentioned dyslipidemic profile in CKD.

In general, CKD patients are a heterogeneous population. In this study regarding inclisiran the underlying etiology of renal impairment is unknown. Effects on both renal function and cardiovascular outcomes may differ between patients who have a predominantly vascular (e.g. hypertension or diabetes) or non-vascular etiology (e.g. glomerulonephritis or polycystic kidney disease etc.). Patients with vascular CKD may be more responsive to the effects of lipid-lowering therapy than patients with non-vascular CKD, in part because lipid-lowering therapy may exert different effects on lipid metabolism when vascular disease is present. Furthermore, since CKD stage is based on the measurement of only one creatinine level at baseline, it is unclear whether patients had actual CKD, or reversible acute kidney injury (AKI). Whether renal function deteriorated or improved over time is unknown.

In conclusion, as patients with CKD are at high risk of MACE and death there is a need for alternative and more effective lipid-lowering therapies. In the short term, inclisiran seems to be an effective and safe option in patients with reduced renal function. In the long term, the efficacy and safety of inclisiran in the setting of kidney disease are yet to be determined. A potentially important benefit includes the sustained pharmacological effect of this agent, necessitating injections only once every six months. In turn, such infrequent dosing may reduce cost, may be more appealing to patients, and may facilitate medication compliance in a group often in need of multiple medications.

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CHAPTER 10.

PCSK9 INHIBITION IN PATIENTS AT HIGH CARDIOVASCULAR RISK

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Based on: PCSK9 inhibition in high-risk patients. Aging (Albany NY). 2019;11(23):10791-2.

EDITORIAL

Cardiovascular disease (CVD) burden is increasing with advancing age, resulting in high mortality and morbidity worldwide. As societies continue to age, >80% of individuals dying from CVD are 65 years or older. Main reason for increased CVD in older people is an absolute increase in atherosclerotic plaque burden. (1) Patients with high plaque burden often have highest clinical benefit from cardiovascular treatment: because absolute risk is high, absolute risk reductions are also relatively high. Therefore, it remains a key point to identify these the subsets of patients with highest plaque burden to provide an optimal treatment strategy with high benefit but low risk. Measuring total plaque burden could provide the most accurate risk stratification. But although various imaging techniques exist, none are currently suitable to implement in routine daily practice. Therefore, surrogate markers of plaque burden should be used, as low-density lipoprotein cholesterol (LDL-C). In addition, patients can be classified according to clinical features that also reflect plaque burden, which provides an easy and cost-effective manner to achieve optimal treatment. Well-known clinical high risk features include patients with chronic kidney disease or diabetes, and also patients with known vascular disease such as a history of coronary artery bypass grafting (CABG) or atherosclerosis in multiple vascular beds (polyvascular disease).

Relatively frail patients are more prone to side effects of treatment, for instance due to an increased bleeding risk. Therefore, finding treatment for primary or secondary prevention with high benefit but low risk of adverse effects is important, especially in older patients. Standard cardiovascular treatment options include medication as aspirin or specific oral anticoagulants, beta-blockers, antihypertensives and lipid-lowering, next to life-style modification, e.g. smoking cessation and regular exercise. Lipid-lowering provides plaque stability and is relatively safe, as the most clinically relevant adverse effect of statins is myopathy. However, especially in older patients statin-associated muscle symptoms can be problematic in daily life. The available evidence from trials indicates that statin therapy produces significant reductions in major adverse cardiovascular events (MACE) irrespective of age, although evidence indicates there is no benefit among patients aged >75 years who do not

already have evidence of occlusive vascular disease. Accordingly, international guidelines recommend statin treatment for patients with established cardiovascular disease as secondary prevention for older people in the same way as for younger patients. (2) However, two other key points in optimal treatment for older patients should be noted. First, life-expectancy should be taken into account depending on the lag time to benefit of treatment. Second, expending life-expectancy is only of interest if quality of life remains acceptable.

Relatively new lipid-lowering drugs are PCSK9 (proprotein convertase subtilisin–kexin type 9) inhibitors. PCSK9 inhibiting provides the opportunity to reduce LDL-C to less than levels achievable with statins in most patients and is therefore a therapeutic option for high-risk patients, or for patients in which current treatment is insufficient due to inadequate effect or intolerance for statins. The ODYSSEY OUTCOMES trial showed that MACE were reduced with the PCSK9 inhibitor alirocumab compared with placebo in 18,924 patients with recent acute coronary syndrome (ACS) and elevated atherogenic lipoproteins despite intensive statin therapy (hazard ratio [HR] of 0.85; 95% confidence interval [CI], 0.78 to 0.93; P<0.001). Furthermore, three recent subanalyses of ODYSSEY OUTCOMES showed high risks of MACE with large absolute reductions in those risks with alirocumab therapy in patients with clinically identifiable high plaque burden, including patients with a history of CABG, diabetes and polyvascular disease. (3-5)

Although ODYSSEY OUTCOMES was not specifically designed for the older population, a subanalysis showed that the beneficial effect of alirocumab was independent of age and without significant safety issues in the 5084 (26.9%) older patients ≥65 years.(6) Of note, only 1007 (5.3%) patients were ≥75 years and 42 (0.2%) ≥85 years, limiting the power to detect differences in these subgroups. Another recent subanalysis of ODYSSEY OUTCOMES showed that alirocumab decreased the risk of any stroke with a hazard ratio (HR) of 0.72 (95% CI 0.57 to 0.91) and ischemic stroke [0.73 (0.57 to 0.93)] without increasing hemorrhagic stroke [0.83 (0.42 to 1.65)]. (7) As primary treatment goal in older patients should be maintaining or improving quality of life,

prevention of strokes is of utmost importance, as stroke can lead to limitations in functional capacity and cognitive function, leading to a significant reduction in quality of life.

In conclusion, it is important to identify subsets of patients for optimal treatment strategies in atherosclerosis, so that efficacy and efficiency are optimized. Monitoring true plaque burden would probably provide the most accurate mechanistic stratification of vascular risk. However, this is clinically not yet feasible in routine practice, in contrast to identifying patients based on easily identifiable risk factors as surrogate plaque marker. Among other established and evolving therapies in atherosclerosis, treatment with PCSK9 inhibitors has high clinical benefit but with few side effects and is therefore potentially suitable also for older patients. Calendar age per se is not a contra-indication for PCSK9 inhibitors, however, the importance of biological age, geriatric impairments and frailty remains to be studied. (8)

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CHAPTER 11.

LEVOTHYROXINE TREATMENT AND CARDIOVASCULAR OUTCOMES IN OLDER PEOPLE WITH SUBCLINICAL HYPOTHYROIDISM

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Based on: Levothyroxine Treatment and Cardiovascular Outcomes in Older People with Subclinical Hypothyroidism: Pooled Individual Results of Two Randomised Controlled Trials. Frontiers in Endocrinology. Accepted.

ABSTRACT

BACKGROUND

The cardiovascular effects of treating older adults with subclinical hypothyroidism (SCH) are uncertain. Although concerns have been raised regarding a potential increase in cardiovascular side effects from thyroid hormone replacement, undertreatment may also increase the risk of cardiovascular events, especially for patients with cardiovascular disease (CVD).

OBJECTIVE

To determine the effects of levothyroxine treatment on cardiovascular outcomes in older adults with SCH.

METHODS

Combined data of two parallel randomised double-blind placebo-controlled trials TRUST (Thyroid hormone Replacement for Untreated older adults with Subclinical hypothyroidism – a randomised placebo controlled Trial) and IEMO80+ (the Institute for Evidence-Based Medicine in Old Age 80-plus thyroid trial) were analysed as one-stage individual participant data. Participants aged ≥65 years for TRUST (n=737) and ≥80 years for IEMO80+ (n=105) with SCH, defined by elevated TSH with fT4 within the reference range, were included. Participants were randomly assigned to receive placebo or levothyroxine, with titration of the dose until TSH level was within the reference range. Cardiovascular events and cardiovascular side effects of overtreatment (new-onset atrial fibrillation and heart failure) were investigated, including stratified analyses according to CVD history and age.

RESULTS

The median [IQR] age was 75.0 [69.7-81.1] years, and 448 participants (53.2%) were women. The mean TSH was 6.38± SD 5.7 mIU/L at baseline and decreased at 1 year to 5.66±3.3 mIU/L in the placebo group, compared with 3.66±2.1 mIU/L in the levothyroxine group (p<0.001), at a median dose of 50 µg. Levothyroxine did not significantly change the risk of any of the prespecified cardiovascular outcomes, including cardiovascular events (HR 0.74 [0.41-1.25]), atrial fibrillation (HR 0.69 [0.32-1.52]) or heart failure (0.41 [0.13-1.35]), or all-cause mortality (HR 1.28 [0.54-3.03]) , irrespective of history of CVD and age.

CONCLUSION

Treatment with levothyroxine did not significantly change the risk of cardiovascular outcomes in older adults with subclinical hypothyroidism, irrespective of a history of cardiovascular disease and age.

TRIAL REGISTRATION

ClinicalTrials.gov Identifier: NCT01660126 (TRUST); Netherlands Trial Register: NTR3851 (IEMO80+)

INTRODUCTION

Subclinical hypothyroidism (SCH) is a common condition in older adults, with a prevalence between 8% and 18% (1). SCH is defined by elevated levels of thyroid stimulating hormone (TSH) with free thyroxine (fT4) within the reference range. Patients with SCH are mostly asymptomatic, although SCH is a possible contributor to various health problems including cardiovascular diseases (CVD) (2). The cardiovascular effect of treating older adults with SCH is uncertain.

The cardiovascular system is sensitive to changes in thyroid hormone concentrations due to thyroid hormone receptors in myocardial and vascular endothelial tissues (3). Associations have been found between SCH and an increase in the number of cardiovascular risk factors (4). In addition, meta-analyses of prospective studies showed that SCH was associated with an increased risk of heart failure, major adverse cardiovascular events (MACE) and cardiovascular death (2,5-7) Although associations have been found between SCH and CVD, data are limited and conflicting regarding the effect of treatment with levothyroxine on cardiovascular outcomes (3) Large randomised controlled trials (RCT) investigating especially cardiovascular outcomes in older patients are limited and most often investigated surrogate markers of CVD, such as cardiovascular risk factors8 or cardiac function and structure (9-11). On the one hand, concerns have been raised regarding a potential increase in cardiovascular side effects from thyroid hormone replacement, such as atrial fibrillation and heart failure. On the other hand, undertreatment may increase the risk of cardiovascular events, especially for patients with CVD or older age.

Two recent RCTs, namely TRUST (Thyroid hormone Replacement for Untreated older adults with Subclinical hypothyroidism – a randomised placebo controlled Trial) and IEMO80+ (the Institute for Evidence-Based Medicine in Old Age 80-plus thyroid trial), reported the absence of beneficial effect of levothyroxine on thyroid specific quality of life related outcomes in older patients with SCH (12,13). For the first time combining all data from the two trials, the aim of the present study is to assess the effect of levothyroxine treatment on cardiovascular outcomes in older adults with SCH.

MATERIALS AND METHODS

This study is a prespecified combined analysis of the TRUST and IEMO80+ studies. These studies were designed and executed as parallel trials with identical study protocols, both investigating whether levothyroxine provides clinical benefits in older persons with SCH.

An Institutional Review Board approved the studies prior to data collection. Written informed consent was obtained from all participants. Data were analysed as one-stage individual participant data (IPD) of these two randomised double-blind placebo-controlled trials. Detailed description and protocols have been published previously (14,15). In summary, older participants (≥65 years for TRUST and ≥80 years for IEMO80+) with SCH, diagnosed by elevated TSH levels (4.6 to 19.9 mIU/L), measured on at least two occasions between 3 months and 3 years apart, with fT4 levels within the reference range, were enrolled in Ireland, Scotland, Switzerland and The Netherlands. Participants were randomized in a 1:1 ratio for levothyroxine or placebo, with titration of the levothyroxine dose according to TSH level every 6-8 weeks and a mock titration schedule with a similar frequency in the placebo group. The levothyroxine group started with a dose of 50µg daily (or 25µg for participants with weight <50kg or a history of coronary heart disease). Participants were followed up for a minimum of 12 months and a maximum of 36 months between April 2013 and May 2018. The final follow-up was on May 4, 2018.

ENDPOINTS

The present analysis reports cardiovascular outcomes, including all-cause and cardiovascular mortality, and both cardiovascular events and cardiovascular side effects. Cardiovascular events are fatal and non-fatal cardiovascular events, including acute myocardial infarction, stroke, amputations for peripheral vascular disease, revascularisations for atherosclerotic vascular disease (including for acute coronary syndrome) and heart failure hospitalisations. Cardiovascular side effects of overtreatment include new-onset atrial fibrillation and new-onset heart failure. Secondary outcomes

include the cardiovascular parameters blood pressure, heart rate and weight, which were measured as positive signals of TSH change.

HISTORY OF CARDIOVASCULAR DISEASE AND AGE

Stratified analyses were executed for patients with or without a history of CVD at inclusion. CVD was defined as ischemic heart disease (both angina pectoris or myocardial infarction), stroke or transient ischemic attack, heart failure, peripheral vascular disease, revascularization or atrial fibrillation. Furthermore, patients were stratified in the 65-80 age range, or ≥80 years old.

STATISTICAL ANALYSIS

Baseline characteristics are presented as mean ± standard deviation (SD) or median [interquartile range (IQR)] depending on the distribution of data, stratified for history of CVD. Hazard ratios (HR) were obtained from a Cox proportional hazard regression model and were adjusted for country, sex, starting dose of levothyroxine and study, similar to previous publications (14,15). Results at 12 months and between-group differences were adjusted for country, sex, starting dose of levothyroxine, study (TRUST or IEMO80+) as random effect and baseline levels of the same variable with the use of linear mixed models. Between-group differences are the value in the levothyroxine group minus the value in the placebo group. The efficacy and safety analyses were carried out in a modified intention-to-treat population, which included participants with data on the outcome of interest. The data were analysed using IBM SPSS Statistics, version 23. P-values were considered statistically significant if lower than 0.05. Interaction analyses were performed between treatment and history of CVD and all secondary endpoints.

RESULTS

In total, all 737 patients from TRUST and all 105 patients from IEMO80+ were included in this combined data-analysis, see Figure 1. Of the 842 participants who underwent randomization, 422 were assigned to receive placebo and 420 to receive levothyroxine. For the baseline characteristics see Table 1. The median age of the 842 participants was 75.0 [IQR 69.7-81.1] years, with 419 (56.9%) participants older than 80 years.

FIGURE 1. FLOWCHART STUDY POPULATION

Combined data of the TRUST and IEMO80+ trials will be examined as one-stage individual participant data of these two randomised double-blind placebo-controlled parallel group trials. Cardiovascular disease (CVD) is defined as ischemic heart disease, stroke or transient ischemic attack, heart failure, peripheral vascular disease, revascularisation or atrial fibrillation. Median follow-up was 17-months.

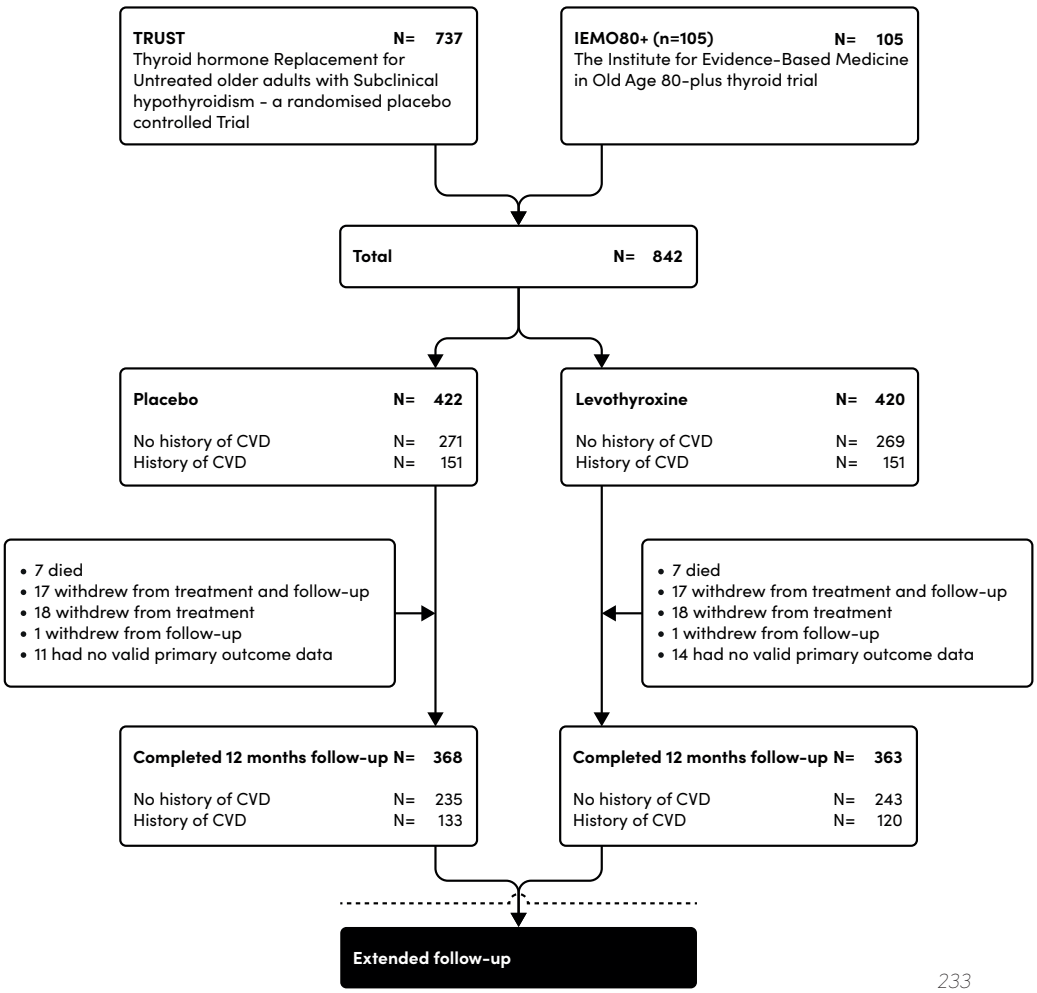


Table 1. Baseline characteristics (n=842)				
	No history of CVD		History of CVD	
	Placebo (n=271)	Levothyroxine (n=269)	Placebo (n=151)	Levothyroxine (n=151)
Age (years), median [IQR]	72.7 [68.6-79.2]	73.6 [68.9-78.8]	79.9 [73.0-84.5]	76.8 [72.0-81.7]
Female sex, n (%)	162 (59.8)	161 (59.9)	62 (41.1)	63 (41.7)
Caucasian[a], n (%)	264 (97.4)	264 (98.1)	150 (99.3)	150 (99.3)
Standard housing[b], n (%)	264 (97.4)	262 (97.4)	142 (94.0)	145 (96.0)
History of cardiovascular disease, n (%)				
Ischemic heart disease[c]			62 (41.1)	63 (41.7)
Stroke or transient ischemic attack			55 (36.4)	33 (21.9)
Peripheral vascular disease			15 (9.9)	20 (13.3)
Revascularisation			46 (30.5)	59 (39.1)
Heart failure			23 (15.2)	15 (9.9)
Atrial fibrillation			53 (35.3)	58 (38.9)
Cardiovascular risk factors, n (%)				
Hypertension	115 (42.8)	133 (49.4)	92 (61.3)	86 (57.0)
Diabetes mellitus	29 (10.7)	40 (14.9)	28 (18.7)	30 (19.9)
Current smoking	25 (9.2)	19 (7.1)	10 (6.6)	13 (8.6)
Former smoking	105 (38.7)	112 (41.6)	76 (50.3)	74 (49.0)
Number of concomitant medicines	3 [1-5]	3 [1-5]	6 [4-6]	6 [4-8]
Clinical parameters				
Body mass index (kg/m^2)	27.2±4.6	27.8±5.2	28.5±4.5	28.5±5.4
Waist circumference (cm)	95.7±12.9	97.1±12.4	100.8±11.4	100.9±12.4

Blood pressure (mmHg)				
Systolic	142±20	143±18	142±20	140±21
Diastolic	75±12	75±11	73±12	72.3±10
Heart rate (beats per min.)	70.4±10.6	69.1±10.6	68.6±13.0	67.6±12.7
Hand-grip strength (kg)	27.3±10.5	27.2±10.3	27.3±11.9	28.5±10.3
Thyroid function[d]				
Thyrotropin (mIU/liter)	6.4±2.1	6.5±2.1	6.2±1.8	6.2±1.7
Median	5.7 [5.1-7.0]	5.7 [5.2-7.0]	5.7 [5.0-6.8]	5.7 [5.0-6.8]
Free thyroxine (pmol/liter)	13.1±1.9	13.4±2.0	14.0±2.0	13.7±2.2
Quality of life[e]				
Hypothyroid Symptoms score	15.4±16.9	17.5±19.2	21.0±20.4	18.9±18.2
Tiredness score	23.0±18.2	25.7±20.7	29.6±22.5	25.5±20.6
EQ-5D descriptive index	0.855±0.18	0.847±0.18	0.804±0.20	0.819±0.22
EQ visual-analogue scale score	77.6±15.9	79.2±15.2	73.3±15.3	76.0±15.1

Values are mean ± standard deviation (SD) or median [Interquartile Range (IQR)]. Cardiovascular disease (CVD) was defined as ischemic heart disease (both angina pectoris or myocardial infarction), stroke or transient ischemic attack, heart failure, peripheral vascular disease, revascularization or atrial fibrillation.a. Race was reported by the patient. b. Standard housing was defined as non-sheltered community accommodation. By contrast, sheltered housing is purpose built grouped housing for older persons, often with an on-site manager or warden. c. Ischemic heart disease was defined as a history of angina pectoris or previous myocardial infarction. d. To convert the values for free thyroxine to nanograms per deciliter, divide by 12.87. e. The Hypothyroid Symptoms score and the Tiredness score from the Thyroid-Related Quality of Life Patient-Reported Outcome (ThyPRO) questionnaire are each assessed on a scale from 0 to 100, with higher scores indicating more symptoms and tiredness, respectively. The minimum clinically important difference for each score has been estimated as 9 points. The EuroQoL [EQ] Group 5-Dimension Self-Report Questionnaire (EQ-5D) scores included both the EQ5D descriptive index (on a scale from -0.59 to 1.00) and the score on the EQ visual-analogue scale (on a scale from 0 to 100); higher scores on each scale indicate better quality of life.

In total, 448 participants (53.2%) were women and 302 (35.9%) had a history of CVD. History of CVD or cardiovascular risk factors did not differ between the placebo or levothyroxine group. Median follow-up was 17 months. A total of 368 participants (87.2%) of the placebo group and 363 (86.4%) of the levothyroxine group completed 12-month follow-up, which did not differ between patients with or without a history of CVD, see Figure 1. In total, 194 (23.0%) patients discontinued the trial regimen and 44 (5.2%) withdrew from follow-up. Most participants (83.8%) started with a dose of 50 µg and 16.2% with 25 µg levothyroxine. Of patients with a history of CVD, 58.9% started with a dose of 50 µg levothyroxine and of patients older than 80 79.5%.

Table 2. Thyroid function and cardiovascular parameters at 12 months for patients with or without a history of cardiovascular disease*

Variable	No history of CVD				
	Baseline		At 12 months		
	Placebo (n=271)	**Levothyroxine (n=269)**	**Placebo (n=235)**	**Levothyroxine (n=243)**	**Difference (95% CI)**
Thyrotropin (mIU/liter)	6.4±0.1	6.5±0.1	5.6±0.2	3.5±0.1	-2.12 (-2.49 to -1.76)
Median [IQR]	5.8 [5.1 to 7.0]	5.7 [5.2 to 7.0]	4.9 [4.6 to 6.6]	3.2 [2.4 to 4.2]	
Range	4.6 to 17.6	4.6 to 17.6	0.1 to 46.0	0.03 to 15.9	
Cardiovascular parameters					
Systolic blood pressure (mmHg)	142±1.2	143±1.1	139±1.2	140±1.1	0.96 (-1.67 to 3.59)
Diastolic blood pressure (mmHg)	75±0.7	75±0.7	74±0.7	74±0.7	0.46 (-1.11 to 2.03)
Heart rate (beats per minute)	70.4±0.6	69.1±0.6	70.1±0.7	69.3±0.7	1.07 (-0.50 to 2.63)
Weight (kg)	74.9±0.9	76.3±0.9	75.0±0.9	76.3±0.9	0.16 (-0.36 to 0.69)

Values are mean ± standard error (SE). Abbreviations: CI, confidence interval; CVD, cardiovascular disease. Results at 12 months and between-group differences are adjusted for stratification variables (country, sex, starting dose of levothyroxine and study as random effect) and baseline levels of the same variable with the use of linear mixed models. Between-group differences are the value in the levothyroxine group minus the value in the placebo group. Interaction analyses were performed between treatment and history of CVD and all endpoints. CVD was defined as ischemic heart disease (both angina pectoris or myocardial infarction), stroke or transient ischemic attack, heart failure, peripheral vascular disease, revascularization or atrial fibrillation.

THYROID FUNCTION

The mean ± SD TSH was 6.38±5.7 mIU/L at baseline, and decreased at 1 year to 5.66±3.3 mIU/L in the placebo group, compared with 3.66±2.1 mIU/L in the levothyroxine treated group (p<0.001), at a median dose of 50 µg. TSH did not differ significantly at baseline or at 12 months between patients with or without a history of CVD (p-interaction=0.31), see Table 2.

	History of CVD					
	Baseline		At 12 months			Interaction
	Placebo (n=151)	**Levothyroxine (n=151)**	**Placebo (n=133)**	**Levothyroxine (n=120)**	**Difference (95% CI)**	**p-value**
	6.2±0.2	6.2±0.1	5.5±0.2	3.8±0.2	-1.63 (-2.17 to -1.11)	0.31
	5.2 [5.0 to 6.8]	5.2 [5.0 to 6.8]	4.9 [3.9 to 6.4]	3.5 [2.7 to 4.4]		
	4.6 to 17.6	4.6 to 14.2	1.9 to 18.0	0.8 to 15.4		
	142±1.6	140±1.7	139±1.7	137±1.9	-1.05 (-5.23 to 3.13)	0.40
	73±1.0	72±1.0	71±1.1	69±1.2	-1.16 (-3.62 to 1.29)	0.28
	68.6±1.1	67.6±1.0	68.7±1.2	67.2±1.2	-0.78 (-3.12 to 1.56)	0.17
	79.3±1.2	80.1±1.4	79.7±1.3	80.6±1.5	0.27 (-0.67 to 1.21)	0.83

CARDIOVASCULAR OUTCOMES

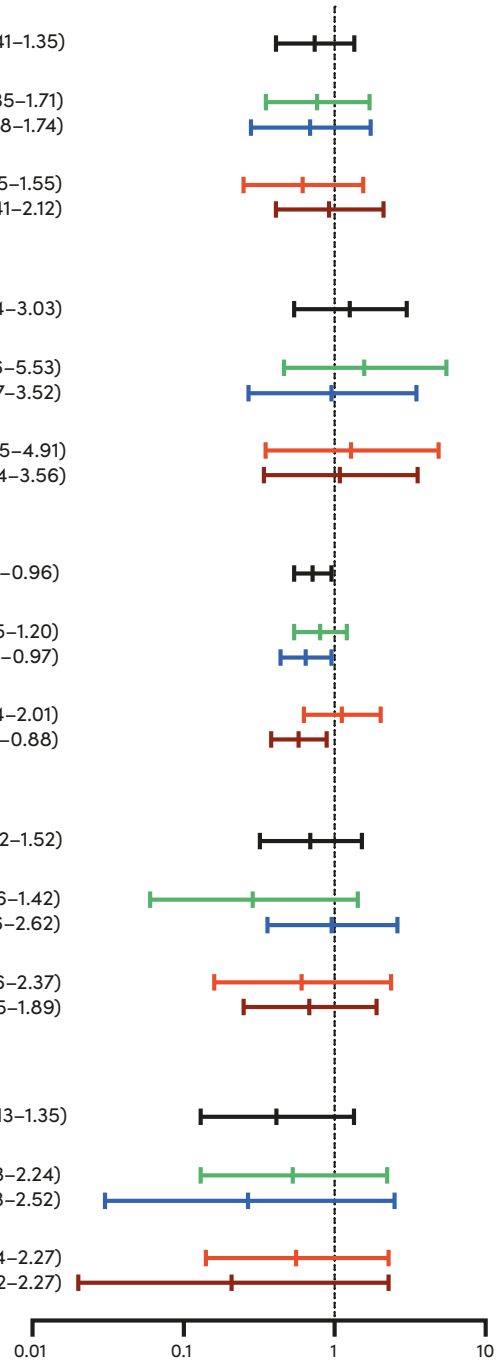
In total, 44 (5.2%) fatal and non-fatal cardiovascular events occurred after a median follow-up of 17 months, which did not significantly differ between placebo and levothyroxine with a HR comparing treatment to placebo of 0.74 (0.41 to 1.35). Comparing cardiovascular side effects of overtreatment risk of new-onset atrial fibrillation was associated with levothyroxine treatment was HR 0.69 (0.32 to 1.52) and HR of new-onset heart failure was 0.41 (0.13 to 1.35). Furthermore, in total, 21 (2.5%) deaths from any cause occurred (of which 4 cardiovascular deaths) with a HR for levothyroxine treatment of 1.28 (0.54 to 3.03). Figure 2 shows a forest plot of all cardiovascular outcomes, comparing placebo to levothyroxine stratified by history of CVD and age, showing that levothyroxine did not significantly change the risk of any of the cardiovascular outcomes, irrespective of CVD history or age (p for interaction all >0.10).

No clinically relevant or statistically significant adjusted differences between levothyroxine and placebo were found at 12 months for blood pressure, heart rate and weight (Table 2). Outcomes did not differ between patients with or without a history of CVD (p for interaction all >0.10).

FIGURE 2. CARDIOVASCULAR OUTCOMES STRATIFIED FOR HISTORY OF CARDIOVASCULAR DISEASE AND AGE

Cardiovascular disease (CVD) is defined as ischemic heart disease, stroke or transient ischemic attack, heart failure, peripheral vascular disease, revascularisation or atrial fibrillation. Hazard ratios for treatment were obtained from a Cox proportional hazard regression model predicting and were adjusted for study, country, sex and starting dose of levothyroxine.

Endpoints and subgroups	Levothyroxine n (%)	Placebo n (%)	HR (95% CI)
Fatal and non-fatal cardiovascular event			
Total population	19 (4.5)	19 (4.5)	0.74 (0.41–1.35)
History of CVD	11 (7.3)	11 (7.3)	0.77 (0.35–1.71)
No history of CVD	8 (3.0)	8 (3.0)	0.70 (0.28–1.74)
Age ≥80 years	7 (6.3)	7 (6.3)	0.62 (0.25–1.55)
Age 65–80 years	12 (3.9)	12 (3.9)	0.93 (0.41–2.12)
Death from any cause			
Total population	12 (2.9)	9 (2.1)	1.28 (0.54–3.03)
History of CVD	7 (4.6)	4 (2.6)	1.60 (0.46–5.53)
No history of CVD	5 (1.9)	5 (1.8)	0.97 (0.27–3.52)
Age ≥80 years	5 (4.6)	4 (2.9)	1.31 (0.35–4.91)
Age 65–80 years	7 (2.3)	5 (1.8)	1.11 (0.34–3.56)
Serious adverse event			
Total population	90 (21.4)	116 (27.5)	0.73 (0.55–0.96)
History of CVD	47 (31.1)	56 (37.1)	0.82 (0.55–1.20)
No history of CVD	43 (16.0)	60 (22.1)	0.65 (0.44–0.97)
Age ≥80 years	33 (29.5)	40 (28.8)	1.13 (0.64–2.01)
Age 65–80 years	57 (18.5)	76 (26.9)	0.59 (0.39–0.88)
New-onset atrial fibrillation			
Total population	11 (2.6)	15 (3.6)	0.69 (0.32–1.52)
History of CVD	2 (1.3)	7 (4.6)	0.29 (0.06–1.42)
No history of CVD	9 (3.3)	8 (3.0)	0.97 (0.36–2.62)
Age ≥80 years	4 (3.6)	6 (4.3)	0.62 (0.16–2.37)
Age 65–80 years	7 (2.3)	9 (3.2)	0.69 (0.25–1.89)
New-onset heart failure			
Total population	4 (1.0)	9 (2.1)	0.41 (0.13–1.35)
History of CVD	3 (2.0)	5 (3.3)	0.53 (0.13–2.24)
No history of CVD	1 (0.4)	4 (1.5)	0.97 (0.03–2.52)
Age ≥80 years	3 (2.7)	6 (4.3)	0.56 (0.14–2.27)
Age 65–80 years	1 (0.3)	3 (1.1)	0.23 (0.02–2.27)



DISCUSSION

In this prespecified combined analysis of the TRUST and IEMO80+ trials, treatment with levothyroxine did not increase or decrease the risk of cardiovascular outcomes significantly in older adults with SCH, irrespective of CVD history and age.

In older patients with SCH, the current European and United States guidelines recommend no routine thyroid hormone therapy (16,17). Especially in older people, treatment should be individualised, gradual and closely monitored. In the oldest old subjects, defined as >80 years old, SCH should be carefully followed with a wait-and-see strategy, generally avoiding hormonal treatment (16). The outcomes of both TRUST and IEMO80+ support this wait-and-see strategy as they showed no consistent beneficial effect of levothyroxine on quality of life, in both older (TRUST) and oldest old subjects (IEMO80+) (12,13) Although experts have pointed out the need, before the TRUST and IEMO80+ studies, randomised trials investigating hard cardiovascular endpoints were lacking (18). Hence we sought to answer the question whether undertreatment may cause cardiovascular events or treatment may cause cardiovascular side effects. We found no significant adjusted differences in cardiovascular parameters and neutral results for all cardiovascular outcomes with wide confidence intervals, although all point estimates were favourable for levothyroxine treatment. Therefore, we found no evidence to support any major short to medium term harmful effect on cardiovascular events of levothyroxine treatment for subclinical hypothyroidism in older people, including in those with known prior cardiovascular disease.

Taken together, our finding that treatment with levothyroxine did not change the risk of all cardiovascular outcomes in older adults with SCH is of incremental value to the limited existing literature. We showed that when treatment with levothyroxine is indicated on an individual basis, treatment should not be initiated especially to prevent cardiovascular events, nor should it be withheld because of potential cardiovascular side effects, irrespective of CVD history. Provided that treatment should be carefully monitored and titrated over time, as was in the trials.

STRENGTHS AND LIMITATIONS

This is a unique combined data analysis of the two largest RCTs to date investigating cardiovascular outcomes in older patients with SCH. Some limitations should be mentioned.

First, it was initially planned in both TRUST and IEMO80+ that cardiovascular events were to be a primary outcome together with thyroid-specific quality of life. Owing to delays and difficulties in recruitment this was changed as it became apparent that both studies would be underpowered for this aspect (13). However, the studies combined enabled the largest data analysis thus far regarding this subject and it is unlikely that a similar experiment will be successful in the near future, especially not a large one. Overall incidence of cardiovascular outcomes after a median follow-up of 17 months in the patients with SCH was still relatively low, only 44 (5.2%) patients had a fatal or nonfatal cardiovascular event. However, 17 months is still relatively short, and does not exclude a substantial cardiovascular 10-year risk. Second, the limited power hampered us to further stratify according to history of CVD and to distinguish between patients with ischemic heart disease, patients with heart failure or patients with vascular disease elsewhere in the body (e.g. cerebrovascular or peripheral artery disease). Furthermore, of the 252 patients with a history of CVD, only 38 subjects had a history of heart failure. Third, of all included older participants, only 251 (29.8%) defined as the oldest old (≥80 years old). Fourth, mean TSH level was not very high at baseline in the total population (6.4±2.0). Fifth, with respect to ethnicity the study population was predominantly white (98%).

CONCLUSIONS

Treatment with levothyroxine did not significantly change the risk of cardiovascular outcomes in older adults with subclinical hypothyroidism, irrespective of a history of CVD.

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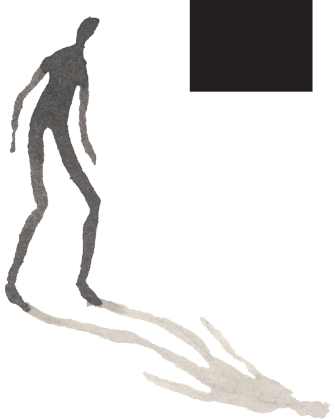

CHAPTER 12.

SUMMARY AND PERSPECTIVES

As the population is aging worldwide, and in spite of all preventive efforts, age-related diseases are increasingly prevalent, such as cardiovascular diseases (CVD) and cognitive impairment. Different vascular risk factors, both the 'traditional' modifiable factors, such as hypertension or diabetes, and non-modifiable factors, such as age or gender, can lead to different kind of intertwined micro- and macrovascular diseases in various or multiple simultaneous organs. There is a growing need for knowledge regarding the interplay between different (co)morbidities, the relation of (co)morbidities with the various underlying pathophysiological mechanisms and treatment options of these (co)morbidities. Therefore, the aims of this thesis were to identify patients at high cardiovascular risk and to optimize treatment for these patients. Below, a summary is given of the main findings of the research described in this thesis. Furthermore, we describe implications of our research and discuss possible directions for future research.

MAIN FINDINGS

PART 1. IDENTIFICATION OF HIGH-RISK CARDIOVASCULAR PATIENTS

In part 1 we investigated various interactions between (poly) vascular diseases and identified high-risk patients.

In chapter 2, we performed a critical review of the diagnostic and therapeutic challenges in patients with suspected coronary microvascular disease. We searched PubMed, EMBASE and Web of Science to examine the differences in microvascular function between women and men, focusing on patients with stable symptoms of ischemic heart disease in the absence of obstructive coronary artery disease. After full text review 13 articles were included, of which six articles met all criteria and seven articles missed angiographic data about coronary artery disease, but concerned mostly healthy volunteers. We found that apart from a higher incidence of microvascular disease in women there are no clinically relevant differences between both sexes, based on coronary flow reserve and myocardial blood flow measurements using various methods, including PET (positron emission tomography) or coronary

angiography. In the past the significance of microvascular dysfunction probably has been underestimated but the full clinical significance of these disorders has yet to be clarified. In the meantime, there is no justification to assume that particularly in women, angina-like symptoms in the absence of obstructive coronary artery disease are probably due to microvascular dysfunction, especially without objective proof. Therefore, there is a need for widely available low-risk non-invasive methods to assess microvascular function. Important questions with regard to the clinical implications of microvascular dysfunction have yet to be resolved in studies involving women as well as men.

The association of (poly) vascular risk factors and diseases with cerebrovascular changes and cognitive impairment in older patients was discussed in chapter 3 till 6. We started in chapter 3 with an evaluation of the role of inflammation by complement receptor 1 gene polymorphisms (CR1 SNPs) and cognitive function in older patients at risk of cardiovascular disease using data from PROSPER (the PROspective Study of Pravastatin in the Elderly at Risk) (1). PROSPER was a double-blind, randomized, placebo-controlled trial, designed to investigate the relationship between statin treatment and the risk of cardiovascular and cerebrovascular events. In total, 5804 older participants (70–82 years) were included if they had a history of, or an increased risk for vascular disease. In this substudy twelve of the 18 investigated CR1 SNPs were significantly associated with cognitive function in multivariate analysis. These data indicate that genetic variation within the CR1 gene is not only associated with Alzheimer's disease, but also with general cognitive function decline during late life. Furthermore, three of these 12 SNPs that were associated with improved cognitive function were associated with lower levels of CRP, which further strengthens previous evidence for a causal role of the complement system in cognitive function.

In chapter 4 and 5 we investigated the interactions between the heart, kidney and brain in their role in cognitive function. In chapter 4 we examined the association of cardiovascular structure and function, measured with cardiac MRI, with cerebrovascular changes and cognitive function in older

patients with end-stage renal disease using data from the Dutch prospective multicenter cohort study COPE (Cognitive decline in Older Patients with End stage renal disease)(2). The COPE study is a unique observational study with comprehensive measurements of cardiovascular function, cerebrovascular changes and cognitive function. The main study objective was to study the association between underlying pathophysiological mechanisms and cognitive decline in older patients (≥65 years) reaching end-stage renal disease, prior to either dialysis or conservative care. Of the 157 patients included in the COPE study, cardiac magnetic resonance imaging (MRI) scans were available for 85 participants, which were included in the current substudy. MRI of the heart was performed measuring aortic stiffness (pulse wave velocity) and cardiac systolic function. Outcomes were MRI-derived cerebrovascular changes and cognitive function in various neurocognitive domains. No statistically significant associations were observed between structural and functional cardiovascular parameters and structural cerebrovascular changes. However, cognitive function was worse in patients with high compared to low pulse wave velocity in all three cognitive domains. Although there were potentially clinically relevant associations of high pulse wave velocity with poor cognition in all domains, after adjustment for age, sex and education only the Trail Making Test A, measuring psychomotor speed, remained statistically significant (p=0.03). In conclusion, these data suggest that a higher pulse wave velocity is associated with lower cognitive function, but not with increased cerebrovascular changes in older patients with end-stage renal disease, suggesting that arterial stiffness may be an underlying mechanism of development of cognitive impairment. In chapter 5 we studied the association of kidney function with cognitive function (both executive functioning and memory) in patients at high cardiovascular risk, again using data of PROSPER. For all cognitive function tests, only severe kidney disease (CKD stage 4), but not mild to modest kidney disease (CKD stage 3a and b), was associated with cognitive impairment at baseline and faster cognitive decline over time. Although the results of the cognitive function tests show the same trend in both cognitive domains, the effect on executive functioning seems larger than the effect on memory. Possible explanation could be that executive functioning seems most often sooner affected than memory, partly due to more sensitive

executive function tests, and memory would be more affected over a longer follow-up period. Furthermore, the association of severe kidney failure with cognitive impairment and decline over time was more outspoken in patients with a history of vascular disease, possibly due to a higher probability of polyvascular damage, in both kidney and brain, in patients with proven CVD.

In chapter 6 we elaborated on the interaction between the heart, kidney and thyroid in a substudy of PROSPER aiming to investigate whether kidney function modifies the association of subclinical thyroid dysfunction and the risk of major adverse cardiovascular events (MACE). Although the association of thyroid dysfunction on MACE has been studied in patients with CKD, this is the first time this association has been studied over different strata of CKD to explore a possible interaction between thyroid and kidney function. No statistically significant relationship was found between subclinical thyroid dysfunction and MACE with adjusted hazard ratios (95% confidence interval) of 0.51 (0.24 to 1.07) comparing subclinical hyperthyroidism with euthyroidism and 0.90 (0.58 to 1.39) comparing subclinical hypothyroidism with euthyroidism. Neither was this relationship present in any of the strata of kidney function, nor did kidney function interact with subclinical thyroid dysfunction in the association with MACE (P-interaction=0.60 for subclinical hyperthyroidism and 0.39 for subclinical hypothyroidism). Therefore, no evidence was found that the potential association between thyroid hormones and CVD was modified by kidney function in older patients with subclinical thyroid dysfunction.

PART 2. TREATMENT OF HIGH-RISK CARDIOVASCULAR PATIENTS

Part 2 focused on optimizing treatment of the high-risk patients as described in part 1.

Chapter 7 and 8 studied treatment with the PCSK9 inhibitor alirocumab in patients with recent acute coronary syndrome and dyslipidemia despite intensive statin therapy using data from ODYSSEY OUTCOMES (Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During

Treatment With Alirocumab), a multicenter, double-blind, placebo-controlled trial in 18,924 patients (≥40 years) (3). Primary data of ODYSSEY OUTCOMES showed that the risk of MACE was reduced with alirocumab compared to placebo (hazard ratio 0.85 [0.78 to 0.93]). In addition, fewer deaths occurred within the alirocumab group. In chapter 7 we determined whether polyvascular disease influenced the risks of MACE's and death and their modification by alirocumab. With placebo, the incidence of MACE's was 10.0% for patients with monovascular (coronary) disease, 22.2% for polyvascular disease in two beds (coronary and peripheral artery or cerebrovascular), and 39.7% for polyvascular disease in three beds (coronary, peripheral artery, cerebrovascular). With alirocumab, the corresponding absolute risk reduction (95% confidence interval) was 1.4% (0.6% to 2.3%), 1.9% (-2.4% to 6.2%), and 13.0% (-2.0% to 28.0%), by respective vascular category, with a significant p-value for interaction (p-interaction<0.001). Therefore, patients with polyvascular disease are an easily identifiable subgroup of patients with recent acute coronary syndrome with high absolute risk of MACE and death. The large absolute benefit of PCSK9 inhibition with alirocumab, when added to high-intensity statin therapy, is a potential benefit for this population of patients. We designed chapter 8 to assess the effect of alirocumab on stroke. Although lowering of atherogenic lipoproteins, including low-density lipoprotein cholesterol (LDL-C), reduces the risk of ischemic stroke, concerns have been raised about very low LDL-C levels and a potential increased risk of hemorrhagic stroke. In our second substudy of ODYSSEY OUTCOMES, alirocumab reduced the risk of any stroke (hazard ratio 0.72 [0.57 to 0.91]) and ischemic stroke (0.73 [0.57 to 0.93]) without increasing hemorrhagic stroke (0.83 [0.42 to 1.65]), irrespective of baseline LDL-C and history of cerebrovascular disease. There was no apparent adverse relation between lower achieved LDL-C and incidence of hemorrhagic stroke in the alirocumab group. Therefore, the present findings indicate that for patients with recent acute coronary syndrome and dyslipidemia despite intensive statin therapy, alirocumab decreases the risk of stroke, over a median follow-up of 2.8 years. The risk of hemorrhagic stroke appears not to be dependent on achieved LDL-C levels within the alirocumab group.

Chapter 9 further elaborates on the efficacy and safety of treatment with another PCSK9 inhibitor inclisiran in patients with CKD. As patients with CKD are at high risk of MACE and death there is a need for alternative and more effective lipid-lowering therapies. In a combined analysis from the phase 1 ORION-7 renal study (n=31) and the phase 2 ORION-1 study (n=247), the pharmacodynamic properties of inclisiran were studied in subjects with normal kidney function, and in subjects with mild, moderate, or severe kidney dysfunction. Wright et al. found that the pharmacodynamic effects and safety profile of inclisiran were similar comparing normal with impaired kidney function (4). Although plasma concentrations of inclisiran are increased in parallel with the degree of kidney dysfunction, circulating inclisiran was undetectable 48 hours after injection in all groups, whereas the LDL-C lipid-lowering effect persists for more than 6 months. Therefore, in the short term, inclisiran seems to be an effective and safe option in patients with reduced kidney function. A potentially important benefit includes the sustained pharmacological effect of this agent, necessitating injections only once every six months. In turn, such infrequent dosing may reduce cost, may be more appealing to patients, and may facilitate medication compliance in a group often in need of multiple medications.

We summarized the potential uses of PCSK9 inhibition in chapter 10, where we describe the treatment of easily identifiable high-risk patients, as identified earlier in this thesis, so that efficacy and efficiency can be optimized. Monitoring true plaque burden would probably provide the most accurate mechanistic stratification of vascular risk. However, although various imaging techniques exist, none are currently feasible to implement in routine daily practice. Therefore, surrogate markers of plaque burden should be used, as LDL-C. In addition, patients can be classified according to clinical features that also reflect plaque burden, which provides an easy and cost-effective manner to achieve optimal treatment. Well-known clinical high risk features include patients with CKD or diabetes, and also patients with known CVD such as a history of coronary artery bypass grafting or polyvascular disease. These patients with high plaque burden often have highest clinical benefit from cardiovascular treatment: because absolute risk is high, absolute risk

reductions are also relatively high. Among other established and evolving therapies in atherosclerosis, treatment with PCSK9 inhibitors has high clinical benefit with few side effects and is therefore potentially suitable also for older patients, who are often more prone to side effects. Although ODYSSEY OUTCOMES was not specifically designed for the older population, a subanalysis showed that the beneficial effect of the PCSK9 inhibitor alirocumab was independent of age and without significant safety issues in the 5084 (26.9%) older patients ≥65 years (5). Of note, only 1007 (5.3%) patients were ≥75 years and 42 (0.2%) ≥85 years, limiting the power to detect differences in these subgroups.

Finally, in chapter 11, the effect of levothyroxine treatment on cardiovascular outcomes was discussed for older patients with subclinical hypothyroidism, using combined data of the randomized double-blind placebo-controlled parallel group trials, TRUST (Thyroid hormone Replacement for Untreated older adults with Subclinical hypothyroidism – a randomised placebo controlled Trial) (6) and IEMO80+ (the Institute for Evidence-Based Medicine in Old Age 80-plus thyroid trial)(7). These studies with identical study protocols both investigated whether levothyroxine provides clinical benefits in older persons with subclinical hypothyroidism, including participants aged ≥65 years for TRUST (n=737) and ≥80 years for IEMO80+ (n=105). No beneficial effect of levothyroxine on thyroid specific quality of life related outcomes were found in both studies separately. In this first combined data analysis levothyroxine did not clearly change the risk of all-cause mortality (hazard ratio 1.28 [0.54-3.03]) or any of the prespecified cardiovascular outcomes, including cardiovascular events (0.74 [0.41-1.25]) and cardiovascular side effects of overtreatment, including atrial fibrillation (0.69 [0.32-1.52]) and heart failure (0.41 [0.13-1.35]). Our finding that treatment with levothyroxine did not clearly change the risk of all cardiovascular outcomes in older adults with subclinical hypothyroidism is of incremental value to the limited existing literature. We showed that when treatment with levothyroxine is indicated on an individual basis, treatment should not be initiated especially to prevent cardiovascular events, nor should it be withheld because of potential cardiovascular side effects, irrespective of CVD history.

IMPLICATIONS AND FUTURE PERSPECTIVES

In this thesis, we further unraveled the complexity of various interacting (poly) vascular diseases, ultimately leading to an increased risk of not only cognitive impairment, but also MACE including death. Identification of these patients and better understanding of the interplay and underlying mechanisms of these diseases is the first step towards preventive strategies. Treating these high risk patients can be a therapeutic challenge, but there is growing knowledge regarding both established and evolving therapies to optimize efficacy and efficiency.

Our studies investigating PCSK9 inhibition and thyroid hormone therapy, emphasize the importance of personalized medicine. An individualized approach is inevitable as the complexity of health-care is increasing, not only to maximize efficacy, but also cost-effectiveness. Looking at treatment with PCSK9 inhibition, although it is known that PCSK9 inhibition provides the opportunity to reduce LDL-C to less than levels achievable with statins in most patients, this treatment is at present relatively expensive. Therefore, it is crucial to prioritize this treatment to maximize its value in selected patient groups for optimal benefit (8). In the future, patents will expire, thus reducing costs of this therapy. In addition, next to the PCSK9 inhibitors that are already on the market, namely alirocumab and evolocumab, a new promising strategy of administrating small interfering RNA targeting PCSK9, such as inclisiran, is currently in development phase III (9). A potentially important benefit includes the sustained pharmacological effect of this agent, requiring injections only once every six months instead of every two weeks, which is necessary for alirocumab and evolocumab. This may reduce cost, may be more appealing to patients, and may facilitate medication compliance in patients often in need of multiple medications, thus being potentially more cost-effective and also improving quality of life. However, long-term efficacy and safety are yet to be determined.

Several key points in cost-effective treatment for high-risk or older patients should be noted. Foremost, life-expectancy should be taken into account depending on the lag time to benefit of treatment. In addition, expanding life-

expectancy is only of interest if quality of life remains acceptable. Calendar age per se is not a contra-indication for the treatments discussed above, however, the importance of biological age, geriatric impairments and frailty have to be taken into account. Treatment may be different for those older people living with frailty compared to those who are very vital up to high ages. Therefore, to optimize treatment, high-risk patients should be screened on functional status and cognitive function. For instance, in blood pressure management cognitive dysfunction has to be considered, as we know that cognitive function can further deteriorate due to low blood pressure. Cognitive dysfunction can also lead to worse medicine adherence, which is particularly problematic in multimorbid patients with polypharmacy. Furthermore, in the case of clinical significant cognitive dysfunction, remaining life expectancy is limited. Important to note is that although general cognitive function can be intact, cognitive function within a specific cognitive domain can be impaired. Meaning that for example a sufficient score on the Mini-Mental State Examination (MMSE), widely used as a screening tool, does not exclude a solely impaired memory function, executive function of psychomotor speed. Therefore, in selective patient groups a comprehensive geriatric assessment is essential. Depending on the results, adaptations of care and extra attention to prevent further decline are required.

A promising component of future therapeutic interventions are potential biomarkers such as metabolomics or miRNAs, used to unravel specific pathophysiological mechanisms in the interactions described in this thesis. For instance, we recently investigated the role of miRNAs in cerebrovascular changes and cognitive function in the COPE study. We showed that older patients reaching end-stage renal disease had an unfavorable angiogenic profile, as indicated by aberrant levels of Angiopoietin-2 and five angiogenic miRNAs (miR-27a, miR-126, miR-132, miR-223, miR-326), compared to healthy persons and patients with diabetic nephropathy. In addition, Angiopoietin-2 was associated with cerebral small vessel disease and cognitive function (psychomotor speed and executive function), while miR-223 and miR-29a were associated with memory function. (12)

The increasing knowledge regarding preventive strategies is of paramount importance, as the Framingham Heart Study already showed that earlier diagnosis and effective treatment of cardiovascular risk factors or proven CVD, can lead to a decline in incidence of dementia (10). Furthermore, next to a vascular approach of preventing cognitive impairment, it would be of great interest to determine whether interventions in other (interacting) organ systems slow down the process of cognitive impairment. For instance, newer agents which seem to meaningfully slow kidney function decline such as SGLT2 (Sodium-glucose Cotransporter-2) inhibitors, may potentially also slow decline in cognitive function, on top of the fact that such agents also decrease risks of CVD (11).

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CHAPTER 13.

NEDERLANDSE SAMENVATTING

Aangezien wereldwijd de bevolking vergrijst komen leeftijdsgebonden ziekten steeds vaker voor, met name hart- en vaatziekten en cognitieve stoornissen. Er is een groeiende behoefte aan kennis over het samenspel tussen verschillende (co)morbiditeiten, de relatie met de verschillende onderliggende pathofysiologische mechanismen en behandelmogelijkheden van deze (co)morbiditeiten. Derhalve waren de doelstellingen van dit proefschrift het identificeren van patiënten met een hoog cardiovasculair risico en het optimaliseren van de behandeling van deze patiënten. Hieronder wordt een samenvatting gegeven van de belangrijkste bevindingen van het onderzoek zoals beschreven in dit proefschrift. Verder beschrijven we de implicaties van ons onderzoek en bespreken we mogelijke richtingen voor toekomstig onderzoek.

VOORNAAMSTE BEVINDINGEN

DEEL 1. IDENTIFICATIE VAN HOOG CARDIOVASCULAIR RISICO PATIËNTEN

In deel 1 hebben we verschillende interacties tussen (poly)vasculaire ziekten onderzocht en patiënten met een hoog cardiovasculair risico geïdentificeerd. De bevindingen van deel 1 staan beschreven in hoofdstuk 2 tot en met 6.

In hoofdstuk 2 hebben we systematisch in de literatuur gezocht om de verschillen in microvasculaire functie tussen vrouwen en mannen te onderzoeken, waarbij we ons richtten op stabiele patiënten met symptomen van ischemische hartziekte bij afwezigheid van obstructief coronairlijden. Na beoordeling van de volledige teksten werden 13 artikelen geïncludeerd. We ontdekten dat er, afgezien van een hogere incidentie van dit syndroom bij vrouwen, geen klinisch relevante verschillen tussen beide geslachten zijn, gebaseerd op de coronaire stroomreserve (coronary flow reserve) en de bloedstroom in het myocard. Dit werd gemeten met behulp van verschillende methoden, waaronder PET (positronemissietomografie) of coronairangiografie. Voorheen werd de relevantie van microvasculaire disfunctie waarschijnlijk onderschat, echter is tot op heden de volledige klinische betekenis van deze

aandoening nog steeds niet bekend. Derhalve is het niet gerechtvaardigd om aan te nemen dat met name bij vrouwen angina-achtige symptomen bij afwezigheid van obstructief coronairlijden het meest waarschijnlijk te wijten zijn aan microvasculaire disfunctie, zeker bij afwezigheid van objectief bewijs. Daarom is er behoefte aan breed beschikbare niet-invasieve methoden om de microvasculaire functie te beoordelen, ook bij mannen.

De associatie van (poly)vasculaire risicofactoren en -ziekten met cerebrovasculaire veranderingen en cognitieve stoornissen bij oudere patiënten werd besproken in hoofdstuk 3 tot en met 6.

We begonnen in hoofdstuk 3 met het evalueren van de rol van ontsteking via complementreceptor 1-gen polymorfismen (CR1 SNP's) en cognitieve functie bij oudere patiënten met een risico op hart- en vaatziekten, gebruikmakend van de PROSPER studie (the PROspective Study of Pravastatin in the Elderly at Risk). Twaalf van de 18 onderzochte CR1 SNP's waren significant geassocieerd met cognitieve functie in een multivariate atanalyse ($p<0.05$). Uit deze resultaten blijkt dat genetische variatie binnen het CR1-gen niet alleen geassocieerd is met de ziekte van Alzheimer, maar ook met algemene achteruitgang van de cognitieve functie bij oudere mensen. Bovendien waren drie van deze 12 SNP's, die geassocieerd waren met een verbeterde cognitieve functie, ook geassocieerd met lagere niveaus van C-reactief proteïne (CRP), wat eerder bewijs van de rol van inflammatie in de cognitieve functie verder versterkt.

In hoofdstuk 4 en 5 hebben we de interacties tussen hart, nieren en hersenen onderzocht. In hoofdstuk 4 onderzochten we de associatie van cardiovasculaire structuur en functie met cerebrovasculaire veranderingen en cognitieve functie bij oudere patiënten met eindstadium nierfalen. Hierbij hebben we data gebruikt van het Nederlandse prospectieve multicenter cohortonderzoek COPE (Cognitive decline in Older Patients with End stage renal disease). Een MRI werd uitgevoerd om de stijfheid van de aorta (pulse wave velocity) en systolische functie van het hart te meten. De eindpunten waren MRI-afgeleide cerebrovasculaire veranderingen en cognitieve functie in verschillende

neurocognitieve domeinen. Er werden geen statistisch significante associaties waargenomen tussen structurele en functionele cardiovasculaire parameters en structurele cerebrovasculaire veranderingen. Echter, de cognitieve functie was slechter bij patiënten met een hoge vergeleken met een lage pulse wave velocity in alle drie de cognitieve domeinen. Hoewel er potentieel klinisch relevante associaties werden gevonden van hoge pulse wave velocity met slechte cognitie in alle domeinen, bleef na correctie voor leeftijd, geslacht en opleiding alleen de 'Trail Making Test A', welke psychomotorische snelheid meet, statistisch significant (p=0.03). Deze gegevens suggereren dat arteriële stijfheid bij eindstadium nierfalen een onderliggend mechanisme kan zijn voor de ontwikkeling van cognitieve stoornissen. In hoofdstuk 5 bestudeerden we de associatie van nierfunctie met cognitieve functie bij patiënten met een hoog cardiovasculair risico, wederom met behulp van gegevens van PROSPER. Alleen een ernstig afgenomen nierfunctie (stadium 4), maar niet milde tot ernstig afgenomen nierfunctie (stadium 3a en b), was geassocieerd met cognitieve stoornissen en snellere cognitieve achteruitgang in de tijd. De associatie van ernstig afgenomen nierfunctie met cognitieve stoornissen en achteruitgang in de tijd was duidelijker aanwezig bij patiënten met een voorgeschiedenis van hart- en vaatziekten, mogelijk als gevolg van een grotere kans op polyvasculaire schade, zowel in de nieren als in de hersenen, bij patiënten met bewezen hart- en vaatziekten.

Hoofdstuk 6 ging dieper in op de interactie tussen het hart, de nieren en de schildklier in een substudie van PROSPER. Het doel was te onderzoeken of nierfunctie van invloed is op de associatie tussen subklinische schildklierdisfunctie en het risico op belangrijke cardiovasculaire gebeurtenissen (major adverse cardiovascular events [MACE]). Er werd geen statistisch significant verband gevonden tussen subklinische schildklierdisfunctie en MACE met gecorrigeerde hazardratio's (95% betrouwbaarheidsinterval) van 0.51 (0.24 tot 1.07) voor de vergelijking tussen subklinische hyperthyreoïdie en euthyreoïdie en 0.90 (0.58 tot 1.39) voor de vergelijking tussen subklinische hypothyreoïdie met euthyreoïdie. Deze relatie was niet aanwezig in een van de verschillende nierfunctie-groepen, noch was er een interactie van nierfunctie met subklinische schildklierdisfunctie in de

associatie met MACE (p-interactie = 0.60 voor subklinische hyperthyreoïdie en 0.39 voor subklinische hypothyreoïdie). Concluderend werd er geen bewijs gevonden dat de potentiële associatie tussen schildklierhormonen en hart- en vaatziekten werd gemodificeerd door de nierfunctie bij oudere patiënten met subklinische schildklierdisfunctie.

DEEL 2. BEHANDELING VAN HOOG CARDIOVASCULAIR RISICO PATIËNTEN

Deel 2 was gericht op het optimaliseren van de behandeling van patiënten met een hoog vasculair risico, zoals beschreven in deel 1. De bevindingen van deel 2 staan beschreven in hoofdstuk 7 tot en met 11.

Hoofdstuk 7 en 8 bestudeerden behandeling met de PCSK9-remmer alirocumab bij patiënten met een recent acuut coronair syndroom en dyslipidemie ondanks intensieve statinetherapie. Hierbij werd gebruik gemaakt van de studie ODYSSEY OUTCOMES (Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab), waarbij hoofdstuk 7 keek of het hebben van polyvasculaire ziekte de risico's op MACE en overlijden beïnvloedden en wat voor effect alirocumab daar op heeft. In de placebo groep was de incidentie van MACE 10.0% voor patiënten met monovasculaire (coronaire) ziekte, 22.2% voor polyvasculaire ziekte in twee vaatbedden (coronair en perifeer of cerebrovasculair) en 39.7% voor polyvasculaire ziekte in drie vaatbedden (coronair, perifeer en cerebrovasculair). Met alirocumab was de overeenkomstige absolute risicoreductie (95% betrouwbaarheidsinterval) 1.4% (0.6% tot 2.3%), 1.9% (-2.4% tot 6.2%) en 13.0% (-2.0% tot 28.0%), respectievelijk per vasculaire categorie, met een significante p-waarde voor interactie tussen de vasculaire groepen (p-interactie<0.001). Derhalve zijn patiënten met polyvasculaire ziekte na een acuut coronair syndroom een gemakkelijk te identificeren subgroep met een hoog absoluut risico op MACE en overlijden. De grote absolute risicoreductie van PCSK9-remming met alirocumab, wanneer toegevoegd aan statinetherapie met hoge intensiteit, levert grote gezondheidswinst op voor deze populatie. Hoofdstuk 8 werd ontworpen om het effect van alirocumab op beroertes te onderzoeken. Hoewel

verlaging van atherogene lipoproteïnen, inclusief lage-dichtheid lipoproteïne cholesterol (LDL-C), het risico op een ischemische beroerte vermindert, zijn er zorgen geuit over zeer lage LDL-C-spiegels en een mogelijk verhoogd risico op een hemorragische beroerte. In onze tweede studie van ODYSSEY OUTCOMES verminderde alirocumab het risico op beroertes (hazard ratio 0.72 [0.57 tot 0.91]) inclusief ischemische beroertes (0.73 [0.57 tot 0.93]) zonder het risico op hemorragische beroertes te verhogen (0.83 [0.42 tot 1.65]), ongeacht de LDL-C concentratie op baseline en een voorgeschiedenis van cerebrovasculaire ziekte. Er leek geen verband tussen een lager bereikte LDL-C concentratie en de incidentie van een hemorragische beroerte in de alirocumab-groep. Concluderend tonen de huidige bevindingen aan dat alirocumab bij patiënten met een recent acuut coronair syndroom en dyslipidemie ondanks intensieve statinetherapie, het risico op een beroerte verlaagt gedurende een mediane follow-up van 2.8 jaar. Het risico op een hemorragische beroerte lijkt niet afhankelijk te zijn van de bereikte LDL-C-spiegels binnen de alirocumab-groep.

Hoofdstuk 9 gaat dieper in op de effectiviteit en veiligheid van behandeling met een andere PCSK9-remmer, namelijk inclisiran, bij patiënten met een verminderde nierfunctie. Aangezien patiënten met een verminderde nierfunctie een hoog risico hebben op MACE en overlijden, is er behoefte aan alternatieve en effectievere lipide-verlagende therapieën. In een gecombineerde analyse van het fase 1 ORION-7-nieronderzoek en het fase 2 ORION-1-onderzoek werden de farmacodynamische eigenschappen van inclisiran bestudeerd bij proefpersonen met een normale nierfunctie en bij proefpersonen met een milde, matige of ernstige nierfunctiestoornis. Wright et al. vonden dat de farmacodynamische effecten en het veiligheidsprofiel van inclisiran vergelijkbaar waren voor een normale versus verminderde nierfunctie (1). Hoewel de plasmaconcentraties van inclisiran parallel toenemen aan de mate van verslechtering van de nierfunctie, was inclisiran niet detecteerbaar in de bloedbaan 48 uur na injectie in alle groepen, terwijl het lipide-verlagende effect langer dan 6 maanden aanhoudt. Derhalve lijkt inclisiran op korte termijn een effectieve en veilige optie te zijn bij patiënten met een verminderde nierfunctie. Een potentieel belangrijk voordeel is het

aanhoudende farmacologische effect van dit middel, waardoor injecties slechts eens in de zes maanden nodig zijn. Een dergelijke dosering kan op zijn beurt de kosten verlagen, kan aantrekkelijker zijn voor patiënten en kan de therapietrouw bevorderen bij deze patiënten welke vaak meerdere geneesmiddelen nodig hebben.

De mogelijke toepassingen van PCSK9-remming zijn samengevat in hoofdstuk 10. Hierin beschrijven we de behandeling van makkelijk identificeerbare patiënten met een hoog cardiovasculair risico, zoals eerder in dit proefschrift geïdentificeerd, zodat werkzaamheid en efficiëntie kunnen worden geoptimaliseerd. Het monitoren van de werkelijke hoeveelheid aderverkalking zou waarschijnlijk de meest nauwkeurige mechanistische stratificatie van het vaatrisico opleveren. Hoewel er verschillende beeldvormende technieken bestaan, is er momenteel geen enkele haalbaar in de dagelijkse routine. Daarom moeten surrogaatmarkers voor aderverkalking worden gebruikt, zoals LDL-C. Bovendien kunnen patiënten worden geclassificeerd op basis van klinische kenmerken die ook de hoeveelheid aderverkalking weerspiegelen, wat een gemakkelijke en kosteneffectieve manier is om een optimale behandeling te bereiken. Bekende klinische kenmerken met een hoog risico zijn onder meer patiënten met nierfalen of diabetes mellitus, en ook patiënten met bekende hart- en vaatziekten, zoals een voorgeschiedenis van een coronaire bypass operatie of het hebben van polyvasculaire ziekte. Deze patiënten met veel aderverkalking hebben vaak het grootste klinische voordeel van een intensieve cardiovasculaire behandeling; omdat het absolute risico hoog is, zijn de absolute risicoreducties ook relatief hoog. Naast andere gevestigde en nog te ontwikkelen therapieën bij atherosclerose, heeft behandeling met PCSK9-remmers een groot klinisch voordeel met weinig bijwerkingen en is daarom mogelijk ook geschikt voor oudere patiënten die meer vatbaar zijn voor bijwerkingen.

Ten slotte werd in hoofdstuk 11 het effect van schildklierhormoontherapie op klinische uitkomsten en cardiale bijwerkingen besproken voor oudere patiënten met subklinische hypothyreoïdie. Hierbij werd gebruik gemaakt van gecombineerde data van de gerandomiseerde dubbelblinde,

placebogecontroleerde, parallelle studies, TRUST (Thyroid hormone Replacement for Untreated older adults with Subclinical hypothyroidism – a randomised placebo controlled Trial) en IEMO80+ (Institute for Evidence Based Medicine in Old Age 80-plus thyroid trial). Behandeling met levothyroxine leverde geen duidelijk voordeel op bij ouderen met een diagnose subklinische hypothyreoïdie. Daarbij verhoogde levothyroxine het risico op cardiovasculaire bijwerkingen niet, zoals een combinatie van cardiovasculaire gebeurtenissen (hazard ratio 0.74 [0.41-1.25]), atriumfibrilleren (0.69 [0.32-1.52]) of hartfalen (0.41 [0.13-1.35]), onafhankelijk van een voorgeschiedenis van hart- en vaatziekten of een hoge leeftijd. Dus indien er klinische indicaties zijn om subklinische hypothyroïdie te behandelen met levothyroxine, hoef je niet beducht te zijn op cardiale bijwerkingen, ook niet bij een voorgeschiedenis van hart- en vaatziekten of een hoge leeftijd.

IMPLICATIES EN TOEKOMSTPERSPECTIEVEN

In dit proefschrift hebben we de complexiteit van verschillende interacties van (poly)vasculaire ziekten verder ontrafeld. Deze ziekten kunnen uiteindelijk leiden tot een verhoogd risico op niet alleen cognitieve stoornissen, maar ook op MACE en zelfs sterfte. Identificatie van deze patiënten en een beter begrip van het samenspel en de onderliggende mechanismen van deze ziekten is de eerste stap richting mogelijkheden tot preventie. Het behandelen van deze patiënten met een hoog risico kan een therapeutische uitdaging zijn, maar er is toenemende kennis over zowel gevestigde als zich nog ontwikkelende therapieën om de werkzaamheid en efficiëntie te optimaliseren.

Onze onderzoeken naar PCSK9-remming en schildklierhormoontherapie benadrukken het belang van gepersonaliseerde geneeskunde ('personalized medicine'). Een geïndividualiseerde aanpak is dingend noodzakelijk bij de toenemende complexiteit van de gezondheidszorg, niet alleen om de werkzaamheid van een behandeling te maximaliseren, maar ook om de kosteneffectiviteit te vergroten. In het geval van behandeling met PCSK-9 remming is bekend dat dit de mogelijkheid biedt om LDL-C verder te verlagen dan meestal haalbaar met statines. Deze behandeling is echter

relatief duur. Daarom is het cruciaal om te prioriteren en om de waarde van deze behandeling in geselecteerde patiëntengroepen te optimaliseren (2). In de toekomst zullen patenten vervallen, waardoor de kosten van deze therapie worden verlaagd. Naast de PCSK9-remmers die al op de markt zijn, namelijk alirocumab en evolocumab, bevindt zich in ontwikkelingsfase III momenteel een nieuwe veelbelovende strategie voor het toedienen van klein interfererend RNA gericht op PCSK9, zoals inclisiran (3). Een potentieel belangrijk voordeel is het aanhoudende farmacologische effect van dit middel, waarbij injecties slechts eenmaal per zes maanden nodig zijn in plaats van elke twee weken, wat nodig is voor alirocumab en evolocumab. Dit kan aantrekkelijker zijn voor patiënten en kan medicatietrouw vergroten bij patiënten die vaak meerdere medicijnen nodig hebben, waardoor het mogelijk meer kosteneffectief is en ook de kwaliteit van leven verbetert. De werkzaamheid en veiligheid op lange termijn moeten echter nog worden bepaald.

Enkele kernpunten in de kosteneffectieve behandeling voor risicovolle of oudere patiënten moeten worden opgemerkt. In de eerste plaats moet rekening worden gehouden met de levensverwachting en daarbij de tijd tot verwacht effect van een behandeling. Daarnaast is de levensverwachting alleen relevant als de kwaliteit van leven acceptabel blijft. Kalenderleeftijd is opzichzelfstaand geen contra-indicatie voor de bovengenoemde behandelingen, maar er moet rekening worden gehouden met het belang van de biologische leeftijd, geriatrische stoornissen en kwetsbaarheid. De behandeling kan anders zijn voor kwetsbare ouderen dan voor diegenen die tot op hoge leeftijd vitaal zijn. Om de behandeling te optimaliseren moeten patiënten met een hoog risico worden gescreend op functionele status en cognitieve functie. Bij bloeddrukmanagement moet bijvoorbeeld rekening worden gehouden met cognitieve stoornissen, omdat we weten dat de cognitieve functie door een lage bloeddruk verder kan verslechteren. Cognitieve disfunctie kan ook leiden tot een slechtere therapietrouw, wat met name problematisch is bij multimorbide patiënten met polyfarmacie. Bovendien is de resterende levensverwachting bij klinisch significante cognitieve stoornissen beperkt. Belangrijk om op te merken is dat hoewel de algemene cognitieve functie intact kan zijn, de cognitieve functie binnen een

specifiek cognitief domein kan zijn aangetast. Dit betekent dat bijvoorbeeld een voldoende score bij de 'Mini-Mental State Examination' (MMSE), dat veel gebruikt wordt als screeningstest, een specifieke stoornis in het geheugen, uitvoerende functies of psychomotorische snelheid niet uitsluit. Daarom is bij selectieve patiëntengroepen een uitgebreide geriatrische beoordeling essentieel. Afhankelijk van de resultaten zijn aanpassingen van de zorg en extra aandacht nodig om verdere achteruitgang te voorkomen.

De toenemende kennis over preventiestrategieën is van het grootste belang, aangezien de Framingham Heart Study al heeft aangetoond dat een eerdere diagnose en effectieve behandeling van cardiovasculaire risicofactoren of bewezen hart- en vaatziekten kan leiden tot een afname van de incidentie van dementie (4). Bovendien zou het, naast een vasculaire benadering om cognitieve stoornissen te voorkomen, interessant zijn om te bepalen of interventies in andere interacterende orgaansystemen het proces van cognitieve achteruitgang vertragen. Nieuwere middelen die de achteruitgang van de nierfunctie significant lijken te vertragen, zoals SGLT2-remmers, kunnen mogelijk ook de achteruitgang van de cognitieve functie vertragen, bovenop de vermindering van het risico op hart- en vaatziekten (5). Daarnaast moet toekomstig onderzoek zich ook richten op andere potentiële biomarkers zoals miRNA's of metabolomics om specifieke pathofysiologische mechanismen te ontrafelen in de interacties beschreven in dit proefschrift, die daarna ook kunnen bijdragen aan mogelijke interventies.

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ABBREVIATIONS

15-WVLT, 15-Word Verbal Learning Test, immediate and delayed;
95% CI, 95% confidence interval;

ACE, angiotensin converting enzyme;
Ach, acetylcholine;
ACS, acute coronary syndrome;
ADP, adenosine diphosphate receptor;
AKI, acute kidney injury;
ARB, angiotensin receptor blocker;
ARR, absolute risk reduction;

Barthel, the Barthel index;
BMI, body mass index;

CABG, coronary artery bypass grafting;
CAC, coronary artery calcium;
CBF, coronary blood flow;
CeVD, cerebrovascular disease;
CFR, coronary flow reserve;
CHD, coronary heart disease;
CI, cardiac index;
CKD, chronic kidney disease;
CMD, coronary microvascular dysfunction;
CO, cardiac output;
COPE, Cognitive decline in Older Patients with End stage renal disease;
CR1, complement receptor 1;
CRP, C-reactive protein;
CVA, cerebrovascular accident;
CVD, cardiovascular disease;
CVR, coronary vascular resistance;

DBP, diastolic blood pressure;

EF, ejection fraction;
eGFR, estimated glomerular filtration rate;
EQ-5D, EuroQol Group 5-Dimension Self-Report Questionnaire;
ESRD, end-stage renal disease;

FFR, fractional flow reserve;
FOURIER, Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Patients With Elevated Risk;
fT4, free thyroxine 4;

GFR, glomerular filtration rate;
GWAS, genome-wide association studies;

Hb, hemoglobin,
HDL-C, high-density lipoprotein cholesterol;
HR, hazard ratio;

IADL, Instrumental Activities of Daily Living;
ICA, invasive coronary angiography;
IEMO80+, the Institute for Evidence-Based Medicine in Old Age 80-plus thyroid trial;
IMR, index of microcirculatory resistance;
IPD, individual participant data;
IQR, interquartile range;

LA, luminal area;
LDL-C, low-density lipoprotein cholesterol;
LDST, Letter Digit Substitution Test;
LDT, Letter-Digit Coding Test;

MACE, major adverse cardiovascular events;
MBF, myocardial blood flow;
MMSE, Mini-Mental State Examination;
MRI, magnetic resonance imaging;

NNT, number needed to treat;
NT-proBNP, N-terminal pro b-type natriuretic peptide;

ODYSSEY OUTCOMES, Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab;
OR, odds ratio;

PAD, peripheral artery disease;
PCSK9, proprotein convertase subtilisin–kexin type 9;
Pd, mean distal coronary pressure;
PLTd, Picture-Word Learning Test – delayed recall;
PLTi, Picture-Word Learning Test – immediate recall;
PROSPER, the PROspective Study of Pravastatin in the Elderly at Risk;
PWV, pulse wave velocity;
QCA, quantitative coronary angiography;

RCT, randomised controlled trial;
RPP, rate pressure product;

SBP, systolic blood pressure;
SCH, subclinical hypothyroidism;
SCWT, Stroop Color Word Test;
SD, standard deviation;
SE, standard error;
siRNA, small interfering ribonucleic acid
SNP, single nucleotide polymorphism;
Stroop, Stroop-Colour-Word Test;

ThyPRO, Thyroid Related Quality-of-Life Patient-Reported Outcome measure;
TIA, transient ischemic attack;
Tmn, mean transit time;
TMTA/TMTB, Trail Making Test A&B;
TRUST, Thyroid hormone Replacement for Untreated older adults with Subclinical hypothyroidism – a randomised placebo controlled Trial;
TSH, thyroid stimulating hormone;
TST, Treat Stroke to Target;

VAT, Visual Association Test;

WMH, white matter hyperintensities;

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CURRICULUM VITAE

De auteur van dit proefschrift werd op 24 april 1992 geboren te Zwolle, waar zij in 2010 haar eindexamen behaalde aan het Gymnasium Celeanum. Aan de Universiteit Leiden studeerde zij geneeskunde. Zij rondde haar studie af met haar semi-arts stage in 2017 op de afdeling Cardiologie van het Leids Universitair Medisch Centrum, waar zij in februari 2018 als ANIOS begon.

Aansluitend heeft zij promotieonderzoek verricht op de afdeling Cardiologie in samenwerking met de Ouderengeneeskunde van september 2017 tot 2020, waarvan de resultaten zijn beschreven in dit proefschrift.

Per 1 september 2020 is zij gestart met de vooropleiding Interne Geneeskunde in het Alrijne Ziekenhuis te Leiderdorp (opleider dr. Hardi) als onderdeel van de opleiding Cardiologie vanuit het Leids Universitair Medisch Centrum (opleider dr. Trines).